Editorial

The year 2021 is marked to the success on Corona vaccines and immunization jabs crossing 108Crplus in December 2021, 40 Cr peoplesi.e.25.9% population fully vaccinated. Friends Immunization is the best protection against deadly diseases as it prevents individual illness and large-scale outbreaks. Immune system has two lines of defence: Innate (nonspecific) immunity.Adaptive (specific) immunity Vaccines are made of dead or alive viruses to get registered in the body and Both stimulate the proliferation of T and B cells, resulting in the formation of effector and memorycells. The body is exposed to weakened or dead pathogenic viruses. The body's immune cells make antibodies to attack the pathogenic viruses. If the body is exposed to the pathogenic viruses again, the body will be prepared with antibodies.
India was successful in developing COVAXIN®, India's indigenous *COVID-19 vaccine* by Bharat Biotech is developed in collaboration with the Indian Council of Medical Research (ICMR) - National Institute of Virology (NIV) The vaccine is developed using *Whole-Virion Inactivated Vero Cell* derived platform technology. Inactivated vaccines do not replicate and are therefore unlikely to revert and cause pathological effects. They contain dead virus, incapable of infecting people but still able to instruct the immune system to mount a defensive reaction against an infection.India was also successful to manufacture resistance boosters from Indian herbs as proactives medicines.

Molnupiravir: First pill to treat Covid gets approval in UK The drug needs to be given within five days of symptoms developing to be most effective. Molnupiravir, like remdesivir, is a nucleoside analogue, which means it mimics some of the building blocks of RNA. But the compounds work in entirely different ways. When SARS-CoV-2 enters a cell, the virus needs to duplicate its RNA genome to form new viruses. Remdesivir is a 'chain terminator'. It stops the enzyme that builds these RNA 'chains' from adding further links. Molnupiravir, on the other hand, gets incorporated into burgeoning RNA strands and, once inside, wreaks havoc. Those RNA strands become faulty blueprints for the next round of viral genomes. When enough mutations accumulate, the viral population collapses. "That is what we term lethal mutagenesis,"
Friends Dengue is another deadly viral disease and effected person rapidly the patients looses platelets from the blood here also Indian herb and neutraceuticals works for rapid growth of platelets in which papaya, blackpepper, harsinghar, mango fruits and goat milk are commonly known to enhance the blood platelets. Research is required to know all this and better products are to be designed. My good wishes to all of you who are participating as an author or as a participant for this great event.

Dr.S.Farooq
Chief Editor (UJPAH)

Contents

Screening And Characterization Of Bioactive Molecules Derived From Medicinal Plants For Antileishmanial Activities For*Leishmania donovani*

[1]Arushdeep Sidana, [1,2*] Umar Farooq , [2]Amir Khan, [1]Shiwani Kausaland [1]Azhar Khan
[1]Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, HP, India
[2]Department of Oral medicine and Allied Dentistry, Faculty of Dentistry, Taif University, Taif.KSA
[*]**Email: ufarooq8"gmail.com**

Abstract-Leishmaniasis is a vector-borne disease caused by genus *Leishmania*.It causes significant morbidity and mortality in the endemic areas of several developing countries. Due to multidrug resistance in *Leishmania spp* and unavailability of an effective vaccine, discovery of new drugs is urgently needed. The aim of the present study was screening of medicinal plants used as Indian traditional medicine for leishmanicidal activity. Promastigote forms of Leishmania parasite were cultured in-vitro in NNN medium and further sub-cultured and maintained in RPMI-1640 medium containing 10% Fetal Bovine Serum for the screening of medicinal plants. A total of 26 medicinal plants were collected and screened for leishmanicidal activity. The methanolic extracts showing antileishmanial activity were subjected to LC-MS analysis to identify the major phyto-constituents in the crude methanolic extracts. The compounds were detected in the LC-MS of active extracts. The plant extract showing maximum antileishmanial activity was further fractionated to isolate the major compound(s).The compound isolated was characterised by IR, Mass spectrometry and subjected to *in vitro* antileishmanial activity against *L. donovani* promastigotes. *In vitro* antileishmanial assay revealed that crudemethanolic extracts of 2/10 plants were active against *L. donovani* promastigotes. Methanolic root extracts of *Inula racemosa were* found active (54.83%) against the parasite while T.*Terrestris* were found least active with such as toxicity, percent inhibition 43.10% at concentration of 500 µg/ml. *Inula racemosa* methanolic extract further fractionated subjected for LC-MS analysis. The compound isolated from the methanolic root extract of *I.racemosa was* isoalantolactone, which did not show any antileishmanial activity against *L. donovani*. The study suggested that crude extract of *Inula racemosa* and *T. Terrestris* have shown potent antileishmanial activity while extracted bioactive molecules does not show efficacy against *Leishmania* parasite. Hence the antileishmanial activity could be due to any other compound which could not be detected, so further study is undertaken.

Key words: *Inula racemosa, T. terrestris, L. donovani,* MDR, LC-MS and IR.

Introduction

Leishmaniasis is a major public health problem caused by an intracellular obligate protozoan parasite of the genus *Leishmania*. Leishmaniasis, by its various clinical forms, causes significant morbidity and mortality in the developing countries of the world every year. It is estimated that 0.2 to 0.4 million new cases of visceral leishmaniasis, 0.7 to 1.3 million new cases of cutaneous leishmaniasis with 20,000 to 30,000 deaths occur every year worldwide (Rama et al 2015). Despite the severity of this parasitic disease till date, there is no vaccine available to efficiently prevent or cure leishmaniasis. The currently used chemotherapy has unpleasant side effects variable efficiency between different species and development of drug resistance in the parasite [Camacho et al 2003, Croft et al 2006]. People living in rural areas of the developing countries are still dependent on traditional medicines toheal theirailments (Chan-Bacab et al 2006). There is a need to explore the potential of naturalproducts obtained from the plants used by traditional healers which may eliminate the parasite from the host without causing any side effects. Several plant species have been screened for leishmanicidal activity after extraction with different solvents like petroleum ether, chloroform, hexane, ethyl acetate, methylene chloride, methanol and

tested *in vitro* and *in vivo* against *Leishmania*parasite (Fournet et al 1996, Pereira et al 2010, Ghosh et al 2011).The discovery and development of leishmanicidal biomolecules is also aided by the biochemical studies of the metabolic pathways of the parasite. In this approach, the enzymes essential for the survival of *Leishmania* are identified and targeted by lead molecules (Ogungbe et al 2013, Sidana et al 2015, 2018). The Indian plants have notbeen screened extensively for leishmanicidal activity and there is a need to search for new therapeutic agent(s) from the diverse range of medicinal plants found in North Indiaagainst *Leishmania spp*. The extracts of different parts of medicinal plants may be screened *in vitro*against *Leishmania spp*. to evaluate their potency and efficacy to inhibit the parasite. The biological activity of plant extracts will be attributed to compounds belonging to diverse chemical groups including alkaloids, flavonoids, phenylpropanoids, steroids and terpenoids (Iwu *et al.,*1994; Rocha *et al.,* 2005; Wang *et al.,* 2009).Thepresent study was aimed to assess the antileishmanial potential of methanolic extract of different medicinal plants *in vitro* against *L.donovani*and further extraction and characterization of bioactive molecules from active plant extract against *L. donovani*to recognize the compounds responsible for the antileishmanial activity.

Material and Methods

Collection of plant material

The medicinal plants used for antileishmanial activity were obtained from Y.S.Parmar University of Horticulture and Forestry, Nauni, HP and Arya Vastu Bhandar, Dehradun, UK, India. A total of 10 authenticated medicinal plants were procured (Table-1).

Table-1 Medicinal plants screened for *in vitro* antileishmanial activity

S. No	Botanical name	Family	Local name	Part used
1	*Acorus calamus*	Acoraceae	Boiye	Leaves
2	*Alstoniascholaris*	Apocynaceae	Chitvan	Leaves
3	*Inula racemosa*	Asteraceae	Pushker	Root
4	*Tribulus terestris*	Zygophyllaceae	Gokhru	Leaves
5	*Aegle marmelos*	Rutaceae	Bael	Dried fruit
6	*Albizia procera*	Fabaceae	Safed Siris	Leaves
7	*Andrographis paniculata*	Acanthaceae	Kalmegh	Stem
8	*Asparagus abscendens*	Liliaceae	Safed musli	Roots
9	*Cassia fistula*	Fabaceae	Amaltash	Fruit
10	*Embeliaribes*	Primulaceae	Vidanga	Fruits

Preparation of plants extracts

The parts of plants were washed with tap water and then with distilled water,followed by drying on absorbing paper at room temperature in open air under shadefor 10-15 days. The dry plant parts were ground to yield coarse powder and stored atambient temperature in amber glass bottles until use. The powder of each plant partwas extracted with methanol using hot Soxhlet extraction for 24 hours. Afterextraction, the extracts were concentrated under reduced pressure using rotaryevaporator. The concentrated extracts were further dried in a desiccator using calciumchloride as a desiccant. The dried extracts were weighed to obtain the percentage yield and stored in air tight bottles at 4°C until use.

In Vitro Assessment of Antileishmanial Activity Parasite stock culture

The Axenic culture of *L. donovani* (LdMIPL-1) was maintained at 25°C in RPMI 1640 (Himedia) medium supplemented with 10% heat inactivated Fetal Bovine Serum (FBS,

Himedia), streptomycin (150 µg/ml), penicillin G (100 µg/ml) and gentamycin (150 µg/ml) at pH 7.2.

Antileishmanial assay

For antileishmanial activity, promastigotes of L. donovani were sub-cultured in Schneider's Insect Medium (Himedia) supplemented with 10% heat inactivated FBS, streptomycin (150 µg/ml), penicillin G (100 µg/ml) and gentamycin(150 µg/ml). The antileishmanial screening was performed in 96-well flat bottom tissue culture plates (Corning Life Sciences, USA). One hundred microliters of cell suspension containing 2 × 106 to 3 × 106 cells/ml was poured in each well of the plate. Four different concentrations of the methanolic root extract i.e. 100, 250, 350 and 500µg/ml dissolved in dimethyl sulfoxide (<0.025% v/v) were added to the culture. The plates were then incubated at 25°C for 24-48 hours. Amphotericin B and sodium stibugluconate were used as positive controls and cell suspension with 0.025% DMSO was used as a negative control. The inhibition of the promastigotes was assessed by measuring the cleavage of 10mg/ml of MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide]. The absorbance was measured by using ELISA plate reader (BioTek, USA) at 595nm. The percent growth inhibition was calculated by the following formula:

$$\% \textbf{ of inhibition} = \frac{\textbf{OD control} - \textbf{OD treated}}{\textbf{OD control}} \times \mathbf{100}$$

IR, Mass spectrometry studies

IR spectrum was taken on an FT-IR spectrophotometer (Model RZX, Perkin Elmer) using KBr pellet. Mass spectrum was recorded on Model Q-ToF Micro (Waters) spectrometer. Isolation and characterization of pure compound from methanolic root extract of *I.racemosa*The methanolic extract of the dried and powdered roots of *I. racemosa* was fractionated by using ethyl acetate and petroleum ether. The fraction in petroleum ether was dried and fine crystals of the compound were obtained. The crystalline white compound was characterized on the basis of its spectral data.

Statistical analysis: The antileishmanial assay was performed in triplicate with three replicates of each concentration tested. The results were expressed as mean ± standard error of mean. The overall variation in a set of data was analysed by one way analysis of variance (ANOVA). A value of P <0.05 was considered significant.

Result

In vitro antileishmanial activity of medicinal plants.A total of 10 methanolic extracts of different parts of medicinal plants used as traditional medicine in India were evaluated for their antileishmanial activity by usingMTT reduction assay. Out of the 10 extracts, only two methanolic extracts of*Inula racemosa*roots,and *Tribulus terrestris*leaves showed significant *in vitro*antileishmanial activity against *L. Donovani*promastigotes (Figure-1 and 2). Theremaining 8 plant extracts have not shown any activity against *L. donovani*, since thehighest concentration (500 µg/ml) of all 8 extracts could not inhibit the growth of the parasite.

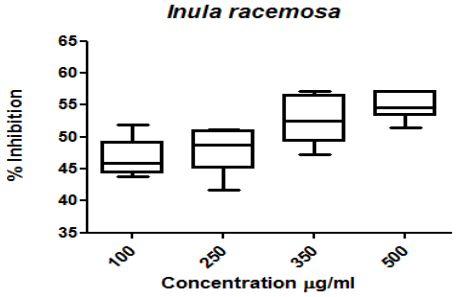


Figure-1 Antileishmanial activity of crude methanolic roots extract of *I. racemosa*

Table-2 Percent inhibition of *L. donovani* promastigotes after 24 hours of incubation with four different concentrations of methanolic root extract of *I.racemosa* and standard error of the mean.

Conc (µg/ml)	100	250	350	500	P value
Mean % Inhibition	46.72	47.91	52.63	54.83	0.0007
SEM	1.224	1.454	1.536	0.892	***

The most active extract was that of the *Inula racemosa*roots which showed54.83% inhibition of *L. Donovani*promastigotes within 24 hours of incubation at 25°C.The inhibition of the parasite was concentration dependent, as it increases with the increasein extract concentration. The means of the percent inhibition were considered significantwith a P value of 0.0007 (P<0.05) (Table-2).Methanolic leaves extract of *Tribulus terrestris* also showed significant activity against *L. donovani promastigotes* with a percent inhibition of 42.77% at a concentration of 500 µg/ml followed by 40.29, 38.14 and 30.50% inhibition at 350, 250 and 100 µg/ml, respectively. The means of percent inhibition were considered significant with a P value of 0.0003 (P<0.05) (Table-3).

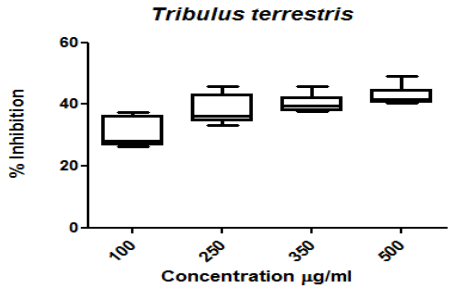


Figure-2 Antileishmanial activity of crude methanolic leaves extract of *T. Terrestris*

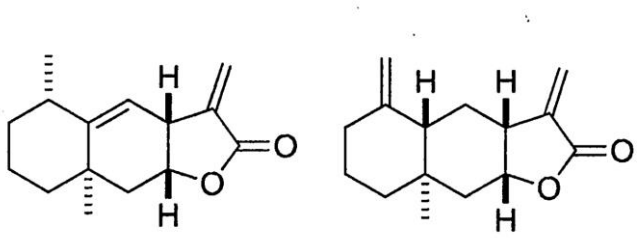
Table-3 Percent inhibition of *L. donovani* promastigotes after 24 hours of incubation with four different concentrations of methanolic leaves extract of *T.Terrestris* and standard error of the mean.

Conc (µg/ml)	100	250	350	500	P value
Mean % Inhibition	30.50	38.14	40.29	42.77	0.0003
SEM	1.978	1.986	1.219	1.324	***

Out of the two active methanolic extracts, minimum antileishmanial activity was observed with the leaves extracts of *Tribulus terestris*. While the methanolic root extracts of *I. racemosa* have shown maximum antileishmanial activity against *L. donovani*. Therefore, the methanolic root extract of *I. racemosa* was further subjected for fractionation for the isolation of compound(s) which may be responsible for antileishmanial activity.

LC-MS analysis of active methanolic extracts

The LC-MS analysis of active methanolic extracts *Inula racemosa* was carried out to identify the major compounds present in the extracts which may be responsible for antileishmanial activity.



A B

Figure-3-Structure of compounds indicated by LC-MS analysis.

The molecular ion peak was present at *Inula racemosa*, the major molecular ion was detected at *m/z* 255 [M^+Na^+]. This molecular mass matched with two major constituents of *m/z*286 [M^+] (Figure 4). In the case of *Inula viz.* alantolactone (**A**) and isoalantolactone (B)

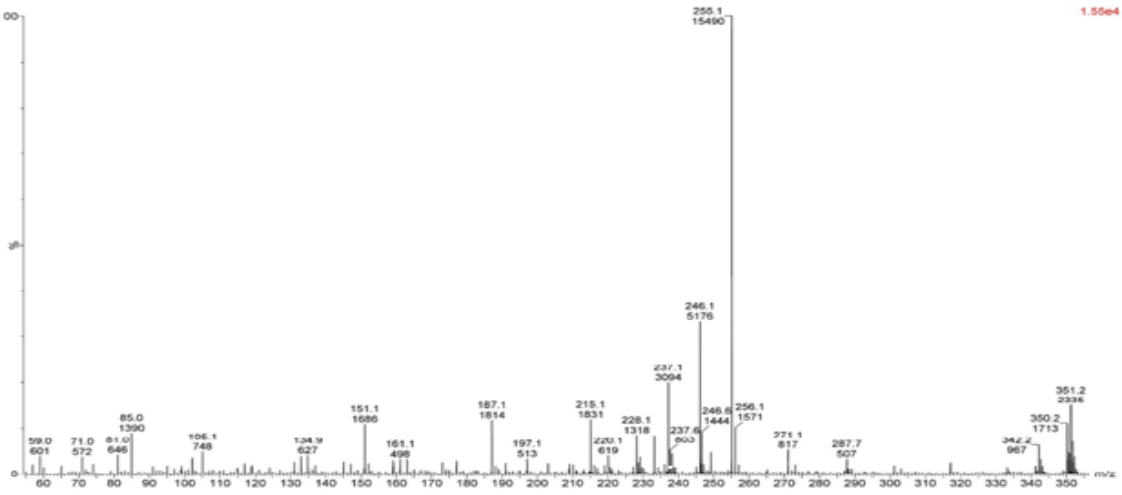


Figure-4 Mass Spectrum of methanolic root extract of *I. recimosa*

Isolation and characterisation of pure compound from methanolic rootextract of *I. racemosa*

The methanolic extract of the dried andpowdered roots of*I. racemosa*was fractionated by using ethyl acetate and petroleum ether. The fraction in petroleum fractionated by using ethyl acetate and petroleum ether. The fraction in petroleumether was dried and fine crystals of the compound were obtained. The crystalline white compound was characterized on the basis of its spectral data(Figure-5).

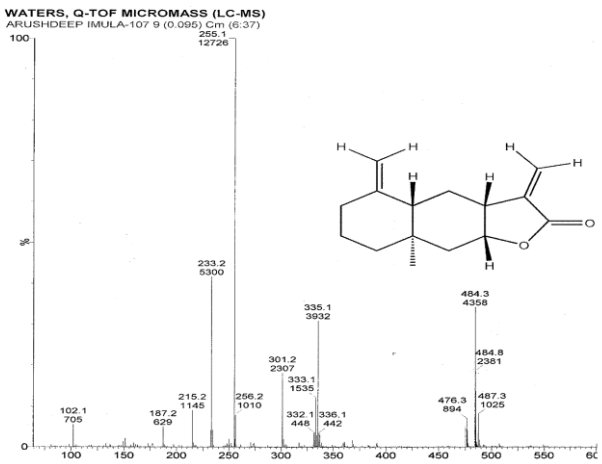


Figure-5 Mass spectrum of Isolantolactone

The IR spectrum indicated the presence of a lactone in thestructure (1752 cm-1). Other functional groups such as hydroxyl, amino andcarboxylic were not present in the IR spectrum. The mass spectrum presented the base peak at 255 [M^+Na^+] and the molecular ion peak at 233 [M^+H^+] depicting.Themolecularmass to be 232. This molecularmasscorresponded to

isolantolactone andlantolactone,the two isomeric lactones reported from *Inula* species (Figure-6).

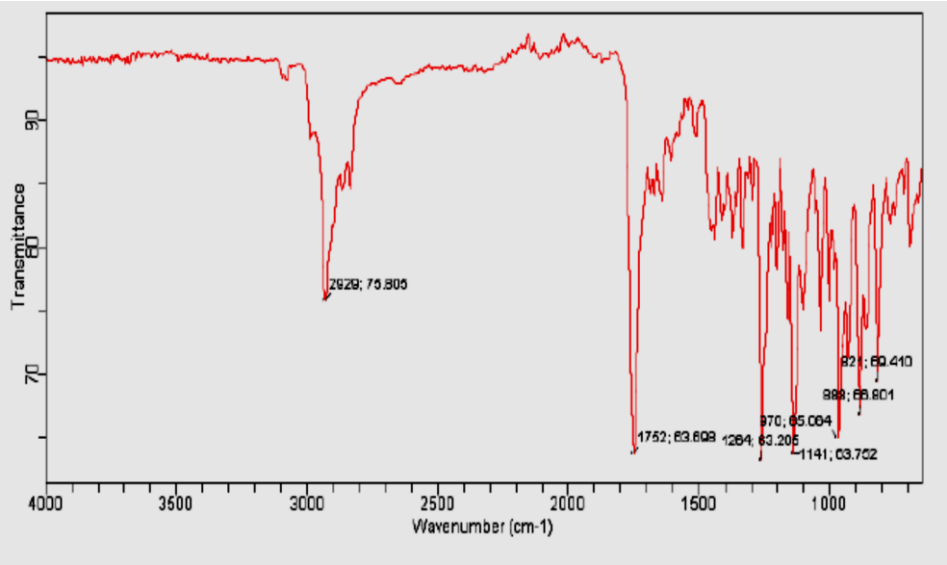


Figure-6 Infrared spectrum of compound isolated from methanolic root extract of *I. racemosa*

Further, the compound was identified by comparison of physical properties and the obtained spectral data with the literature values of isoalantolactone (Table-4)

Table-4 Physical properties of the compound isolated from methanolic roots extract of *I. racemosa.*

S. No	Physical Properties of Compound	Observation
1	Color	White
2	Melting Point	112° C
3	Solubility	Methanol

Discussion

Leishmaniasis, being prevalent in developing countries, creates a worldwide health problem. Control of the disease becomes more difficult due to lack of an effective vaccine and safe and economical antileishmanial drugs. The current available antileishmanial drugs including pentavalent antimonials, liposomal formulations of amphotericin B, pentamidine and miltifosine, all of these are associated with one or more drawbacks which limit their uses (Santos et al 2008). In the search of new antileishmanial drugs, research is going on worldwide and is more concentrated towards the use of natural products rather than synthetic chemicals. Biological molecules extracted from plant and marine natural products represent an important source of novel therapeutics against various forms of leishmaniasis (Rocha et al 2005, Tempone et al 2011).The survey of the plant kingdom for finding a potent antileishmanial agents is going on from decades and several active biomolecules have been exposed with strong antileishmanial effect (Sen and Chatterjee, 2011). The present study was aimed to screen medicinal plants used in traditional medicine in India for antileishmanial activity against *L. donovani*. A total of 26 methanolic extracts were prepared from different parts of the plants. Out of the 26 crude methanolic extracts, two have shown significant leishmanicidal activity by inhibiting 35-55% promastigotes within 24 hours of application.

Tribulus terrestris (Zygophyllaceae) has shown 42.77% inhibition of the *L. donovani*

promastigotes at a concentration of 500 µg/ml. In earlier studies, extract of *Peganum harmala* (Zygophyllaceae) was reported to show potent *in vitro* antileishmanial activity against *L. major* (Mirzaie*et al.,* 2007). Similarly, potent *in vitro* and *in vivo* antileishmanial activities of crude hydro-alcoholic extract of *Peganum harmala* seeds were observed against *L. major* (Rahimi-Moghaddam *et al.,* 2011). The ethanolic fruit extract of *T. Terrestris* was

reported to have very potent antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Proteus vulgaris* and *Corynebacterium diphtheria* (Al-Bayati and Al-Mola, 2008). Similarly, we have also reported potential antileishmanial activity of crude methanolic extract of *Acorus calamus, Alstoniascholaris*and *Berberis aristate* (Sidana et al 2015).

The methanolic root extract of *Inula racemosa* (Asteraceae) was found most active extract with 54.83% inhibition of the *L. donovani* promastigotes. Similarly, the ethanolic root extract of *Echinacea purpurea* of family Asteraceae has been reported to possess potent antileishmanial activity against promastigotes of *L. major* (Soudi*et al.,* 2007).The dichloromethane extracts of aerial parts of *Acanthospermumhispidum* (Asteraceae) has also been reported to show potent *in vitro* antileishmanial activity against *L. Mexicana* (Bero*et al.,* 2011). The LC-MS analysis of methanolic root extract of *I. racemosa* revealed the presence of alantolactone and isoalantolactone. The antileishmanial activity of these compounds has not been reported so far. In the present study, owing to the potent antileishmanial activity of *I. racemosa*, we have fractionated the methanolic root extract of this plant to isolate the major compound(s) present in it which may be responsible for the antileishmanial activity. The isolated compound was then characterized by its physical properties and spectral studies like IR, Mass and NMR.

The compound obtained was white in colour with molecular mass of 232 and melting point of 112°C. The structural formula of the compound was C15H20O2. The isolated compound was identified as isoalantolactone. Further, this compound was subjected for *in vitro* antileishmanial activity against promastigotes of *L. donovani*. However, it did not show any antileishmanial activity. Contrary to this, the isomer of this compound i.e. alantolactone has been reported to exhibit potent antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus cereus* (Lokhande *et al.,* 2007). Both alantolactone and isoalantolactone have also been reported to show antifungal and anthelmintic activities (Satyawati *et al.,* 1987). This suggests that the strong antileishmanial activity of the crude methanolic root extract of *I. racemosa* may not be due to these two compounds; however, it may be due to some other compound which could not be detected in the LC-MS analysis and could not be isolated by fractionation.

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Conclusion

We discovered that the crude extracts of*Inula racemosa*and *T. terrestris* showed substantial antileishmanial activity in this investigation. Furthermore,isoalantolactone, a bioactive Component derived from the methanolic root extract of *I. racemosa* has no antileishmanial action against *L. donovani*. As a result, the antileishmanial activity could be attributed to any other chemical that was not found, hence more research is needed.

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Evaluation Of Essential Oil Extracted From *Acorus calamus* Rhizomes And Leaves

[*1]Versha Parcha, [1]Sukanya Chhetri, [1]Deepak Kumar and [2]Rajendra Rana
[1]Department of Chemistry& Pharmaceutical Chemistry Dolphin (PG) Institute of Biomedical & Natural Sciences, Manduwala, Dehradun, India
[2]Uttarakhand, Science Education & Research Centre, Dehradun, India
*Email: vershaparcha@gmail.com

Abstract-Essential oils are volatile complex compounds which are characterized by a strong odour and are formed naturally by aromatic plants as secondary metabolites. The practical value of essential oils in daily life is explored through the study of numerous physicochemical characteristics (*Parthiban* et al., 2011). Physicochemical characteristics indirectly influence the quality of the essential oils. The commercial importance of oils mostly depends on these physicochemical characteristics, which provide baseline data to determine its suitability for consumption. Therefore, the present study was taken to evaluate characteristics of the essential oil extracted from the leaves and rhizomes of *Acorus calamus* which is a wild aromatic plant species growing in the lower Himalayan region and the essential oil extracted from the plant is used frequently in skin care and aromatherapy to uplift mood and relieve stress. Essential oil was extracted from both rhizome and leaves (Ac R and Ac L) of *Acorus calamus* and are analysed for characteristics like yield, specific gravity, viscosity, refractive index, acid number, saponification value, etc. Results indicated that that essential oil Ac R is more superior to Ac L in quality enhancing its commercial importance and can be taken up for further studies.

Keywords: *Acorus calamus* , Specific gravity, Viscosity, Refractive index, Acid number

Introduction

Essential Oils are basically a natural oil, obtained from distillation and having a distinctive smell, of the plant or other sources from which it is extracted. They are rich sources of biologically active compounds (*Bishop* et al., 1997)[5]. Essential oils from a broad spectrum of plant species have shown antinociceptive, anti-inflammatory, antimicrobial, antiviral, antitumoral and antioxidant activities (*Rufino* et al., 2010)[6]. Moreover, recently there has been a profound interest in the antimicrobial characteristics of extracts from aromatic medicinal plants, specifically essential oils.

Essential oils are garnering a lot of attention because of their many uses as antioxidants, antifungals, and antiseptics. The exploration of various properties investigates the practical importance of herbal oils in daily life (Parthiban et al., 2011)[7]. Physical characteristics of oil like colour, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, carbonyl percentage, test for phenol and solubility etc. indirectly influence the quality of both essential and fixed oils. The overall productivity of oils is primarily determined by their physicochemical properties, which provide standard data to determine their suitability for consumption. The objective of the study is to evaluate characteristics of the essential oil extracted from the leaves and rhizomes of *Acorus calamus* commonly known as sweet flag is wild aromatic plant species growing in lower Himalayan region.This perennial herb is common on the banks of streams and in damp marshy places.*Calamus* is an essential herb in Ayurvedic medicine, and it is used as a "rejuvenator" for the brain and nerve system, as well as a digestive system remedy. The rhizomes and leaves of *Acorus calamus* contain aromatic oil that has been used medicinally since ancient times and has been harvested commercially. The rhizomes are thought to have antispasmodic, carminative, anthelmintic, nauseate, nervine, sedative, and stimulant properties and are used to treat epilepsy, mental illnesses, chronic diarrhoea, essential oil Ac R and Ac L extracted dysentery, andabdominal pain.Here we are from Rhizome and leaves which indirectly affects the quality of oil.

Material and Methods

Preparation of substrate

The whole *Acorus calamus* plant was collected from a local mark of Dehradun authenticated by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. The leaves and the rhizomes were cut into small slices followed by oven drying at 60 °C - 80 °C for 48 hrs. The dried parts were then stored at room temperature and then used for the experiment.

Extraction of oil from leaves and rhizomes of *Acorus Calamus*

600 gm of dried leaves and 300 gm of rhizomes of *Acorus calamus* was subjected to hydro-distillation in a Clevenger's apparatus. The volatile fraction, thus obtained after hydro-distillation for 6 - 8 hours, exhibited two distinct layers: an upper aromatic oily layer and a lower colourless aqueous layer.These layers were transferred into separating funnel with addition of diethyl-ether in it. The upper layer was collected and dried over anhydrous sodium sulphate.

Took the weight of small dry empty beaker and pour the solution into it and then put it over water-heater till all the ether evaporated away and only oil was left. Took the weight of the beaker with oil. By this, we can calculated the amount of oil obtained.The obtained oil was stored at low temperature (4 - 6°C) for further use[8].

Studies of characteristics of extracted oil[9]

Characteristics study of an oil provide a base line for aptness of oil. These characteristics of both oils Ac R & Ac L were studied in terms of colour , Specific gravity, Specific Viscosity, Refractive index, Acid number, saponification number[10,11].

Specific gravity

A cleaned and dried empty pycnometer was taken and weighed.

The pycnometer was filled upto the mark with double distilled water and weighed again.

The weight of water was recorded.

After removing the water the pycnometerwas dried in the oven and filled upto the mark with the essential oil under experimentation.

The weight of pycnometer with oil was calculated using the following formula.

$$\text{Specific gravity} = \frac{\text{wt. of the oil}}{\text{wt. of an equal volume of water}}$$

Specific Viscosity

1. An Ostwald viscometer was cleaned and dried. Ten ml of an oil under experimentation was filled in the bulb of the viscometer.
2. The solution was sucked upto upper mark and the viscometer left as much.
3. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.
4. The process was repeated by filling the viscometer with pure water. The specific viscosity of the oil was calculated as per following formula:

$$\eta_2 = \frac{\rho 2 \times t2}{\rho 1 \times t1} \times \eta_1$$

where,

ρ_1 = density of water (g/ml)

ρ_2 = density of an oil (g/ml)

η_1= Viscosity of water (cp)

η_2= Viscosity of an oil (cp)

t_1 = mean time flow of water within the mark

t_2 = mean time flow of oil within the mark

Refractive index

Abbe type of refractometer was used to determine the refractive indices of the oils.

The double prisms of the apparatus were cleaned with alcohol and one dropof theoil was placedbetween them.

1. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minute to equate the temperature of the oils and the apparatus.

2. The alidade of the refractometer was moved backward or forward to get a broader line which was a band of colour.
3. A sharp colourless line was obtained by rotating the screw heads of the compensator. Finally, the line wasadjusted in such a way that it fell on the point of intersection of the cross-hairs.
4. The refractive index was head directly on the scales of the sector.

$$\text{Acid number} = \frac{\text{Volume of 0.1 N Alkali consumed}}{\text{Weight of 1ml essential oil}} \text{ X } 5.61$$

Acid number

1. One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask.
2. Three drops of 1% phenolpthaline were added to the contents of the flask and it was titrated against 0.1 N sodium hydroxide solution.
3. The first appearance of pink colouration that did not fade with in 10 second was considered as the end point.
4. Another set without oil was also run parallel to treatment and the difference in the amount of alkali used while titrating the treatment and the set without oil gave the amount ofalkali consumed for determination of the acid number of the oil.
5. The acid number was calculated by
6. the following formula :

$$\text{Acid number} = \frac{\text{Volume of 0.1 N Alkali consumed}}{\text{Weight of 1ml essential oil}} \text{ X } 5.61$$

Saponification number

1. One ml of the essential oil was taken into a 100 ml saponification flask. Ten ml of 0.5 N alcoholic sodium hydroxide solution was added to the flask and an air cooled glass condenser (1 meter in length and 1 cm in diameter) was attached to it.
2. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature.
3. The contents were the titrated against 0.5 N aqueous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator.
4. Another set, without oil was also run parallel to the treatment set and the difference in the amount of acid consumed for the determination of saponification number of the oil, which was calculated by the following formula:

$$\text{Saponification number} = \frac{\text{Volume of 0.5 N Acid consumed}}{\text{Weight of 1ml essential oil}} \text{X } 5.61$$

Results and Discussion

Extraction of essential fromrhizomeandleaves is carried out using K Lelvengers apparatus and results of yield are summarized Table -1.

Table-1 Yield percentage of Extracted Oil

Part of the plant used	Initially amount taken (in gm)	Oil obtained (in gm)	% oil yield(w/w)
Leaves (Ac L)	600	3.2850	0.54%
Rhizomes (Ac R)	300	1.9068	0.66%

Table-2 Characteristics of Essential oil of *Acorus calamus*

Parameter studied	Ac L	Ac R
Colour	Dark yellow	Pale yellow
Specific gravity	0.843	0.878
Specific viscosity	0.094	0.104
Refractive index	1.501	1.553
Acid number	14.582	12.756
Saponification number	33.982	35.346

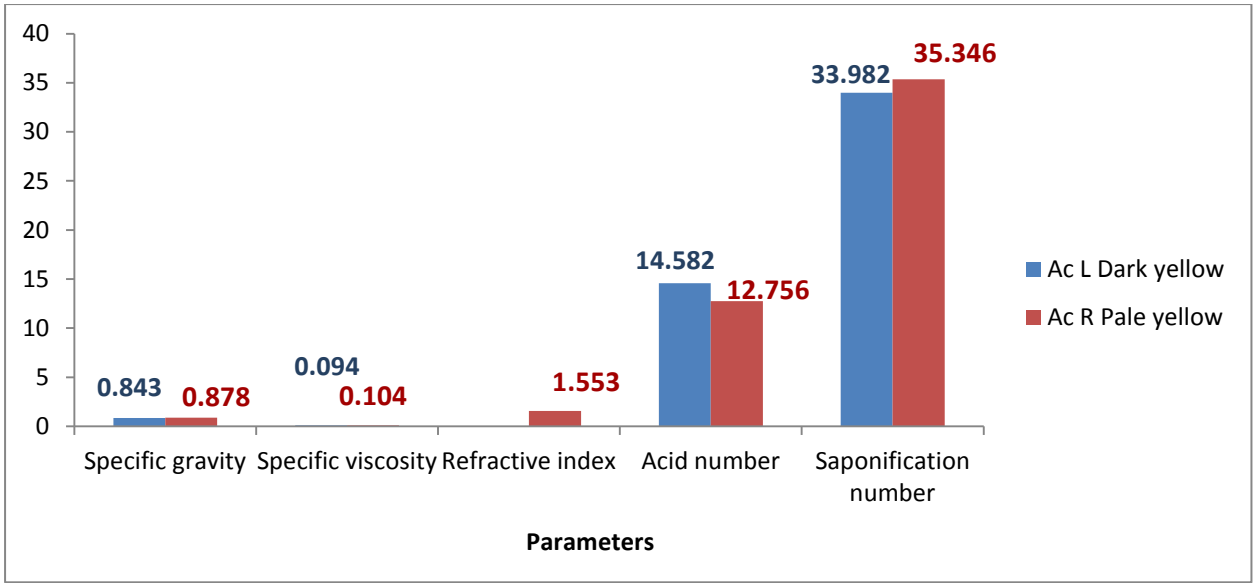

Figure-1 Characteristics of Essential oil of *Acorus calamus* Rhizome and leaves

The essential oil of leaves and rhizomes of *Acorus calamus* was found to be dark-yellow and pale-yellow respectively. Specific gravity is defined as the ratio of the density of a respective substance to the density of water at 4°C[12]. For most of the findings, specific gravity values of oils are less than 1 except few containing oxygenated aromatic compounds[13]. Our results are little inconsistent with Elert (2000), who described that most of the oils are characterized with specific gravity ranges from 0.9100 to 0.9400.

The acid number is the number of milligram of KOH required to neutralize the free fatty acids in one grame of oil. The acid number quantifies the amount of acids in an oil. Acid value is an indirect method for determining the amount of free fatty acid in oil samples and their edibility[14]. Oil with a low free fatty acid content is more widely preferred[15].

The number of milligram of KOH which is required to completely saponify one gram of oil sample is called saponification value[16]. The saponifiaction value of an essential oil of AcR is found be to little bit higher than that of the essential oil of AcL as well as the essential oil of AcR is proved to possess low acid number value than the essential oil of AcL which suggests that the quality of the oil and the suitability for consumption of essential oil from AcR is superior to AcL.

Conclusion

It has been concluded from the above study that the essential oil of Ac R is more suitable for the usage purposes on commercial scale than Ac L. Many research had already been performedwithin the past to assess the traits of essential oil from rhizomes of *Acorus calamus*, however the above study evaluate the characteristics of oil from both leaves and rhizomes and shows that the oil from rhizome is advanced to that of the leaves.

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Aloin Content Determination In*Aloe Vera* With Its Physicochemical Properties Along With Its Physicochemical And Phytochemical Evaluation In *Piper nigrum*

*Hritika Sinha

*Department of Biotechnology, IILM College of Engineering and Technology, Greater Noida, India

*Email: hritikasinha24@gmail.com

Abstract-India has a vast diversity of herbs. Around 3,000 years before, these herbs were acknowledged and use as medicinal plants and helpful for treating people. New scientific research has established that some plants and herbs have presence of many active compounds and possess specific pharmacological properties. *Aloe vera* (ghritkumari) and *Piper nigrum*(black pepper or maricha) are the oldest plants which are used as a medicinal herb as well as in household. *Aloe vera* or Ghritkumari species are currently used by cosmetic and pharmaceutical industries. *Aloe vera* has antibacterial, antiviral, and antiseptic properties which help to treat skin-related problems.*Piper nigrum*is a species that is used in various medications since very old times. They have also been used as domestic medicine against various infections, a quality attributable to the existence of certain chemical moieties in them. Physicochemical analysis and bioactive compound evaluation of the medicinal plants in essence of*Aloe vera*and*Piper nigrum* was undertaken in this study.In physicochemical analysis of *aloe vera*, many parameters were tested against pulp extract and leave extract (i.e., pulp and leave); parameters are color, odour, taste, total solid, total ash value, total dissolved solid, specific gravity, P_H, refractive index. For aloin content evaluation, High-Performance Layer Chromatography techniques was used in the *Aloe vera* plant. In physicochemical analysis,many standardization parameters like alcohol soluble extractive values, water-soluble extractive values, loss on drying,total ash value, acid insoluble ash and total ash value of *P. nigrum* fruits were analysed. For phytochemical screening, following a 6-stage extraction process is performed, the extracts collected from*P.nigrum* were exposed to a number of preliminary biochemical and phytochemical tests. The presence of tannins, saponins, terpenoids, flavonoids, and alkaloids wastested in the chloroformand aqueous extract which is used in the following investigation.However, from the aggregation of all our results, we concluded that the presence of these phytochemicals and bioactive component inthese medicinal plants indicates potential therapeutic properties for welfare of humans.

Keywords: Aloe vera, Piper nigrum, Aloin content evaluation, Phytochemical screening, HPLC.

Introduction

India is known for its ancient medicative systems-Ayurveda, Siddha, and Unani. Medical systems are found mentioned even within the ancient Vedas and different scriptures. The ayurvedic concept was regarded and evolved between 2500 and 500 BC in India. Understanding the essential fundamentals of Indian Ayurveda is to spend longer with nature and observe nature, the plants, and herbs. Every plant or herb features a specific quality and might be used to treat a multitude of ailments and diseases. Medicative plants like Aloe vera, black pepper, haldi, tulsi, elaichi, and adrak, satavri are unremarkably utilized in an exceeding form of Ayurvedic home remedies and are considered to be the foremost effective aid among fighting ailments related to throat and skin. As a rich source of nutrients, antibacterial and antioxidant properties, Ayurvedic herbs are non-toxic for humans so the product or remedies created using them are usually recommended for their high therapeuticvalue.

'Kumari' is also known as *Aloe vera* and it belongs to the family 'Liliaceae'. It is also known as Ghritkumari. The plant grows in a semi-wild state throughout the dried parts of India. The plant grows 30-60 cm in height, is perennial, with a short stem. The leaves are large, 40-50 cm long, thick, fleshy, lance-shaped, with a sharp apex and spiny margins. The whole plant is bitter and sweet in taste (rasa), sweet in the post-digestive effect (vipaka) and cold in potency (virya). The fresh gel or its mucilage or its solid extract is used for medicinal purposes. In inflammatory conditions, associated with pain and swelling, the external application of its leaf extract, is very beneficial. Ghritkumari is useful in number of diseases. In small doses, it is an effective appetizer, digestant, liver stimulant and in large doses, it works as an

anthelmintic and purgative. It is very useful as a blood purifier, hence valuable in skin disease and jaundice due to viral hepatitis. Kumari is a valuable herb in the treatment of tumours also[1]. Now a days*Aloe vera*is used in allopathic, homeopathic and ayurvedic medication systems. Not only as medicine now have people also used it as a food. Ghritkumari contains all beneficial vitamins, minerals, enzymes, amino acids, as well as it has natural sugar. It has various Bioactive compounds with emollient, purgative, anti-microbial,antioxidant, anti-helminthic,antifungal, antiseptic, anti-inflammatory and cosmetic values for health care. *Aloin is an anthraquinone-C-glycoside present in Aloe vera. This compound is extremely variable among different species and highly depends on the growing conditions of the plants. Currently, HPLC analysis requires aloin extraction by procedures using various solvents. The most commonly used solvents are methanol;Methanol is the most frequently used solvent for the extractionofanthraquinones[2].

Piper nigrum (P. nigrum) (black pepper) is a precious medicinal plant that belongs to the own circle of relatives Piperaceae. Black pepper is a critical and maximum generally used spice and appeared as "the king of spices'' amongst diverse spices. *P.nigrum* is grown in diverse tropical areas like India, Indonesia, and Brazil.*P.nigrum* is also known as "kali mirch" (Hindi and Urdu), "usana" (Sanskrit), "Golmorich" (Bengali), "Black pepper" (English). Black pepper is likewise used as a medicine, a preservative, and a flavouring agent in perfumery.Marica one of the oldest names of P.nigrum consists of the fully mature dried fruit of Pippernigrumlinn. a climber, cultivated from north Konkan Kerala and also in Assam. Marica was held in high esteem by ancient sages of India. It is one of the herbs mentioned in allayurvedic scriptures. Marica is one of the ingredients of 'TRIKATU' – three pungent {sunthi (Zingiberofficinale), marica and pippali (Pipperlongum)} which alleviates cold, asthma, and body fats, it also improves the taste sensation, reduces flatulence and anorexia, and also is diaphoretic. Marica is used both, internally as well as externally. In the form of an external paste with sesame oil, it is beneficial in the skin diseases like scabies, leukoderma. The paste application helps in reducing the swelling and the pain. In case of tooth decay and aches, the marica powder is used for brushing the teeth. The lively thing in Piper nigrum is the alkaloid piperine. It promotes digestion with the aid of using stimulating the secretion of the digestive enzymes from the pancreas. In addition to this, it additionally obstructs in vitro oxidation with the aid of using extinguishing loose radicals at the side of the inhibition of enzymatic biotransformation of medication withinside the liver. Apart from these, pepper is used to alleviate pain, flu, colds, chills, rheumatism, fever, and muscularaches[1].The objectives of the research were physicochemical analysis of Aloe vera and itsaloin content evaluation through HPLC (High-Performance Liquid Chromatography); along with physicochemical and phytochemical screening of *Piper nigrum.*

Material And Methods

Aloe vera

Plant Material: Fresh leaves of aloe vera is collected from Himalaya drug company, Dehradun, India from which the pulp and leaves were separated and covered in aluminium foil and kept in air tight jar for further use in refrigerator.

Piper nigrum

Plant Material: Fresh dried fruit of black pepper was collected from Himalaya drug company, Dehradun, India. The collected black pepper was cleaned properly under running tap water to make them free from soil and dust and then kept in hot air oven at 60 degree Celsius for 24 hours.

Crude Extraction: 20 grams of coarse powder of black pepper is soaked in 100 ml of Chloroform, and aqueous at room temperature for 3 days inside an Iodine Flask with occasional shaking. The solvents were then filtered using Whatman ™ no.1 filter paper and concentrated using a water bath. The extracts were then kept in glass bottles for further usage.

Identification Test

The individual sample was subjected to the physicochemical and qualitative phytochemical screening. Phytochemical tests were carried out adoptingstandard procedures (Trease et.al 1983, Kokate et.al 1997, Hegde et.al 2010).All of the reagents were made by adoptingstandard procedures (Indian Pharmacopoeia 2014).

Aloe vera

Physicochemical Analysis

Total Solid[3]

Weigh accurately or measure an accurate quantity of the substance under examination stated in the individual monograph, place in a tared dish, evaporate at as low a temp as possible until the solvent is removed and heat on a water-bath until the residue is apparently dry.

Transfer to an oven and dry to constant weight at105 degree, unless otherwise stated in the mono graph.Owing to the hygroscopic nature of certain residues, it may be necessary to use dishes provided with well-fitting covers and to cool in a desiccatorand weigh the dish.Calculated the content of extractable matter in mg per g of air-dried material.

Total Ash Value[3]

Heat a silica crucible to red heat for 30 mins, allow to cool in a desiccator and weigh.

Weigh the accurately about 2g of the substance under examination and evenly distribute it in the crucible.

Dry at 100 degree to 150 degree for 1 hr and ignite to constant weight in a muffle furnace at 600 degree.

Allow the crucible to cool in a desiccator after each ignition.

Calculate the percentage of ash on the dried basis.

Refractive Index[3]

The Abbe refractometer is convenient for the most measurements of refractive index but other refractometer of equal or greater accuracy may be used.

Place the sample on the refractometer and spread it evenly, set the sodium light.

And check the refractive index from the eye piece.

Write refractive value of sample.

P_HValue[3]

The P_H value of a solution is determined potentiometrically by means of glass electrode, a reference electrode and a P_H meter either of the digital or analogue type.

Calibrate the apparatus using buffer solution D as the primary standard, and buffer solution A for adjusting the meter to read the appropriate $P_H$value.

Dip the glass electrode into sample and wait for 5 min.

Take the reading.

Specific Gravity[3]

Select a scrupulously clean, dry pycnometer that previously has been calibrated by determining its weight.

Fill the pycnometer with the sample and clean the excess liquid from the pycnometer.

Weigh the pycnometer with sample and subtract the tare weight of the pycnometer from the filled weight.

Total Dissolved Solid[3]

5gm sample is weighed and dissolved in 75ml water and place the beaker in the magnetic stirrer at 700- 1000c for 10 min.

Make up the sample in 100 ml and the filter it with Whatman filter paper 1.

Place the sample in petri dish and place it in water bath for 1 hr and then place the petri dish to hot air oven for complete moisture free.

Weight the petri dish and calculate the dissolved solid in it.

Quantification AloinContent

Extract purification: Three different samples of the aloe vera plant were taken i.e the pulp, the peel and the whole leaf. All the three samples were homogenizedin a blender separately and the resultant samples were subjected to HPLC to determine the aloin content in the sample.

Sample preparation: For all the 3 sample, approx 250mg sample weighed and mix with 50ml of methanol. Then sonicate the solution for proper mixing. And through nylon filter fill the vial of the HPLC tray.

Chromatographic condition: Samples were analyzed in Shimadzer LC 20A0 liquid chromatograph system with SPD-M20AuV detector in isocratic mode. The elution was carried out with gradient solvent system with flow rate 1 ml/min at 40°C temperature. The mobile phase consisted of acetonitrile (22%) and water (78%) (v/v) basis and phosphate buffer. The sample was injected at 20 µl. Deuterium (D2) Lamp Beckmann was used at wavelength 220 nm for detection of aloin.

Aloin content evaluation through HPLC Chemicals

Acetonitrile (HPLC grade), Ortho-phosphoric Acid (0.1%), Methanol (Chromatography Grade), Ultrapure Distilled water (Filtered with 0.22-micron filter), Aloin standard (52.3 % Pure), aloe vera sample (pulp, leave, leave + pulp).

Chromatographic System and instrument

A Shimadzu® LC-2010CHT HPLC system was used for analysis. With a spectrophotometric detector set at 420 nm, a column oven (set at 40 ˚C), a reverse-phase c18 column (250mm × 4.6 mm). The mobile phase was a 50:50 (v/v) mixture of acetonitrile and 0.1% ortho-phosphoric acid at the flowrate of 1 ml per minute.

Standard and Working Solution

Standard solution: 50.3mg of Aloin was dissolved in methanol in a 50 ml volumetric flask to a final concentration of 1.0 mg per ml and sonicated for 10 minutes in an ultrasound bath and completed to the final volume.

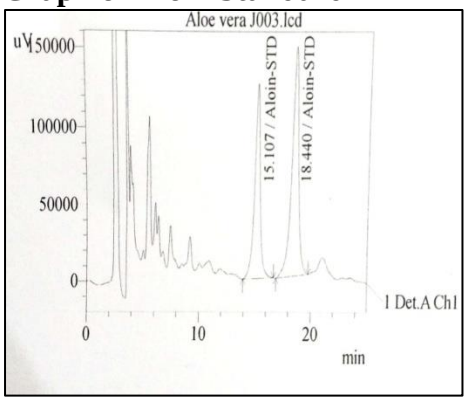
Test Solution

Test Solution was prepared by adding 500 mg of sample in 50 ml of methanol in a volumetric flask. The solution is then placed in ultra sound bath for mixing the sample.

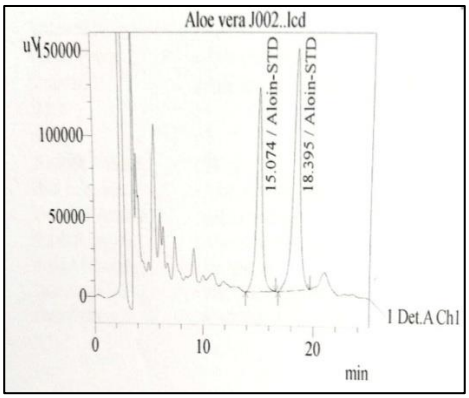
Procedure

All the solutions (Test, Standard, and Methanol blank) were then placed in the system. A method was designed to take 22% solvent as acrylonitrile and 78% ortho-phosphoric acid with 1 cycle being 25 minutes long, 7 cycles were run in total with first cycles of methanol blank, second, third and fourth of Standard and fifth, sixth and seventh of the samples. The following graphs were obtained:

Graph of Aloin Standard



Graph 1-Standard aloin-1



Graph 2-Standard aloin-2

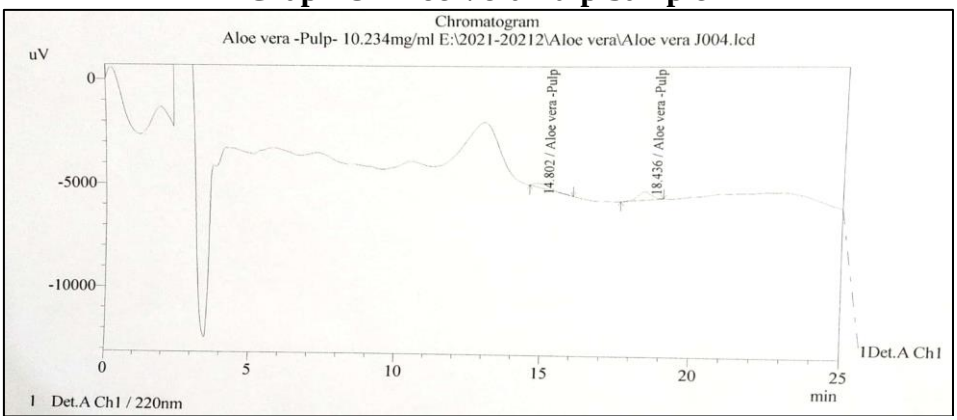
Aloin standard-1

Title	Sample name	Sample id	Ret. time	Average	Height	Conc.
Aloe vera j002	Aloin std-1	Aloin std-1	15.074	4261976	124576	0.00
Aloe vera j003	Aloin std-1	Aloin std-1	15.107	43317756	126131	0.00
Average			15.091	4289866	125354	0.00
%RSD			0.153	0.919	0.877	0.00
Maximum			15.107	4317756	126131	0.00
Minimum			15.074	4261976	124576	0.00
Standard deviation			0.023	39442	1100	0.00

Aloin standard-2

Title	Sample name	Sample id	Ret. time	Ave.	Height	Conc.
Aloe vera j002	Aloin std-1	Aloin std-1	18.395	6139179	145926	0.00
Aloe vera j003	Aloin std-1	Aloin std-1	18.440	6214683	147655	0.00
Average			18.418	6176931	146791	0.00
%RSD			0.174	0.864	0.833	0.00
Maximum			18.440	6214683	147655	0.00
Minimum			18.395	6139179	145926	0.00
Standard deviation			0.031	53390	1223	0.00

Graph Of Aloe Vera Pulp Sample



Graph-3Aloe vera Pulp graph

Peak table

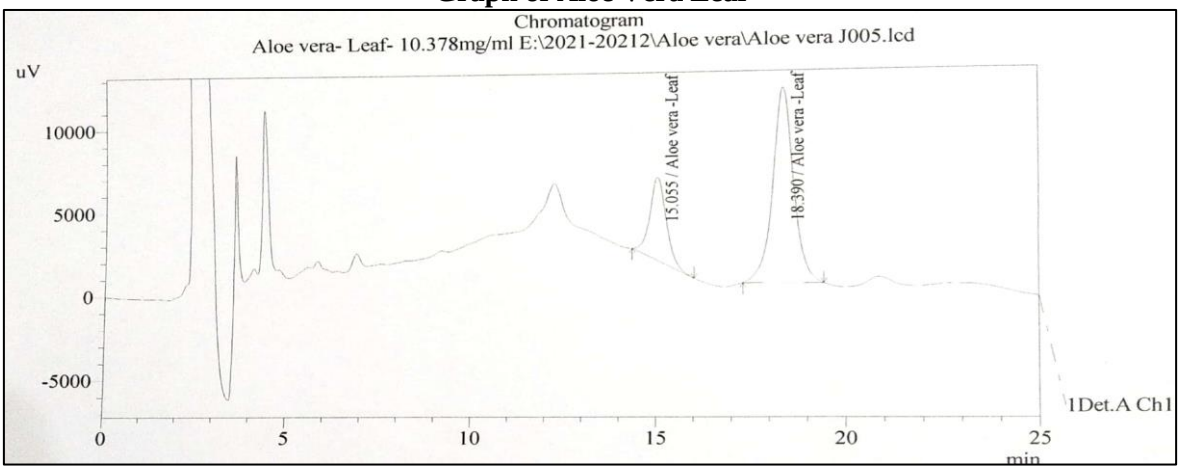
Peak#	Name	Ret. Time	Area	Height	Area%	Height
1	Aloe vera- pulp	14.802	8040	185	38.970	31.684
2	Aloe vera- pulp	18.436	12591	398	61.030	68.316
Total			20631	583	100.00	100.00

Calculation

Area of Sample **Concentration of Sample**
---------- X ---------- X Purity of standard
Area of Standard **Concentration of Standard**

$$\frac{\mathbf{20631}}{\mathbf{10466797}}\mathbf{X}\frac{\mathbf{11006}}{\mathbf{10.234}}\mathbf{52.3} = \mathbf{0.010}\%$$

Graph of Aloe Vera Leaf



Graph 4-Aloe vera leaf graph

Peak table

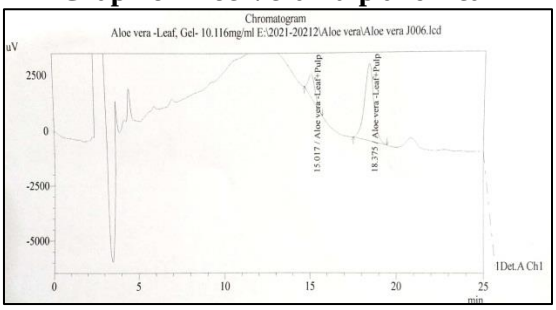
Peak#	Name	Ret. Time	Area	Height	Area%	Height
1	Aloe vera- leaf	15.055	152168	4949	25.739	30.048
2	Aloe vera- leaf	18.390	439026	11522	74.261	69.952
Total			591194	16472	100.00	100.00

Calculation

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \text{ X } \frac{\text{Concentration of Sample}}{\text{Concentration of Standard}} \text{ X Purity of standard}$$

$$\frac{591194}{10466797} \text{X} \frac{1.006}{10.234} 52.3 = 0.286\%$$

Graph of Aloe Vera Pulp and Leaf



Graph 5-Aloe vera pulp and leaf graph

Peak table-

Peak#	Name	Ret. Time	Area	Height	Area%	Height
1	Aloe vera- leaf and pulp	15.017	28142	1020	18.232	22.871
2	Aloe vera- leaf and pulp	18.375	126215	3441	81.768	77.129
Total			154358	4461	100.00	100.00

Calculation

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \text{ X } \frac{\text{Concentration of Sample}}{\text{Concentration of Standard}} \text{ X Purity of standard}$$

$$\frac{154358}{10466797} \text{X} \frac{1.006}{10.234} 52.3 = 0.0766\%$$

Piper nigrum

Physicochemical Analysis

Determination of foreign organic matter[3]

To determine the foreign matter, content material was spread on a thin layer on a A4 sheet.

Inspect the sample with the eye or with the use of a 6x lens and separate the foreign organic matter manually as completely as possible.

Weigh and determine the percentage of foreign organic matter from the weight of the drug taken.

Loss on Drying[3]

LOD is the loss of mass expressed as percent w/w.

To estimate the LOD, 1gm of the air-dried crude drug is accurately weighed in a flat dried weighing bottle and then dried to constant mass, the final weight is noted and the LOD calculated by formula.

LOD=$\frac{FinalWt.}{IntialWt.}$**x100**

Determination of Total Ash Value[4]

For determination of total ash 1gm of the ground air dried material, accurately weighed, in a crucible (usually silica or platinum) and spread in an even layer

It is then ignited by gradually increasing the heat to 500-600 degree Celsius until it is white, indicating the absence of carbon.

The residue is allowed to cool in a desiccator for 30 minutes, and weighed without delay. The content of total ash is calculated in mg per g of air-dried material.

Determination of Acid Insoluble Ash[4]

Boil the ash obtained in total ash determination for 5mins with 25ml of 2M hydrochloric acid

Collect the insoluble matter in an ashless filter paper.
Wash with hot water and ignite to constant weight.
The content of acid insoluble ash is calculated in mg per g of air-dried material.

Determination of Alcohol Soluble Extractive[4]

Macerate 5g of the air-dried drug, coarsely powdered, with 100ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing to stand for 18 hours.
Filter rapidly, taking precaution against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105 degrees Celsius, to constant weight and weigh.
Calculated the content of extractable matter in mg per g of air-dried material.

Determination of Water-SolubleExtractive[4]

For determination of number of active constituents extracted with water from a given amount of sample.
Similar procedure was adopted as described above for total alcohol extractive except replacing ethanol by water.
Calculated the content of extractable matter in mg per g of air-dried material.

Phtochemical Screening[5]

Test for Tannins

2ml of each extract was added separately to 4 ml of water and a few drops of 0.1% $FeCl_3$were added to the extracts to form a blue coloured solution.

Test for Terpenoids

Salkowaski Test

5ml of extracts was taken in different test tubes.
To each of them 2ml of chloroform was added, along with it 3ml of concentrated sulphuric acid was added slowly to form a layer.

Test for Saponins

1ml of the extract was added to 20ml of distilled water in a test tube and was shaken vigorously for 15minutes.
Formation of the foamy layer indicated the presence of saponins.

Test for Cardiac Glycosides

Keller-Killani Test
2ml of glacial acetic acid was added to 5 ml of extracts containing a drop of $FeCl_3$solution followed by the addition of 1ml of concentrated sulphuric acid.
A greenish ring may form just above ring and gradually spreads throughout this layer.

Test for Flavonoids

Aqueous filtrate along with concentrated sulphuric acid was taken in a test tube;
5ml of dilute ammonia solution was added.
To all other filtrates few drops of 1% aluminium solution was added.
The presence of flavonoids was indicated by the development of yellow colour.

Test for Alkaloids

Mayer's Test

1ml of the extracts and 10ml of acid alcohol were boiled and filtered.
To 5ml of filtrates, 2ml of dilute NH_3 and 5ml of $CHCL_3$ were added and shaken to extract the alkaloidal base.
The chloroform layer was extracted with 10ml of acetic acid.
Positive test with Mayer's reagent gives a cream-coloured solution.

Test for Phenolic Compounds[6]

Ferric chloride test

Extract was diluted to 5ml of distilled water and filtered.
To the filtrate 5% of ferric chloride was added.
Dark green colour indicates the presence of phenolic compounds.

Results and Discussion

Aloe vera

Physicochemical analysis
Physical and chemical test are performed on Aloe vera. 2 samples of Aloe vera are undergone for physicochemical analysis, 1 Aloe vera pulp and 2 Aloe vera leave.

Aloe vera Pulp Extract Aloe Vera Leave Extract

Table-1 Physicochemical analysis of Aloe vera pulp extract vera leave extract

Parameter	Experiment Result
Colour	Offwhite
Odour	Characteristic
Taste	Bitter
Total Solid	1.54%
Total Dissolved Solid	0.704%
Total Ash Value	0.1267%
Refractive Index	1.334
P_H Value	4.48
Specific Gravity	Not Applicable

Table 2-Physicochemical analysis of Aloe Aloin content

Parameter	Experiment Result
Colour	Green
Odour	Characteristic
Taste	Bitter
Total Solid	1.933%
Total Dissolved Solid	0.976%
Total Ash Value	0.2612%
Refractive Index	1.334
P_HValue	4.58
Specific Gravity	1.009g/ml

Through the graph the aloin content iscalculated in pulp, leaf and pulp, leaf sample. Results are as followed:

Table-3 Aloin content

Aloe Vera Sample	Amount Percent
Pulp	0.0101%
Leaf	0.286%
Pulp And Leaf	0.0766%

Piper nigrum

Physicochemical analyses Physical and chemical test are performed on blackpepper. Black pepper sample completely stand on standardized parameters. Result is as followed:

Table-4 Physicochemical analysis of black pepper

Parameters	Experiment Yeild	Standard Report
Foreign Matter (%)	0.132%	Not More Than 2.0%
Loss On Drying (%)	10.86%	Not More Than 12.0%
Total Ash Value (%)	4.72%	Not More Than 5%
Acid Insoluble Ash (%)	0.28%	Not More Than 0.5%
Alcohol Extractive (%)	8.736%	Not Less Than 6%
Water Extractive (%)	7.752%	Not Less Than 6%

Phytochemical analyses

The presence of active phytochemical constituents such as tannins, saponins, terpenoids, flavonoidsand othersare the principal reasons for a plant to exhibit medicinal activity. Flavonoids are well knownantioxidants and cardiac glycosides exhibit positive as well as negative effects on the heart. Saponins act as anti-feed ants in plants and likewisetannins shield the plants from phytophagousinsects and herbivores. Terpenoids provide plants their phytoalexin property. All these Phyto-active compounds in conjunction elevate the therapeutic, commercial and economic values ofsuch plants.The results forall the phytochemicalscreenings aredisplayed inTables-2 for black pepper.

Table-5 Phytochemical analysis of black pepper

Test	Chloroform Extract	Aqueous Extract
Tannins	Absent	Present
Saponins	Absent	Present
Terpenoids	Present	Present
Cardiac Glycosides	Absent	Absent
Flavonoids	Present	Absent
Alkaloids	Absent	Absent
Phenolic Compounds	Absent	Absent

Conclusion

From the above research we can conclude that *Aloe vera* is a herb which have optimum amount of Aloin in it, which can either be used to make antibiotic derivatives or can be used as skin care product. *Piper nigrum*isa herb rich in phytochemicals and use for many disease internally and externally. Phytochemical present in piper nigrum are tannins, saponins, terpenoid, and flavonoids which are helpful for therapeutics and used as safe substitute to many of the chemical drugs which are present in market presently. This medicinal plant gives future scope for creating drug without side effect on humans.

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Indian Pharmacopoeia, 2014, Volume I.The Ayurvedic Pharmacopoeia of India, part1 volume III (first edition, $P_{p.}$234-235).Preliminary phytochemical and antimicrobial screening of Syzygiumaromaticum, *Elettaria cardamomum and Piper nigrum* extracts.

Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum L*.) against some human Pathogens. P.Ganesh*, R. Suresh Kumar and P. Saranraj, Department of Microbiology, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Chemical Studies OnFatty Oil Of *Trewia nudiflora Kernels*

[*1]Rashmi and [2]Shikha Baskar
[1]Sardar Vallabh Bhai Patel University of Agriculture & Technology ,Modipuram, Meerut , India
[2]YSB Foundation, Garden View Apartments, Panditwari, Dehradun, Uttarakhand, India
[*]**Email:** rashmi@icfre.org

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Abstract-*Trewia nudiflora* Linn belongs to family Euphorbiaceae, sub-family Acalyphoideae and it is one of the important medicinal plants in Indian systems of medicine like Ayurveda, Siddha, etc. It has numerous phytochemical and pharmacological significance. The kernels of*Trewia nudiflora*were chemically examinedand fatty oil was isolated and characterized by Gas Liquid Chromatography. The fatty oil content in the kernels was found to be 20.99% on moisture free basis. Arachidic acid (40.25%) and Linolenic acid (30.26%) were identified as major constituent. Physico-chemical analysis of fatty oil was also carried out.

Keywords: Euphorbiaceae, *Trewia nudiflora*, Fatty acid composition, Gas – Liquid Chromatography.

Introduction

Trewia nudiflora (Family : Euphorbiaceae) is a rapidly growing, soft wooded versatile dioecious tree which grows within the semi-evergreen and moist tropical forests. It is commonly known as tumri, and distributed in India, Srilanka and in Indo Malaya region. The tree is naturally found along the foot hills and river banks, where moisture is assured. It flourishes in the Terai and Bhabar Division of UP. Flowers are green in colour usually solitary axillary. Male and female flowers appear on separate trees, males are yellow in long lax drooping inflorescences while females are green, solitary or 2-3 together in the leaf Axis. Fruits are pale green, drupaceous, obscurely quadrangular, 2.5-3.8 cm in diameter, borne in large numbers. Seeds are black, usually four and surrounded by yellowish fleshy arils[1] (figure-1). The bark contains Taraxerone and β-sitosterol. A poultice prepared from the roots is applied in gout and rheumatism. The roots contain resinous matter and fats. Decoction of the shoots is said to relieve flatulence and is also used for the treatment of swelling. The oil is pale yellow in colour and very similar to tung oil. An alkaloid, nudiflorine (1-methyl-5-cyano-2-pyridone) has been reported in the leaves [2-3]. A minor unprecedented diterpene, 3β, 17-dihydroxycleistantha-12,15-dien-2-one, two known triterpenes (glutin-5-en-3-ol and Olean-18-en-3-one (Germanic one)) and three known Sterols (22*E*,24*R*)-5α, 8α-epidioxyergosta-6,22-dien-3β-ol, (22*E*,24*R*)-5α, 8α-epidioxyergosta-6, 9 (11), 22-trien-3 β-ol, and (22*E*,24*R*)-6-methoxyergosta-7, 22-dien-3,5-diol) was isolated from pericarp of *Trewia nudiflora*[4]. Some maytansinoids isolated from *T.nudiflora*seeds are tumor inhibitors and may be responsible for the resistance of the seeds to fungal degradation[5-6]. Powell and his colleagues isolated two novel compounds namely trenudine and treflorine which contain two fused macro cyclic rings and these compounds fully retain activity against KB cells and P388 Lymphocytic leukemia[7].Seed oil of *T.nudiflora* is known to contain glycerides of α-Kamlolenic acid (18-hydroxy-cis-9, trans-11, trans-13-octadecatrienoic) acid [8].

Figure-1 Flowers and fruits of *Trewia nudiflora*

Material and Methods

Ripped fruits of *Trewia nudiflora*were collected from the campus of *Forest Research Institute*, *Dehradun*. The kernels were removed and crushed to obtain a coarse powder. The powdered kernels were extracted with petroleum ether (60-80^{0}C) by using soxhlet apparatus. Removal of the solvent under reduced pressure gave pale yellow coloured fatty oil. The physico-chemical properties of the fatty oils were determined using standard methods[9].

The oil was saponified with 0.5N alcoholic potassium hydroxide for 2hr and mixture of fatty acids were isolated following normal procedure. Fatty acid methyl esters were prepared by refluxing the mixture of fatty acid with 1% Sulphuric Acid /MeOH on water bath for 4hr, cooled and usual work up yielded methyl esters. The analysis of fatty acid methyl esters was carried out on Chemito Gas Liquid Chromatography fitted with FID (240^0). The temperature of the injector was maintained at 230^{0}C. Capillary column (25m, BPX 70, 0.22 mm ID, 0.25µm) was used. Nitrogen used as carrier gas (40ml/min.). Split was maintained at 60ml/min and purge was maintained at 2ml/min.The oven temperature was programmed from 150-230^{0}C (with increase in temperature 3^{0}C) followed by a final hold up of 25ml/min. Methyl esters were identified by comparing the retention times of standard fatty acid methyl esters and also by their co-injection. The percentages were considered as weight percentage.

Table-1 Physico-chemical Characteristics of *Trewia nudiflora* Seed Oil

Characteristics	*Trewia nudiflora*
Oil (wt. %)	20.99
Specific gravity (d^{19})	1.1805
Refractive Index (η_D^{20})	1.509
Acid value	34.782
Saponification value	204.765
Ester value	166.056
Unsaponifiable matter (wt. %)	0.7317
Protein content	20.985

Table-2 Fatty Acid Composition (wt %)of *Trewia nudiflora* Seed Oil

Fatty acid	*Trewia nudiflora*
C:9	1.06
C:10	0.11
C:15	9.75
C: 18:0	0.51
C: 18:1	10.65
C:18:3	30.26
C:20	40.25
C:21	2.77
C:22	2.41
C:24	2.11
Unidentified	0.12

Results and Discussion

Trewia nudiflora fatty oil content was determined on the moisture free basis and it was found to be 20.99%. Their physico-chemical properties are given in Table-1. GLC analysis of a mixture of methyl esters of the fatty acids prepared from the fatty oil showed the presence of eleven fatty acids in the oil. Out of which, ten were characterized (Table-2). The identified fatty acids constituted 99.88%

of the mixture of fatty acids obtained from the fatty oil. The fatty acid composition (Table-2) indicated that Arachidic acid (40.25%) is the major constituent of the followed by Linolenic acid (30.26%), Oleic acid (10.65%) and Pentadecanoic oil acid (9.75%)while Heneicosanoic acid (2.77%), Behenic acid (2.41%), Lignoceric acid (2.11%), Nonaoicacid (1.06%) and Capricacid (0.11%) are the minor constituents.Literature survey revealed that in an earlier report T.nudifloraseed oil was reported to contain α-elaeosteraic acid (39.50%), Linoleic acid (25.13%), Oleic and saturated acids (35.37%) as the chief constituent 1 but in this study Arachidic acid and Linolenic acid are present in good amount. The fatty oil may be of drying nature due to higher content of Arachidic acid, Linolenic acid and other unsaturated acids. Linolenic acid is an important component of lipids.[10-13]

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Combined Effect Of *Asparagus Racemosus* And Ecliptaalba As Anticandidal Drug And Its Underlying Pharmacognostic Properties

***Prgya Gupta and Aparna Pandey**
Department of Biotechnology, IILM College of Engineering and Technology,Greater Noida, India
*Email :prgyagupta10@gmail.com

Abstract-Ecliptaalba and Asparagus racemosus is widely used in Ayurveda to cure various ailments. Plants and plant derived preparations have been used as traditional remedies and in medicines for the different treatments. The in vitroanticandidalactivity of Shatavri the "queen of herbs" and Eclipta alba "False daisy'' and "King of hairs" was tested against Candida Albicans and various extract of both the plants were evaluated for pharmacognosticstudies. Ecliptaalba is an important medicinal plant used in treatment of various health problems including digestion, asthma, cough, headache, and skin color.It also shows antimicrobial activity against candida and a very promising result against the fungal strain. Also the extract of Asparagus racemosus showed high degree of activity in case the same strain and both the plants were found similar with that of standardantibiotics use. This research paper throws light on the above pharmacognostic and antimicrobial properties. Both the plants were used as therapeutic agent for human disease.

Keywords: Eclipta alba,Asparagus racemosus, Candida albicans, Anticandidalactivity.

Introduction

Plants are parallel to human beings and no doubt are much more safer medicine options, and therefore the use of herbal medicines in recent years for primary health care is increasing worldwide, especially in developing countries. Medicinal plants are of great importance to the health of individuals and communities. The medicinal values lie in chemical active substances that produces specific physiological action on human body.Medicinal plants produces a diverse range of bioactive molecules, making them rich source of different types of medicines. According to WHO about more than 80% of world population use herbal medicines. Worldwide about more than thousands of plants are been used as medicine, the plant extracts from Asparagus racemosus and Ecliptaalba have been tested for antimicrobial activity against Candida albicans.

Shatavri also known as the queen of herbs has been a boon to mankind, as it is said prevention is better than cure, Shatavri is used in various tonics, health supplements owing to its medicinal properties. It is used in treatment of ulcer, gout, asthma, diuretics and is also been found to have anticandidalactivity .Candida being a really harmful pathogenic fungal to human causes gut infection skin infection.

Ecliptaalba belongs to the family Asteraceae. This weed basically grows in tropical and sub-tropical regions. Ecliptaalba has various names include bhangara in Hindi, maakaa in Marathi, bhangaro in Gujaratkesuriya in Bengali and galagara in Telegu. It contains many bioactive componentsi.e. triterpenes, flavonoids,steroids, polypeptides,polyacetylenes and thiophene derivatives. It is used in the treatment of gastrointestinal disorders, respiratory tract disorders, fever, hair loss and liver disorders. In Ayurveda, its leaf extract is considered a powerful liver tonic, rejuvenate, and especially good for the hair.

Candia albicans is a polymorphic fungus, a member of the normal human microbiome. In most individuals, Candida albicans resides as a lifelong, harmless commensal. Under certain circumstances, however, C. albicans can cause infections that range from superficial infections of the skin to life threatening systematic

infections. Several factors and activities have been identified which contribute to the pathogenic potential of this fungus. Pure culture of Candida albicans were made separately and maintained at on nutrient agar. The pure culture was used for testing antifungal activity.

Material And Methods

Plant Material

Whole plant of Ecliptaalba and Asparagus racemosus was collected. The leaves of Ecliptaalba and roots of Asparagus racemosus was collected from the Himalaya Drug Company, Dehradun, India. The collected leaves and roots were cleaned properly under running tap water to make them free from soil and dust and then dried for 24 hours and then oven dried at 105 degreesCelsius for 6 hours. Dried leaves and roots were chopped and ground to coarse powder using an electronic grinder. identified, collected, washed, shade dried for 24 hours and then oven dried at 105 C for 6 hours.

Preparation Of Plant Extracts

The powder of both the plants was prepared separately by mechanically grinding the roots of Asparagus racemosus and leaves of Ecliptaalba. The powder was sieved by using 60 mesh.

Different extract was prepared by dissolving the powder in various solvents. 5 gm powder

in 100 ml of solvent was kept for 24 hrs. and shaken at regular intervals, then filtered. The filter cake was discarded and filtrate was collected, and concentrated to 20 ml by evaporating it using hot water bath.

Aqueous extract of Shatavri

5gm of Shatavri powder weighed by electronic balance is added to 100 ml of distilled water in stoppered conical flask along with 4-5 ml of chloroform, is mixed and shaken at regular intervals for 24 hrs. Chloroform is used to as to prevent contamination since fungal growth is common in water.

5gm of Ecliptaalba powder weighed by electronic balance and added to 100 ml of distilled water in stoppered conical flask and 4-5 ml of chloroform in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

Chloroform extract

5gm of Asparagus Racemosus powder weighed and added to 100 ml of chloroform in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

5gm of Ecliptaalba powder weighed and added to 100 ml of chloroform in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

Methanol extract

5gm of Asparagus Racemosus powder weighed and added to 100 ml of methanol in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs. 5gm of Ecliptaalba

powder weighed and added to 100 ml of Methanol in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

Ethanol extract

5gm of Asparagus Racemosus powder weighed and added to 100 ml of ethanol in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

5gm of Ecliptaalba powder weighed and added to 100 ml of ethanol in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

Acetone extract

5gm of Asparagus Racemosus powder weighed and added to 100 ml of Acetone in stoppered conical flask, mixed and shaken at regular intervals for 24 hrs.

All the extracts were filtered using whatsman filter paper no. 41 .the filter cake was discarded and the filtrate was collected and concentrated. The volume is made upto 20 ml by evaporating is using hot water bath.

Media preparation

The media was prepared by dissolving nutrient agar in distilled water and was autoclave at 121 C for 15 minutes. 20 ml of sterile agar media plates were poured in sterilized petri dish and allow to solidify

which were used for testing antifungal activity. 300ml of Nutrient broth
was prepared and inoculated by pre inoculated candida albicans strain. 14 Nutrient agar plates were prepared. The activity is tested by well diffusion method.

Anticandidal activity assay

A well of size 5 mm diameter is punched using borer. Each extract sample was loaded in the well separately and incubated at 24 C for 24 hrs. As this is the best temp for growth of fungus.

ResultsAnd Discussion

Phytochemical screening

The chloroform, methanol, ethanol, acetone and aqueous (water) extracts of Ecliptaalba leaves and roots of Asparagus racemosus were subjected to phytochemical screening for the presence of alkaloids, terpenoids, flavonoids, tannins, saponins according to standard procedures.

The qualitative phytochemical screening revealed the presence of various phytoconstituents in different extracts of plants. It is evident that the methanol extract recorded the presence of maximum number of chemical constituents including terpenoids, flavonoids, saponins, alkaloids and tannins. Terpenoids were detected in all the extracts whereas saponins, alkaloids, and tannins were absent in chloroform extract.

Presence of diverse range of secondary metabolites in both the plants is indicative of significant therapeutic activity. The presence of flavonoidsare considered to be good free-radical scavengers, indicate that the plant may have anti-oxidant properties and they are accountable for biological actions. Terpenoids also play an active role in wounding healing, strengthen the skin, increase the concentrations of antioxidant in wounds, and restore inflamed tissues by increasing blood supply. Saponins have the property of coagulating and precipitating red blood cells and hemolytic activity.Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with the compounds.

Qualitative analysis of *Asparagus Racemosus* roots extracts

Phytochemicals	Extracts	
	Ethanol	Acetone
Sterols	+	+
Alkaloids	+	+
Flavonoids	+	-
Amino acids	+	+
Tannins	+	+

Qualitative analysis of *asparagus Racemosus* roots extract

Phytochemicals	Extracts			
	Ethanol	Aqueous	Chloroform	Methanol
Terpenoids	+	+	+	+
Alkaloids	+	+	-	+
Flavonoids	+	+	+	-
Saponins	+	+	-	-
Tannins	+	-	-	+

Anti-Fungal Activity Assay

The antifungal activity of methanol, ethanol,chloroform, aqueous and acetone extracts of Shatavri and Eclipta alba leaves was determined against Candida albic and

fungi by the agar well diffusion method. The results of antifungal activity assay clearly show the extracts have antifungal activity against the tested pathogenic fungi Candida albicans. The growth inhibitory activity of all the extracts against the tested fungi are summarized in the table 3 and 4. Fungal growth inhibition results shown in table 3 clearly indicate that methanol, ethanol, chloroform and acetone extracts of Shatavri roots and Eclipta alba leaves exhibited varying degrees of antifungal activity against the test fungi result show that growth inhibition in the fungi is highest with acetone extract in Shatavri and ethanol extract in Eclipta alba, suggesting highest antifungal activity as compared to other extracts tested. Acetone extract of Shatavri shows growth inhibitions nearly at par with positive control.

The minimum inhibitory concentration of Shatavri leaves extracts was found in range 12-20 nm and of Ecliptaalba was found in range 13-14 nm whereas the positive control shows 30 nm range.

Extracts (Shatawri)	Candida Albicans
Methanol	**12 Mm**
Ethanol	**13 Mm**
Acetone	**20 Mm**
Chloroform	**12 Mm**
Aqueous	**Not Detected**

Extracts (Eclipta Alba)	Candida Albicans
Methanol	**11 Mm**
Ethanol	**14 Mm**
Chloroform	**13 Mm**
Aqueous	**Not Detected**
+Vecontrol	**30 Mm**

Conclusion

The chemical drug areused to control fungal infection in humans caused by candida albicans, this study shows that Asparagus racemosus can be potent herbal medicine for the treatment of candida albicans infection. The bioactive compounds secreted from the plants with acetone extract and ethanol extract of Ecliptaalba targetscandidaalbicans. Pharmacognostic evaluation of both plants is made, compared and added accordinglytodevelop a bioactive extractat par with positive control.

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The Ayurvedic Pharmacopoeia of India, Part 1, Volume 2555 Medicinal Plant By Dr.S.Farooq Phytochemical, physio-chemical and anti-fungal activity of *Eclipta alba* Iqbal Hussain[1'"], Naeem Khan', RiazUllah', Shanzeb', Shabir Ahmed', Farhat Ali Khan and Sultan Yaz Pharmacognostic and Phytochemical Evaluation of Roots of Ecliptaprostrata.*Ambika Sharma, Mittal Arun, Aggarwal Sushma, Gupta Anil Kumar and Sardana Satish.Department of Pharmacognosy, Hindu College of Pharmacy, Sonepat (Haryana). India.

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Phytochemical Analysis of Leaf Extract of *Ecliptaalba* (L.) Hassk by GC-MS Method[*] Anand.D1, John Wyson.W1, Saravanan. $P_{p.}$1, Rajarajan.S2Pharmacognostical and phytochemical analysis Of Bhringraj (Eclipta Alba Hassk.)Dr. Ritu Sharma[1], Dr. Sumit Naithani[2].

Effect Of *Cannabis Sativa* On Spermatogenesis Of Male Albino Rats

*[1]S.P. Singh, [2]M.K. Purohit, and[3]Shruti Saxena
*[1]Department of Zoology, D.B.S. (P.G.) College, Dehradun, (UK.), India
[2]Department of Zoology, S.G.R.R. (P.G.) College, Dehradun, (UK.), India
[3]Department of Zoology, S.G.R.R. University, Dehradun,(UK.), India
[4] Department of Chemistry, D.A.V. (P.G.) College, Dehradun, (UK.), India
*Email: drspsingh1949@gmail.com

Abstract-Herbaceous plants have been used as food and medicine since time unknown. Excess of everything is harmful. Many plants may also be harmful if taken for long time and may impair function of reproductive organs i.e. testis and ovary. *Cannabis sativa* Linn. (Hemp plant), commonly known as "Bhang" or "Marijuana", now cultivated all over India, found wild in Himalayan region. Its preparations mainly 'cannabinoids' used as narcotic and psychotropic (medicinal) drugs. In the present communication, the effect of *C. sativa* (leaf powder) on spermatogenesis is reported. The leaf powder as aqueous suspension at doses of 50, 100 and 200 mg/kg/day were fed to three groups (dose wise) with a control group (vehicle treated) of male albino rats for 60 days. On day 61st, all the rats were sacrificed. The reproductive organs were taken out from body and processed for histological examination. Both initial and final body weight were recorded. The weight of organs were also taken before autopsy. The weight of reproductive organs were significantly reduced at higher doses. The spermatogenesis was arrested in testes. The seminiferous tubules were disfigured and reduced in size. Their lumen filled with cellular debris. The Leydig's cells were atrophied. The epididymes and vasa deferentia were devoid of spermatozoa. It is concluded that *Cannabis sativa* is harmful to male reproductive status of animal and human beings.

Keywords: *Cannabis sativa*,Medicinal plants, Reproductive organs,Anti spermatogenesis, Herbal drugs, Fertility and Sterility.

Introduction

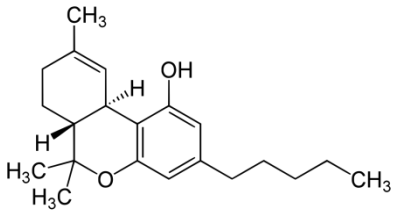
Successful reproduction is essential for continuity of species including human beings. Herbaceous plants have been used as food and medicine since time immemorial. Excess of everything is harmful. Many plants may also be harmful if taken for long period of time and may impair function of of reproductive organs i.e., testis and ovary. *Cannabis sativa* Linn (Hemp plant), commonly known as "Bhang" or "Marijuana", now cultivated all over India, found in Himalayan region also. The plant is nitrophilic, blooming well in nitrogenouswastes of soil near human habitation. Its preparations mainly "Cannabinoids" are used as narcotic and psychotropic (medicinal) drugs. In traditional medicine of India, particularly *Cannabis sativa* has been used as hallucinogenic, hypnotic, sedative, analgesic and anti-inflammatoryagent (Bonini*et al.*, 2018).

Many plants have been reported to impair reproductive function including libido and fecundity of animals if feeding them for long period i.e., chronically administration. Notable research work was reported by Nelson and Patanelli (1965), Pakrashi and Pakrashi (1977), Das (1980), Joshi *et al.* (1981), Dixit and Joshi (1982) and Khanna*et al.* (1986). in this regard. Effect of long term feeding of *Cannabis sativa* was also reported earlier by Dixit *et al.* (1974, 1978) and Singh and Singh (2017). Due to its psychoactive properties, it is commonly used as a drug of abuse and said to affect reproductive function. Considering the above point, the present study was done by authors and same is reported in this communication.

Phytochemistry

The main psychoactive constituent of *Cannabis sativa* is Tetrahydrocannabinol (THC). The plant is known to contain more compounds. They are commonly called as *Cannabinoids*. The constituents have been studied by various techniques *viz.,* paper chromatography, thin layer chromatography, gas-liquid chromatography, spectro-photometry etc. According to Sharma *et al.* (1972), the following are present – Tetrahydrocannabinol (T.H.C.) whose chemical structure is given. It is most active cannabinoid. Other compounds are

Cannabinol (C.B.N.). It is pharmacologically not active. Cannabidiol (C.B.D.) is also not very active. Podder and Ghosh (1974) have also reported same compounds in *Cannabis* samples from other states – U.P., M.P., Orissa, West Bengal. Krishnamurthy and Kaushal (1974) have reported compounds T.H.C. C.B.N. and C.B.D. in the samples of *Cannabis sativa* collected from Northern Indian regions and concluded that T.H.C. was in rich amount and found most active pharmacologically.



Tetrahydrocannabinol (THC)

Material and Methods

Experimental Animals

The experiment was done on swiss male albino rats (*Rattusrattusnorvegicus*). Healthy, adult, colony-breed Swiss albino rats, four-five months old weighing between 150- 200 gms were selected and acclimatized to the laboratory condition for seven days prior to commencement of experiment. The rats were kept in polypropylene cages (60 cm × 45 cm × 45 cm) under normal conditions of photoperiod and room temperature. Four groups of male rats were made, each containing 05 male rats. First group served as control (05 rats) vehicle treated, while three groups (05 rats in each group) were treated as drug treated group (dose wise). The vehicle was gum acacia powder dissolved in water (20%) by weight and volume (w/v). All the rats were fed twice a day with balance laboratory diet (Hindustan lever limited, Mumbai). Tap water was provided them *ad libitum*. All the rats were maintained as per U.G.C. guidelines and supervised by the members of Animal Ethical Committee appointed by the then principal of the college.

Plant Material

The leaves of *Cannabis sativa* were collected from surrounding areas of Haridwar and Dehradun, Uttarakhand. The plant was identified at Botanical Survey of India (Northern circle) Dehradun, India during June and July months. The leaves were dried in shade and after that kept in oven at 30°C for two days. Then, these were grinded mechanically to a fine powder, filtered through muslin cloth and stored in sealed glass bottles and labelled. This dried powder fed to experimental male rats.

Doses and Administration

Male rats of groups II, III and IV were orally fed with three doses 50, 100 and 200 mg/kg leaf powder respectively for 60 days. Each dose was dissolved in distilled water until it changes into a homogenous mixture. The volume was adjusted in such a way that 01 ml of this solution containing 50 mg powder. The rest of doses 100 and 200 mg were prepared and administered to rats of group III and IV respectively for 60 days. The administration of doses were done orally with specially designed knobbed feeding needle fitted into a syringe. The vehicle was administered in similar way to male rats of control group i.e. group I.

Record of body weight and organ weight

The initial and final body weight of the control and treated male rats were taken dose wise. The weight of reproductive organs i.e. testes, epididymes etc. noted (after dissected rats) in a semi-micro balance. The weights were recorded in tabular form of both control and treated rats. The data were statistically analysed using student 't' test. The values were expressed as mean ± standard error (S.E.). The significance of difference of weights between control and treated rats was taken $P < 0.05$ as significant.

Histological Studies

For histological studies, the testes, epidermes and vasa deferentia were taken out from body after dissection of all groups of male rats on day 61st. These organs were fixed in Bouin's fixative. After removing the fixative, these organs were dehydrated in graded series of ethanol, cleared

in xylene and embedded in paraffin wax. The organs embedded in paraffin wax were sectioned at 05µ (micron) using "Rotary Microtome" and mounted on glass slides. The mounted section of organs were stained with dyes haemotoxylene and eosine. Stained slides of testes etc. were examined under the microscope and photographed.

Results and Observations

Oral administration of aqueous suspension of leaf powder of *Cannabis sativa* in treated male rats revealed the effect on reproductive organs.

Effect on body and organ weight Table displays the changes in the body and reproductive organs weight. The male rats of control group did not show any change (reduction) in body weight. It was maintained throughout the experimental period i.e. 60 days. Treated male rats administered with leaf powder aqueous suspension of *Cannabis sativa* showed some reduction in the body weight at higher doses i.e. 100 and 200 mg/kg doses for 60 days. The higher doses i.e. 100 and 200 mg/kg doses for 60 days caused significant reduction in the weight of reproductive organs i.e. testes and epididymes in comparison to control male rats.

Histopathological changes

The administration of leaf powder as aqueous suspension through oral route for 60 days caused pathological changes in reproductive organs i.e. testes, epididymes and vasa deferentia were observed and compared with controls. The higher doses i.e. 100 and 200 mg/kg caused most deleterious (harmful) effects.

Effect on testes

Control-The testes of control male rats showed the normal histological features. The seminiferous tubules were rounded in shape, wide and lined with germinal epithelium which appeared with normal germinal cells and large sized sertoli cells. All stages of spermatogenesis were clearly indicated on seminiferous tubules. The spermatogonia with nuclei of moderate size. Spermatocytes and spermatids are present in normal position. The tubular lumens filled with spermatozoa. The Leydig's cells were present in space between seminiferous tubules known as interstitium. Vascularity appeared normal (Figure-1).

Treated-The administration of *Cannabis sativa* leaf powder at 50 mg/kg dose for 60 days caused insignificant histological changes in the testes. Various spermatogenic cell types in the seminiferous tubules were normal as in control rat testes. The administration of 100 mg/kg and 200 mg/kg doses for 60 days caused degenerative changes. The spermatogenesis was arrested at spermatid stage in majority of seminiferous tubules. Germinal epithelium appeared normal. The degeneration in the testes consisted of damaged spermatocytes, spermatids and spermatozoa. Deshaped seminiferous tubules were also noted. The interstitium (space between two seminiferous tubules) contained atrophied Leydig's cells. Vascularity was also increased (Figure-2).

Effect on Epididymes

Control- The epididymes of the control male rats after 60 days showed a normal histological structure. The epithelium showed columnar epithelial cells with basal nuclei. The lumen of the ductules were wide and filled with numerous spermatozoa. The well organisedstereocilia were present at the border of epididymalductules. The intertubular connective tissues with vascularity were normal. The spermatozoa are matured and stored in epididymes. These were present in epididymes (Figure-3).

Treated- The administration of of*Cannabis sativa* leaf powder as aqueous suspension at 50 mg/kg dose for 60 days caused no changes in histological structure of epididymes. The columnar epithelial cells appeared normal with basal nuclei. The epididymal tubular lumen was without spermatozoa. The stereocilia were normal. The higher doses i.e. 100 and 200 mg/kg administered for 60 days caused nuclear dysplasia in columnar cells of epithelium of the ductules of epididymes. The lumen of epididymalductules were devoid of spermatozoa. There were no spermatozoa at all. The stereocilia were distorted at certain points and adhered to each other. Intertubular spaces were increased (Figure-4).

Effect on vas deferens

Control-The vas deferens of control male rats after 60 days showed normal histological picture of its organisation. The cellular layers i.e., external longitudinal muscles and internal circular muscles were normal. Lamniapropria

and innermost layer, the mucosal lining which surround the lumen appeared normal with stereocilia and folds. The lumen was full of spermatozoa. The vas deferens is a passage for spermatozoa (Figure-5).

Treated – The administration of *Cannabis sativa* leaf powder as aqueous suspension to male rats for 60 days did not cause any untoward effect on vasa deferentia at 50 mg/kg dose. The structural details resembled to control rat's vas deferens. The higher doses i.e. 100 and 200 mg/kg administration for 60 days caused notable changes in this part. Histological structure was not much changed except the innermost layer i.e. mucosal lining which showed distortion of stereocilia and cellular organization. The lumen appeared empty. There were no spermatozoa (Figure-6).

Discussion

Cannabis sativa leaf powder as aqueous suspension was administered orally at doses of 50, 100 and 200 mg/kg for 60 days to male albino rats. The purpose behind this study was to assess the effect of long term feeding of *Canabis sativa* on spermatogenesis that occur in male reproductive organs i.e. testes, epididymes and vasadeferentia. The result showed marked effect on male reproductive organs with total arrest of spermatogenesis. Varying degree of damage caused in different testicular elements i.e. testes and androgenic male hormone producing cells, the Leydig's cells were mostly atrophied. The weight of the testes and epididymes was also reduced at higher doses i.e. 100 and 200 mg/kg doses. The effect appeared to be anti-androgenic.

In the male albino rat, the testes are scrotal and the complete spermatogenic cycle requires about 65 days (Jackson, 1966). During the first 45 days, the differentiation and maturation of spermatozoa occur in the testes. During the last 20 days, the spermatozoa are transported through the epididymes to the vas deferens for ejaculation. The sperms are stored in the caudaepididymes for further maturation from the testes.

The follicle stimulating hormone (F.S.H.) secreted from pituitary gland, is directly related to the weight of testes. Heavier will be the testes, more will be secretion of F.S.H. It is confirmed that both non-steroidal (plant product etc.) and steroidal agents inhibit pituitary gonadotropin either acting directly on pituitary or through the hypophyseal axis. The reduced secretion of F.S.H. causes significant decrease in the weight of testes and accessory reproductive organs of male rats (Dorfman*et al.,* 1963). Paul *et al.,* (1953) had demonstrated the reduction of weight of testes and accessory reproductive organs in absence of spermatids and spermatozoa. The change in weight of testes also corresponds to the presence or absence of post-meiotic cells in the testes. The physiology of reproductive organs (also known as genital organs) are androgen dependent (Nelson and Patanelli, 1965).

In the present study, the chronic administration of *Cannabis sativa* caused arrest of spermatogenesis in male albino rats. It also caused reduction of weight of testes etc. Similar findings by chronically administration of flower extract of *Malvaviscusconzattii* in male albino mice were made by Joshi *et al.* (1981). The anti-spermatogenic effect of the extract of *Aristolochiaindica* in male mice was reported by Pakrashi and Pakrashi (1977). A marked reduction in population of spermatozoa was observed when aqueous suspension of seed powder of *Carica papaya* was administered to adult male rats (Das, 1980). The leaf extract of *Vincarosea* caused significant histological changes in the testes, epididymes and also decreased weight of reproductive organs (Chinoy and GeethaRanga, 1983). Similar effects of long-term feeding of Tulsi leaves, *Ocimum sanctum* caused reduction of testicular weight and decrease in sperm population (Khanna*et al.,* 1986). The long and short term administration of flower extract of *Hibiscus rosasinensis* (Kholkute and Udupa, 1974) resulted in the significant decrease in the weight of male reproductive organs i.e. testes, epididymes and seminal vesicles in male albino rats under experiment. Singh and Singh (2017) reported that *Solanumxanthocarpum* (seeds) caused similar effects in male Guinea pigs also.

Dixit *et al.* (1974) reported that the testicular function was arrested with the chronically administered *Cannabis* extract to male mice. Dixit *et al.* (1978) also reported the adverse effect of *Cannabis* extract on testicular function of Toad, *Bufoandersonii*Boulenger. The above-mentioned studies of *Cannabis sativa* leaf on arrest of male reproductive functions strongly

support our studies as reported in this communication.

Conclusion

Cannabis sativa Linn.is a herbaceous plant commonly known as "Bhang", "Hemp" and "Marijuana". It has been used as a psychotropic drug since time immemorial. It has therapeutic effect on many psychosomatic disorders. Its preparations are narcotic also. The most effective phytochemical constituent is "Tetrahydrocannabinol" (T.H.C.). It is said that long term use of the *Cannabis* cause sexual weakness. According to present study, the *Cannabis* leaf powder when fed to male albino rats for 60 days, the doses 100 and 200 mg/kg caused anti-spermatogenic effects.

Acknowledgement

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Table-1 Effect of *Cannabis sativa* leaf powder on Body Weight (gm) and Genital organ Weight (mg) of male rats administered at different doses for 60 days. 05 rats were used in each group (control and treated). Values are mean ± S.E.

Treated	Doses (mg/kg)	Body weight (gm)		Genital organ wt. (mg)	
		Initial	Final	Testes	Epididymes
Control	–	156.10 ± 19.24	172.15 ± 13.20	698.12 ± 10.65	198.35 ± 23.07
Aqueous suspension	50	156.20 ± 17.15	157.15 ± 12.18	696.44 ± 7.73	191.10 ± 17.43
	100	156.10 ± 23.20	157.10 ± 13.12	690.46 ± 13.12	190.25 ± 13.25
	200	154.15 ± 16.43	155.25 ± 27.10	295.17 ± 11.10*	117.16 ± 12.75*

*P < 0.05

Effect of Cannabis sativa on Spermatogenesis of Male Albino Rats

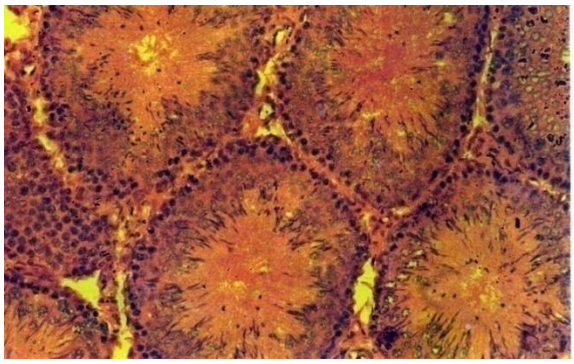

Figure-1 T.S. of testis of control male rats. Note all spermatogenic elements including organized germinal epithelium, spermatocytes, spermatids and spermatozoa in seminiferous tubules and Leydig's cells in the interstitium X 400.

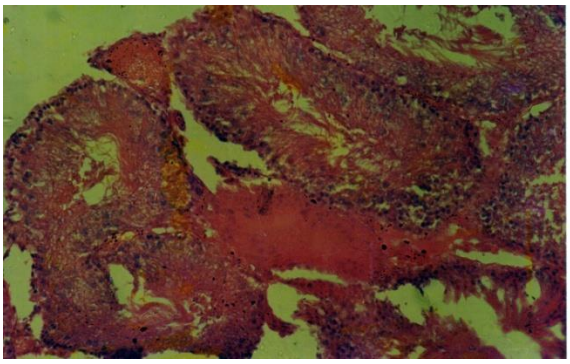

Figure-2 T.S. of testis of treated male rat with *C. sativa* leaf powder suspension at 200 mg/kg dose for 60 days. Note arrest of spermatogenic activity in damaged seminiferous tubules and absence of Leydig's cells. X 400

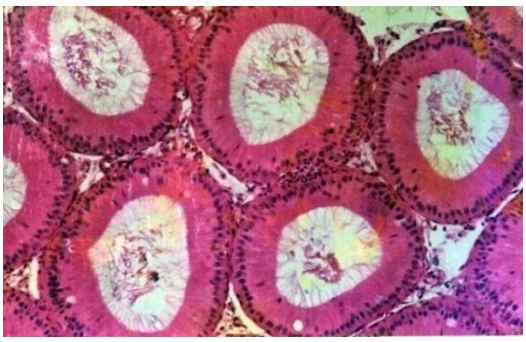

Fig. 3 : T.S. of epididymis of control male rat. Note the normal histology with organized epididymal epithelium and spermatozoa in the epididymalductules and normal stereocilia. X 400.

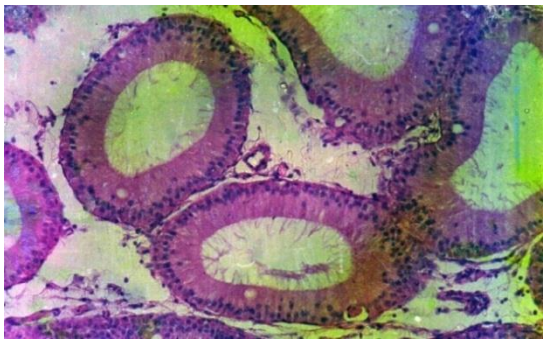

Fig. 4 : T.S. of epididymis of treated male rat with *C. sativa* leaf powder at 200 mg/kg dose for 60 days. Note the vacuoles in epididymal epithelial cells, distorted stereocilia and empty epididymalductules. X 400

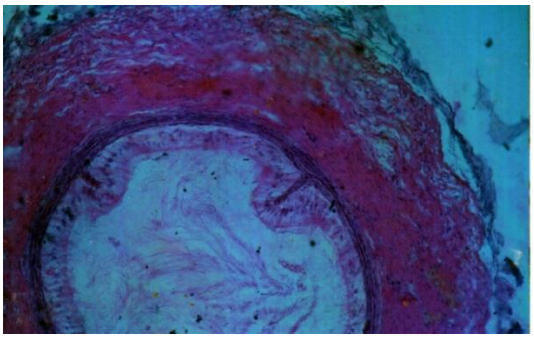

Figure-5 T.S. of vas deferens of control male rat. Note the normal histoarchitexture including mucosal lining with epithelial cells, folds and stereocilia. Plenty of spermatozoa in the lumen. X 400

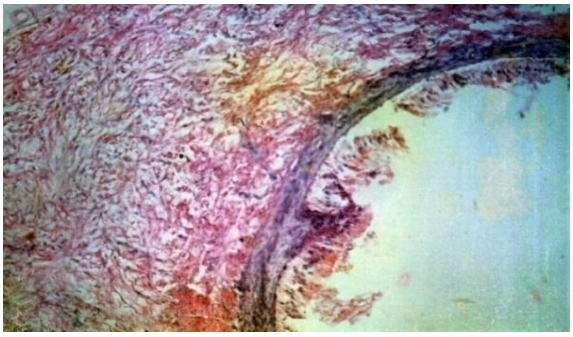

Fig. 6 : T.S. of vas deferens of treated male rat with *C. sativa* leaf powder at 200 mg/kg dose for 60 days. Note the absence of spermatozoa in the lumen, distorted luminal epithelium and mucosal layer and loss of stereocilia. X 400

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Phytochemical Screening and Anti-microbial Activity Of Leaves And Rhizomes of *Acorus calamus* Linn.

*[1]Ragib Ali and [2]Mirza Azim Beg
[1& 2]QA/QC Department, Himalaya Wellness Company, Faridabad, HaryanaIndia
*Email:ragibali94@gmail.com

Abstract-The present communication attempts to evaluate the comparative Phytochemical screening and Anti-microbial activity of leaves and rhizomes of *Acorus calamus* Linn. (Araceae family). Acorus calamus Linn .is a well-known medicinal plant in traditional medical systems having various ethanopharmacological uses. As the official source of plant was roots and rhizomes and it had been studied extensively. Previously leaves of Acoruscalamuswere not regarded as useful part of plant, but now-a-days there is growing interest in leaves of this plant as there is no detailed work reported so far on its leaves. Antimicrobial activity was performed using methanolic and aqueous extract through cold percolation method against Staphylococcus aureus,Pseudomonas aeruginosa, Escherischia coli and Salmonella typhi. The extract was found to have positiveresults against all.

Keywords: *Acorus calamus* Linn., Methanolic extract aqueous extract, Antimicrobial activity.

Introduction

Acorus calamus Linn.is semi-aquatic herb with creeping rhizomes and sword shaped long leaves, found near marshy places, river banks and lake.[1]It is up to 6 feet tall, aromatic, sword shaped leaves bearing small yellow/green flowers and branched rhizome. It is widely distributed throughout India and Ceylon, in marshes, wild or cultivated, ascending the Himalayas up to 6000 feet in Sikkim, marshy tracts of Kashmirand Sirmoor in Manipur and Naga Hills.[2]The roots and rhizomes *Acoruscalamus*Linnare used medicinally since ancient times. They possess antispasmodic, carminative and anthelmintic properties and are also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, fever and glandular and abdominal tumours.[3,4] They are also employed for kidney and liver troubles, rheumatism, sinusitis, eczema and anti-cellular activities.[5] Recently roots and rhizomes identified as antibacterial agent against fish pathogen[6] and also shows insulin sensitizing activity.[7] Whereas mature green leaves exhibit various activities including insect repellent, when cut and stored with dry foods[8], antihyperlipidemic activity, anti-diabetic activity[9], antipsychotic activity[10], antimicrobial and analgesic actions.[11]As we all know that it is increasingly being realized that majority of the diseases today are due to the shift in the balance of pro-oxidant and the antioxidant homeostatic phenomenon in the body. Keeping in view of the above observations, in the present study, methanolic and aqueous extract of leaf and rhizome of the plant were subjected to evaluate the comparative Phytochemical Screening and Anti-microbial activity.

Material and Methods

Leaves and rhizomes of *Acorus calamus* Linn. were collected from locality of Faridabad, Haryana. Plant material was authenticated by **Dr. Maya Ram Uniyal** (Ex. Advisor medicinal plant UP/UK).

Chemicals-10%aqueous (DMSO) Dimethyl sulfoxide , (Ofloxacin (4µg/ml).

Methodology[15]

Zone of inhibition (Diffusion Method)-The dried extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 500mg/ml and sterilized by filtration through a 0.45µm membrane filter. Antibacterial activity was determined by agar well diffusion method. Bacteria were cultured at 30°C for 24 hrs in Muller Hinton Broth (MHB, Hi-media). An inoculums consisting of 10^6CFU/ml was used. Antibiotics such as Ofloxacin (4µg/ml) and solvent 10% DMSO without the test compound were used as positive and negative controls respectively. The tests were conducted in triplicate.

Determination of minimum inhibitory Concentration

The minimum inhibitory concentration (MIC) of the methanol extract was evaluated by Tube dilution method. The methanol extract MIC was determined by dilution of the extract to various concentrations (15.625-500mg/ml). All the tubes were incubated to suitable temperature for 18- 24hrs. The tubes were observed for any growth. The MIC was interpreted as the lowest concentration of the extract that did not show any visible growth when compared with control tubes.

Results And Discussions

Results obtained from the present study show that the leaf and rhizomes of Acorus calamusLinn. contain alkaloids, saponins, terpenoids, flavonoids, resins, essential oil, carbohydrate and tannin,. Results are reported in Table-1 and 2. Phytochemical screening of successive fractions from Soxhlet: (+) shows presence, and (-) shows absence of content.

Table-1 Phytochemical Screening of Leaf of *Acorus calamus* Linn.

S. No.	Constituents	Tests	Hexane	Chloroform	Ethylacetate	Methanol	Water
01.	Carbohydrates	Bendict's test	+	+	+	+	+
		Molisch's test	+	+	+	+	+
		Caramelistation	+	+	+	+	+
02.	Glycosides	Fehling test	-	+	+	+	+
03.	Steroids	Libermannburchard test	+	+	+	-	-
		Salkowski reaction	+	+	-	-	-
04.	Proteins & Amino-acids	Biurete test	-	-	-	+	+
		Ninhydrin	-	-	-	-	+
05.	Saponins	Foam test	-	-	-	+	+
06.	Tannins	$FeCl_3$ test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanilin hydrochloride test	-	-	-	+	+
07.	Triterpenoids	Libermannburchard test	+	+	+	-	-
08.	Alkaloids	Dragndroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
09.	Resin	Resin	-	-	-	-	-
10.	Flavonoids	Alkaline reagent test	-	+	+	+	+
		Shinod'a test	-	-	+	+	+

Table-2 Phytochemical Screening of Rhizome of *Acorus calamus* Linn

S. No.	Constituents	Tests	Hexane	Chloroform	Ethylacetate	Methanol	Water
01.	Carbohydrates	Bendict's test	+	+	+	+	+
		Molisch's test	+	+	+	+	+
		Caramelistation	+	+	+	+	+
02.	Glycosides	Fehling test	-	+	+	+	+
03.	Steroids	Libermannburchard test	+	+	+	-	-
		Salkowski reaction	+	+	-	-	-
04.	Proteins & Amino-acids	Biurete test	-	-	-	+	+
		Ninhydrin	-	-	-	-	+
05.	Saponins	Foam test	-	-	-	+	+
06.	Tannins	$FeCl_3$ test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanilin hydrochloride test	-	-	-	+	+
07.	Triterpenoids	Libermannburchard test	+	+	+	-	-
08.	Alkaloids	Dragndroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
09.	Resin	Resin	-	-	-	-	-
10.	Flavonoids	Alkaline reagent test	-	+	+	+	+
		Shinod'a test	-	-	+	+	+

Antimicrobial Activity (Cup Plate Method)

Table-3 Zone of inhibition (mm)

Sample	***E. coli***	***Staph. aureus***	***P. aeruginosa***	***Sal. typhe***
Methanolic Extract of leaves	20	23	18	16
Aqueous Extract of leaves	14	19	17	15
Methanolic Extract of rhizome	22	30	20	17
Aqueous Extract of rhizome	12	17	15	11
Ofloxacin (+Ve Control)	28	30	24	22

Table-4 Minimum Inhibitory Concentration (mg/ml) of Extract

Sample	***E. coli***	***Staph. aureus***	***P. aeruginosa***	***Sal. typhe***
Methanolic Extract of leaves	20	23	18	16
Aqueous Extract of leaves	14	19	17	15
Methanolic Extract of rhizome	22	25	20	18
Aqueous Extract of rhizome	12	14	16	12

Conclusion

It could be concluded that the methanolic and aqueous extract of *Acoruscalamus*Linn leaves and rhizomes have potentially good antimicrobial activity.The tested extracts of *Acoruscalamus*have shown antimicrobial efficacy against most of microbes examined.Differential antimicrobial activity of extracts against different bacteria might be due to present of different active phyto-chemicals. Among those antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids are very important compounds in antimicrobial effects. Further study is required to determine the different active compounds from these under-utilized tropical fruits and their full spectrum of efficacy. These resources have the prospect of finding new clinically efficient antimicrobial compounds and the knowledge can be extended for future investigation into the field.

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PreliminaryPhytochemical ScreeningAnd Qualitative Estimation ofHerb (Moss Rose)

*[1]Shweta Tyagi, [2]I.P. Pandey, [1]Ashish Kumar
*[1]D.A.V.(PG) College,Muzaffarnagar, U.P., India
[2]Professor Emeritus Dehradun, India
*Email-shewtatyagi@gmail.com

Abstract-The four differentvarieties (white, yellow, pink and red) of *Portulaca grandiflora (Moss Rose)*were screened for their *preliminary phytochemical estimation*. These plants were extracted by diethyl ether, acetone and ethanol, respectively. The results showed that all the different extracts having considerable amount of all the phytochemicals except steroids and anthraquinone. Ethanolic extract of all plant varieties show maximum qualitative estimation while the extract prepare with diethyl ether shows minimum.

Keywords: Portulaca grandiflora, Phyto-chemical, Moss rose, Qualitative.

Introduction

Portulaca grandiflora is a drought and heat tolerant annual native to hot, dry plains in India. This herbaceous plant in the purslane family (Portulacaceae) is cultivated throughout the world as a garden annual for its showy flowers that bloom all summer long with little care. Moss rose is a semi-succulent plant that stores water in its fleshy leaves and stems approximately 10-30cm high, leaves about 12-35 mm in length and 1- 4 mm in width, linear-subulate, thick, and fleshy and spirally arranged. The bright green leaves are oblong to cylindrical with pointed tips. Common names embrace nonvascular plant Moss rose in English, Nonia in Hindi, Pung mapan satpi in Manipuri and Gul-e-Shama in Urdu, portulaca, and Sun plant.(Dkhil *et al*.,2011)Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Prince and Prabakaran, 2011).Phytochemicals are naturally occurring in the medicinal plants leaves, vegetables and roots that have defence mechanism and protect from various diseases(Ncube, N.S. et al.,2008). Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al,* 2007). Terpenoids are very important in attracting useful mites and consume the herbivorous insects (Kappers, 2005).The main objective of our research work was to analyse the presenceor absence of different phytochemicals in the selected plants used for healing and curing of various diseases.

Materials and Method

Plant materials

P. grandiflora or moss rose was obtainedfrom Rama Nursery,Roorkee Road, Muzaffarnagar. The plants were harvested at maturity, and during or prior to their flowering period. Four varieties (orange, red, pink and white) of the same species were used in this study.

Preparation of plant extracts

Preparation of sample and extraction were carried out as described by Saha *et al.* with slight modifications. The whole fresh plant samples (stem, flower and leaf) were washed with distilled water and cut in to small pieces, shade- dried under for 1 week and followed by complete drying at 40 °C in oven. Then grinned to from powder.10 g of these dried sample from each variety was extracted separately with 100 mL of three different solvents: acetone, di ethyl ether and ethanol for 24 h in ashaker at 100 rpm at temperature 30 °C. The extracts were filtered using Whatman filter paper and filtrates were used as an extract. Extracts

were kept at 4 °C until the bioassay analyses.

Phytochemical screening (Qualitative method)

Preliminary phytochemical tests were carried out on the different extracts (acetone,diethyl ether and ethanol) of*Portulaca grandiflora* using standard procedures to identify the constituents as described by (Sofowara, 1993; Trease and Evans,1989; Harborne,1973 and 1984).

Test for Tannins: 1ml ofevery sample is boiled in 20 ml ofdistilled water in a test tube and then filtered separately. Acouple of drops of 0.1% ferrous chloride are additional anddetermined for brownish green or a blue-black colouration.

Test for Saponins: 2 ml of every sample is boiled in 20 ml ofdistilled water in a water bath and filtered separately. 10ml of the filtrate is mixed with 5 ml of distilled water and jolted smartly for a stable persistent froth. The frothing is mixed with three drops of olive oil and jolted smartly, then determined for the formation of emulsion.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a little of the liquid filtrate of every plant extract followed by addition of targeted H_2S0_4. A yellow colouration determined in every extract indicated the presence offlavonoids. The yellow colouration disappeared on standing.

Test for Steroids: 2 ml of acetic anhydride is added to 1mlof extract of every sample with 2 ml H_2SO_4.The colour modified from violet to blue in some samples indicating the presence of steroids.

***Test for Terpenoids (Salkowski test*)**: 5 ml of every extract is mixed in 2 ml of chloroform, and targeted H_2SO_4 (3 ml) is vigorously additional to create a layer. A venetian red colouration of the interface points out positive resultsfor the presence of terpenoids.

Test for Triterpenoids: One ml of every extract is addedto 1 ml of chloroform; 1 ml of acetic anhydride wasadded following the added of 2 ml of diluted H_2SO_4. Formation of blood-red violet colour indicates the presence of triterpenoids.

Test for Alkaloids: Mayer's test: To a couple of (one) ml of every extract, a drop of Mayer's chemical agent was added. A creamy or white precipitate indicatedthe presence of alkaloid.

Test for Anthraquinones: 5ml of every extract solution was hydrolysed with diluted H_2SO4 extracted with benzene. 1 ml of dilute ammonia is added in this solution. Pink coloration indicated the positive response for anthraquinones.

Test for Polyphenols: Plant product (4 ml) is added to every extract (1ml) and also the ensuing resolution is transferred in take a look at tubes and warmed in a water bath(15 minutes). 3 drops of freshly ready ferrous cyanide resolution were added to the extract solution. Formation of a blue colour indicated the presence ofpolyphenols.

Test for Glycosides (Keller-Killani test): Five ml of every extract was treated with 2 ml of glacial acetic acidcontaining one drop of ferrous chloride resolution. This is oftenunderlayed with 1 ml of targeted H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristicss of cardenolides.A violet ring appeared below the brown ring, whereas within the carboxylic acid layer, a green ring could be observed step by step throughout skinny layer.

Results and Discussion

This study revealed that the presence of all the phytochemicals considered as active medicinal chemical constituents except anthraquinone and steroids as shown in table. Important medicinal phytochemicals such as terpenoids,triterpenoids, flavonoids, alkaloids, tannins, glycosides, polyphenol and saponin were present in considerable amount of all the samples. The phytochemical screening and qualitative estimation of all four plants studied showed that the whole plant (leaves, stem and flower) was rich with important

phytochemicals. Ethanol extract of all the four varieties showed maximum presence while diethyl ether showed minimum effect with all varieties. Moss rose varieties were found rich with polyphenol, alkaloids, glycosides and saponin content, Saponins are therapeutically important because they lower bad fats in the body and have anticancer potentials. Saponins help in lowering cholesterol which will subsequently reduce the risk of cardiovascular diseases such as hypertension which usually leads to stroke. Saponins are known to produce inhibitory effect on Inflammation (Olayinka Temitayo Ogunmefun, 2018).Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial (Mahato and Sen,1997). Plants having alkaloids areused in medicines for reducing headache and fever. These are attributed for antibacterial and analgesic properties (Pietta, 2000).Alkaloids are useful as central nervous system (CNS) stimulants in pharmacological application.

Table- Preliminary Phytochemical screening and qualitative estimation of the four varieties (red, yellow, white and orange) of *Portulaca grandiflora* whole plant extracted with different extracts (acetone, di ethyl ether and ethanol)

S.No.	Phytochemicals	plant varieties	Acetone	Di Ethyl Ether	Ethanol
1	Tannins	Red	+	+	+++
		Yellow	+	+	++
		Pink	+	+	++
		White	+	+	+++
2	Saponins	Red	++	++	++
		Yellow	+	++	++
		Pink	+++	++-	++
		White	+++	++	++
3	Flavonoids	Red	++	+	+++
		Yellow	++	+	+++
		Pink	+	+	++
		White	++	+	++
4	Steroids	Red	+	-	-
		Yellow	-	-	-
		Pink	-	-	-
		White	-	-	-
5	Terpenoids	Red	++	+	+
		Yellow	+	+	++
		Pink	+	+	++
		White	++	++	++
6	Triterpenoids	Red	++	++	+++
		Yellow	+	+	+++
		Pink	+	++	++
		White	++	++	++
7	Alkaloids	Red	+++	+	+++
		Yellow	++	+	++
		Pink	+++	+	+++
		White	++	+	+++
8	Anthraquinones	Red	-	-	-
		Yellow	-	-	-
		Pink	-	-	-
		White	-	-	-
9	Polyphenols	Red	++	++	++
		Yellow	++	++	+
		Pink	+	++	++
		White	++	+++	+++
10	Glycosides	Red	++	+	+++
		Yellow	+	+	+++
		Pink	++	++	++
		White	+++	+	+++

Alkaloids also find its usefulness as pain relievers. In industries anthraquinones are used for washing of bowels (laxatives) and in dye production.Flavonoids function to reduce the risk of coronary heart diseases and possess anticoagulant, anti-inflammatory, and aphrodisiac properties(Olayinka Temitayo Ogunmefun, 2018).

Conclusion

The selected plants varieties of moss rose plant is the rich source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, and glycosides etc. Medicinal plants play a vital role in preventing various diseases. The phytochemical analysis of these plants is also important and have commercial interest in both research institutes and pharmaceuticals companies for manufacturing of the new drugs for treatment of various diseases.

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Comparative Study On Antimicrobial Activities Of *Ziziphus jujube* and Ziziphus*nummularia*Fruits Extract

*[1]Mirza Azim begand [2]Ragib Ali
[1 and 2] QA/QC Department, Himalaya Wellness Company, Faridabad Haryana India
Email: azim_0088@yahoo.com

Abstract-The present study was aimed at detecting the phytochemicals and evaluating comparative antimicrobial activities of *Ziziphusjujuba,* and Z. *Nummularia* fruits extract known for their medicinal properties in folk medicine. The comparative assessment of antifungal activity was performed in terms of percentage of radial on solid medium (potatoes dextrose agar PDA) against *Aspergillus flavus, M.gypseum, T. flavurusclem* and *Penicillium expansum.* The antibacterial effect was studied by the agar direct contact method using *Staphylococcus aureus*, *Pseudomonas aeruginosa B.cereus, B.pumilus, M.luteus* and *Escherichia col strains.* The results revealed that the methanolic extract and the acetonic extract exhibited significant antimicrobial activity of concentration of 100-500 µg/ml against tested organisms respectively.

Keywords: *Ziziphusjujuba,* Z. *nummularia*fruits extract, Antimicrobial activity

Introduction

Ziziphusjujuba

Ziziphus jujube Lam. is also called as Badari, Baer, Bogari is belonging to the family Rhamnaceae[1]. It is a small sub deciduous tree with dense spreading crown, commonly 6 - 8 m high. The Plant is distributed throughout India, Iran, Afghanistan and in China. The bark is blackish to grey or Brown, rough, regularly and deeply furrow, the furrows are at about 1.2 cm apart. Blaze 9;13 mm, Branches usually armed with spines, mostly in pairs, one straight ,the other with curved[2-6]. Leaves 3-6.3 by 2.5-5 cm., oblong or ovate, usually minutely serrulate or apex distinctly toothed, obtuse, base oblique 3; nerved, nerves depressed on the glabrous shining upper surface. A survey of literature on Ziziphus jujube Lam revealed a few pharmacological activity on the plant were reported, antisteroidogenic activity[7]anti obesity activity[8] anticancer activity[9], anxiolytic activity[10-12]. The plant is reported to contain alkaloid Jubanine-E[13] and sedative flavonoids[14] such as swertish and spinosin[15]. Triterpenic acids have been isolated from the fruits of *Zizyphusjujuba*. Betulin, Betulinicacid ,Ursolic acid Ceanothic acid are triterpenes reported by Shoei et al.[16-21]

Ziziphusnummularia

Ziziphusnummularia is a common plant of central India and its root, leaves and seed are used by tribes for curing different diseases like allergy, scabies, eczema and pyorrhea etc. The roots of *Ziziphusoxyphylla*Edgew and juice of fresh leaves of *Z. Mauritiana*L are used for curing jaundice[22]. A cold suspension of dried roots powder of *Ampelozyziphusamazonicus*is used to prevent malaria[23]. Traditionally, *Origanummajorana*L is used in asthma, indigestion, headache, rheumatism and protect against hydroquinone induced cytogenesis and histological changes[24-25] . The methanolic extract of *Zizyphispinosi*semen, over a concentration range of 0.05–5 µg/ml, prevents N-methyl- *D*-aspartate (NMDA) induced neuronal cell damage *in vitro*[26]. The seeds of *Z. jujuba*have been used as analgesic, tranquilizer, convulsant and have been prescribed for the treatment of insomnia and anxiety in Asia[27].Furthermore, traditionally, jujube is used prophylactically for liver diseases[28]. The fruit being mucilaginous is also very soothing to the throat and decoctions of *jujube* have often been used in pharmacy to treat sore throats. *Z. jujuba*extracts exhibited protection against hydroquinone induced cytogenesis[25]. Theasinensin a polyphenol obtained from fruits of *Z. jujuba* suppressed the antibiotic resistance of Methicillinresistant*Staphylococcus aureus*[29]. Extracts of *Z.jujuba*fruits and seeds exhibited moderate activity against *Lycoriellaingenua*and *Coboldiafuscipes*, which are important mushroom pests[30].

Materials and Methods

Collection of *Ziziphus jujuba* and Z. *nummularia* fruits

Fruits of *Ziziphus jujube* and Z. *nummularia*fruits were collected from locality of Faridabad, Haryana. Plant material was authenticated by **Dr. Maya Ram Uniyal**(Ex. Advisor medicinal plant UP/UK).

Extraction of *Ziziphusjujuba*and Z. *nummularia*fruits in different solvents (Nonpolar to Polar)

The collected plant material was washed with water to remove other undesirable material and then dried under shade. The air-dried fruits (200 gm) of allwere crushed and remove the seed separately. The crushed fruits extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone, methanol by hot percolation method using soxhlet apparatus. The extract was then evaporated till dryness to obtain residue. These using were concentrated under reduced pressure. The extract was used for antimicrobial activity.

Anti-microbial activity of different extracts[31]

The **anti-microbial activity** of the fruits of *Ziziphusjujube* and Z. *nummularia*wascarried out. The fruits extracts were screened for anti bacterial and anti fungal activities.

Anti bacterial activity of fruits extract

In this study, the anti bacterial activity was studied against the micro organismand the bacterial cultures used in the study were: 1. *Escherichia coli,* 2. *Pseudomonas aeruginosa and 3. Bacillus cereus.*These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37^0C for about 18-24 hours and then stored at 4^0 C as stock for anti bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at 37^0 C overnight. To test anti bacterial activity, the well diffusion method was used.

Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-Media Laboratories, Mumbai. The media used for anti bacterial activity Muller- Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121ºC at 15psi for 15-20 minutes autoclave.

Plate preparations

25 ml of pre autoclaved Muller-Hinton agar(MHA) was poured into 90 mm diameter pre sterilized Petri-plates. These Petri-plates were allowed to solidify at room temperature.

Well diffusion method

After the plates solidified, the freshly prepared microbial growth culture suspension (about 20µl) was spread over the Muller – Hinton agar (MHA) media using L shaped sterilized glass spreader separately under the aseptic condition using laminar air flow. Then, well were made in each plate with the help of borer of 8 mm diameter .In these well, about 100 µl of each fruits extract individually was loaded. This method depends upon the diffusion of fruits extracts from hole through the solidified agar layer of Petri-dish to such an extent that the growth of added microorganism is prevented entirely in a circular area or Zone around the hole containing fruits extract.

Incubation: Petri plates were incubated for overnight at 37^0C ± 0.5^0C in the incubator.

Inhibition Measurement of Zone of inhibition

After incubation, the diameter of clear zone of incubation produced around the well or holes were measured in mm by ESR Tube and compared with standard drug.

Results

Table-1 Antibacterial activity of different extracts of *Ziziphus jujube* and standard drug Chloramphenicol, Streptomycin, Ampicillin.

S.No.	Test Organism	Inhibition zone in mm					
		Pet. Ether	Chloroform	Methanol	Standard Drug		
					Ampiciline	Streptomycin	Chloram-phenicol
1	E.coli	-	3	12	25	20	24
2	Bacillus cereus	-	5	30	20	20	20
3	pseudomonas	-	15	10	-	22	15

Table-2 Antibacterial activity of different extracts of *Ziziphusnummularia*and standard drug Chloramphenicol

S.No.	Test Organism	Inhibition zone in mm						
		Pet. Ether	Chloroform	Acetone	Methanol	Standard Drug		
						Ampiciline	Streptomycin	Chloram-phenicol
1	E.coli	15	24	22	-	22	18	30
2	Bacillus cereus	20	18	27	-	15	17	36
3	B.pumilus	-	20	26	24	-	17	22
4	M.luteus	-	11	15	18	32	25	28

Table-3 Antifungal activity of different extract of *Ziziphusjujuba*and standard drug Amphotericin-B and Clotrimazole.

S.No.	Test Organism	Inhibition zone in mm				
		Pet. Ether	Chloroform	Methanol	Standard Drug	
					Amphotericin-B	Clotrimazole
1	*Aspergillusniger*	-	-	20	-	12
2	*Sclorotium*	-	10	8	-	-
3	*Candida- albicans*	-	-	25	-	14
4	*Rhizopus*	-	-	9	-	-

Table-4 Antifungal activity of different extract of *Ziziphusnummularia*and standard drug Amphotericin-B and Clotrimazole.

S.No.	Test Organism	Inhibition zone in mm					
		Pet. Ether	Chloroform	Acetone	Methanol	Standard Drug	
						Amphotericin-B	Clotrimazole
1	*Aspergillusniger*	-	-	-	-	-	12
2	*M.gypseum*	20	16	21	-	-	-
3	*T.flavurusclem*	14	13	23	-	-	11

Discussion

The antimicrobial activity of fruits extracts of *Ziziphusjujuba*was found active against E.coli, Bacillus cereus, Pseudomonas in chloroform and methanol extracts whereas leaves extracts of Pt.ether was found less active against E.coli, B. cereus, Pseudomonas. The leaves extracts of methanol was found highly active against *Candida albicans and Aspergillusniger*while less active against *Rhizopus and Sclorotium.*

The leaves extracts of Pt. ether did not find any activity. The results revealed that the **methanolic extract** has shown more degree of anti microbial activity than other extract when compared to the standard drug. It is due to presence of chemical constituents like carbohydrates, phenolic compounds,tannins, triterpenoids, saponins,terpenoids, proteins and aminoacids, this was confirmed by phytochemical studies. While in case of ***Ziziphusnummularia*** the**acetonic extract** showed good antibacterial activity against B.cereus and B.pumilus and good anti-fungal activity against *T. flavurusclem*in comparison to the standard drug.

Conclusion

The findings of the present study revealed that *Ziziphus jujube &Z. nummularia*different fruit extract contain potent antimicrobial property. The results also support the folkloric usage of the studied plants. These resources have the prospect of finding new clinically efficient antimicrobial compounds and the knowledge can be extended for future investigation into the field of pharmacology for better drug discovery.

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Antimicrobial activity Of *Curcuma longa* Along With Its Total Polyphenolic And Curcuminoid Content

[1]Suman LataChhimwal, [*2] Nilesh Gupta,[3]Iqra and Sonakshi Chandra
[1]The Himalaya Drug Company,Sahranpur Road Dehradun,UK, India
[*2] Department of Biotechnology IILM College of Engineering and Technology, Gr. Noida (201306) U.P., India
[3]M J P Rohilkhand,University, Bareilly, India
[*]Email: nileshgupta300@gmail.com

Abstract-Turmeric or Haldi, a spice derived from the rhizomes of the plant *Curcuma Longa*. *Curcuma longa* is a member of the Zingiberaceae family or the ginger family. The bright yellow color of turmeric comes mainly from compounds known as Curcuminoids. Curcuminoid is a group of fat-soluble polyphenolic compounds includes curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the primary curcuminoid in turmeric, it has powerful anti-inflammatory effects and is a very strong antioxidant and is approximately 77% of the curcuminoid content. Turmeric is also a source of polyphenols, which is a category of plant compounds that offers various health benefits. They can act as antioxidants, meaning they can neutralize harmful free radicals that would otherwise damage cells, regularly consuming polyphenols is thought to boost digestion and brain health, as well as protect against heart disease, type 2 diabetes, and even certain cancers. Plants show medicinal properties because of the phytochemical present in them. In order to extract these essential phytochemicals out of the rhizomes of turmeric, Acetone, Methanol, Ethanol, and Chloroform solvents were used and further analysis were done by Qualitative Phytochemical Screening, Analysis of Polyphenolic component using UV-Visible Spectrophotometer, analysis of curcuminoid content using HPLC (High-Performance Liquid Chromatography) and anti-microbial testing via well diffusion method on *S.aureus* (Gram +ve) and *E.coli* (Gram -ve).

Keywords: Phytochemicals, Polyphenols, Curcuminoids-HPLC.

Introduction

Curcuma longa L. (turmeric) of ginger family (Zingiberaceae) is one of oldest cultivated spice plants in the south-east Asian countries. For many years rhizomes of the plant has been used as a safe and active drug for the treatment of various chronic diseases. Curcuma longa is a perennial herb with orange, tuberous pulpy roots that grow to about 60 cm in length. India produces about 400,000 tons per year or about 80% of the world's supply of commercial turmeric[1].

Figure-1 Plant of Turmeric

Figure-2 Diagram of Plant

Figure-3 Fresh Rhizomes

Figure-4 Dried Rhizomes

The yellow color, which is characteristic of the turmeric rhizome, is due to the presence of 3–5% of curcuminoids. The curcuminoids include curcumin, demethoxycurcumin, bis-demethoxycurcumin, and curcumin of which curcumin is the major bioactive constituent[2,3.]

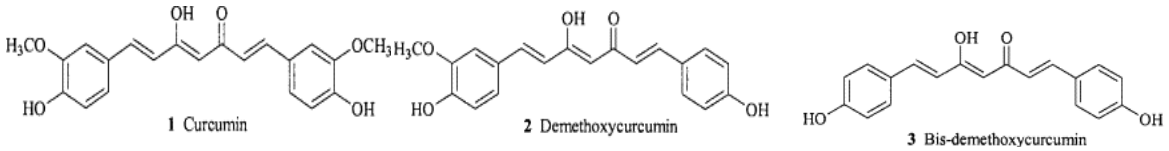

Figure-5 Different Curcuminoids

It is recommended for treating high cholesterol, abdominal pains, Wounds, eczema, psoriasis, Jaundice, menstrual disorder, Inflammations, diabetes, Cancerous Symptoms, and as a blood purifying activity[4]. Many species of Curcuma are traditionally used for their medicinal properties, Anti-inflammatory Antibacterial, and Antifungal activity has been reported for species such as *C.longa, C. zedoria, C. aromatica, and C. amada*[5].Curcuminoids exhibit properties like free-radical scavenging, antioxidant activity and also act as inhibitors of human immune deficiency virus type 1 (HIV-1) integrase enzyme.

Plant extracts and oils have been used for a wide variety of purposes since ancient times. One of their purposes is as a source of medicine as they contain a range of organic compounds with therapeutic values. The majority of people depend on traditional medicine as their primary healthcare. About 80% of people in this world depend on herbs for health. According to WHO, medicinal plants will be the best source to obtain a variety of drugs. Herbal products are highly effective for treating a wide range of diseases and infections[6].

Besides serving medicinal purposes, plant extracts are also used as herbs and spices. These spices and herbs are considered effective and safe against certain ailments. Long-term consumption of these substances is also guaranteed not to cause any side effects. Herbs like Ashwagandha, Triphala, and Shatavari have been used as medicine in traditional Indian medicine or Ayurveda. Ayurvedic medical systems have wide uses for both fresh and dried preparations. The dried powders are used to treat distinctly different ailments via using them as pastes or plant juices[7].

There is a common concept among people that herbal medicines being natural in origin have no side effects and are safe. Herbal remedies used in traditional medicine provide an interesting and still largely unexplored source for the creation and development of potential new drugs for chemotherapy which might help to overcome the growing problem of resistance and also the toxicity of the currently commercially available antibiotics. Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and ant malarial activities[8,9].

Plant phenolics are important constituents that contribute to functional quality, color, and flavor and have significant roles both as singlet oxygen quenchers and free radical scavengers, helping to minimize molecular damage[10].These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans[11,12].

The objectives of the research were to extract the phytochemicals out of the *Curcuma Longa* rhizomes using organic solvent (Acetone, Chloroform, Ethanol, Methanol). Qualitative screening of the Solvent extract for presence of different phytochemicals, to test the microbial activity of the extracts against *E. coli* (Gram Negative) and *Staphylococcus aureus* (Gram Positive) , analysis of poly phenolic compounds and analysis of curcuminoid compound using High-Performance Liquid Chromatography (HPLC).

Material and Methods

Plant Material: Dried turmeric rhizomes were collected from the quality assurance department of The Himalaya Drug Company (Dehradun) which was then further dried under shade for 5 days. It was then grounded into a coarse powder. The powder was then kept in an air tight jar for further usage.

Crude Extraction: 20 grams of coarse powder is soaked in 100 ml of Acetone, Chloroform, Ethanol and Methanol at room temperature for 3 days inside an Iodine Flask with occasional shaking. The solvents were then filtered using Whatman ™ no.41 filter paper and concentrated using a water bath. The extracts were then kept in glass bottles for further usage.

Identification Test: The individual extract was subjected to the qualitative phytochemical screening forth presence of some chemical constituents. Phytochemical tests were carried out adopting standard procedures[13,14,15]. All of the reagents were made by adopting standard procedures[16].

Alkaloids

Mayer's Test: In which the alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added.

Flavonoids: Themethanolic extract was warmed with metal Mg and added 5-6 drops of conc. hydrochloric acid. The red color was observed for Flavonoids.

Tannins: In which 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

Steroids:1 ml of extract was dissolved in 3 ml of Chloroform Equal volume of Concentrated H_2SO_4 was added into the test tube (from the side of the test tube slowly). The appearance of green fluorescence in the acid layer and pink colour in chloroform layer confirms the presence of steroids in the extract.

Saponins: 2ml of extract was taken in a test tube and 8 ml of distilled water was added into it.The test tubes were then shaken vigorously for 5 minutes.Formation of stable foam confirms presence of saponins in the extract.

Phenols:1 ml of sample was taken in a test tube. A few drops of Alcoholic $FeCl_3$ were added into the extract.Appearance of blue-black colour indicates presence of phenols in the extract.

Phytosterol

Salkowski's Test: 2 ml of extract was taken in a test tube then 2 ml of chloroform was added and filtered.The filtrate was treated with a few drops of Conc. H_2SO_4,Shaked and allowed to stand. Appearance of golden red colour indicates presence of phytosterols in the extract.

Carbohydrates

Iodine Test: 2 ml of extract was taken into a test tube and few drops of iodine solution were addedinit.Appearance of blue colour indicates the presence of carbohydrates in the extract.

Reducing Sugar

Fehling Test: 2 ml of extract was taken in a test tube. The extract was hydrolysed with a few drops Dil. HCl and neutralized with a few drops of Dil. NaOH.

1 ml of Fehling A was added following by 1 ml of Fehling B. The test tube was then gently heated on a water bath. Formation of brick red ppt indicates the appearance of reducing sugars in the extract

Total Polyphenol Content Estimation

Reagent Preparation

Sodium Carbonate Solution: Add 29 gm of Sodium carbonate in 100 ml of water

Phosphomolybdotungstic Reagent: Mix Folin&Ciocateu's Phenol reagent (2N) (Make LobaChemie) with water in ratio 1:1

Standard Solution Preparation

Stock Pyrogallol Standard Solution: Add 50 mg of pyrogallol in 100 ml water.

Working Pyrogallol Standard Solution: Add 5 ml of pyrogallol standard solution in 100 ml of water.

Test Solution Preparation: Take 1 gm of sample in 250 ml flat bottom flask. Add 150 ml water in it and place it on the water bath at 97^0C for 30 minutes**.** Cool the flask under running water and allow the residue to settle **.**Transfer the supernatant in a 250 ml volumetric flask. Repeat the process with 50 ml and 25 ml respectively**.** The whole extract was transferred after the last cycle.The

volume was then bought up to 250 ml**.** The extract was then filtered **.**The filtrate was

Method

Test Solution

Pipette out 2.0 ml of solution in 25 ml volumetric flask

Add 1.0 of Folin&Ciocateu's Phenol Reagent

Add 10.0 ml of water

Bring the volume to 25 ml with Sodium Carbonate solution

After exactly 30 minutes take absorbance standard at 760 nm using water as compensation liquid

Calculation

$$\frac{A_1}{A_2} * \frac{W_1}{V_1} * \frac{V_2}{W_2} * \frac{25}{100} * \text{Purity of Standard Solution}$$

A_1 = Sample Absorbance

A_2 = Standard Absorbance

W_1 = Weight of Standard in mg

W_2 = Weight of Sample in mg

V_1 = Volume of Standard made (100 ml)

V_2 = Volume of Sample made (250 ml)

HPLC analysis of Curcuminoids content

HPLC Instrument

The methanolic extract was concentrated and analyzed using HPLC of *Curcuma longa* was analysed as per standard method[17]with some modifications. The extract was filtered through membrane syringe filter (0.20 m) and 20 µl of filtrate was used for analysis in the HPLC.

Preparation of standard solution

Standard solution: Standard Curcumin was dissolved in methanol in a 50 ml volumetric flask to a final concentration of 1.0 mg/ml and sonicated for 10 minutes in an ultrasound bath and completed to the final volume.

Working Solution: (0.01 mg/ml) This solution was prepared by adding 1 ml of standard solution in 100 ml of volumetric flask and then kept for further processing.

Standard Solution

Pipette out 2.0 ml of working standard in a 25 ml volumetric flask

Add 1.0 of Folin&Ciocateu's Phenol Reagent

Add 10.0 ml of water and bring the volume to 25 ml with Sodium Carbonate solution

After exactly 30 minutes take absorbance standard at 760 nm using water as compensation liquid.

HPLC conditions

Column : C18 phenomenexluna (250×4.6 mm 5µ)

Mobile phase : Orthophospheric acid (0.1%) in water : Acetonitrile (50:50)

Flow rate - : 1 ml/minute

Wavelength : 420 nm

Injection : 20 µl

Temperature : 35°C

Run time : 20minute

Procedure

Stabilized the instrument with the mobile phase till the baseline is satisfactory then injected the standard solution of three times and then recorded the chromatogram. The % RSD between the results should be less than 2 % and then injected the sample solutions and recorded the chromatogram.

make the volume up to the mark with methanol.

Sample preparation (5.0mg/ml)

Test Solution was prepared by adding 100 mg of sample in 50 ml of methanol in a flat bottom flask. The solution was then heated on a water bath for 30 minutes at 800C. The solution was then cooled down to room temperature and makes the volume up to the mark with methanol.

Calculation of Curcuminoid

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \times \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}} \times \text{Purity of standard}$$

Anti-bacterial Assay

Microbial Samples: The microbial samples used for the procedure were obtained from the microbiology unit of department of quality assurance and quality control, The Himalaya Drug Company. Microorganisms used were *Staphylococcus aureus* (Gram

Positive) and *Escherichia coli* (Gram negative) They were subculture in recommended media purchased from Hi-Media, India private Ltd, Mumbai and stored in 4ºC for further use.

Culture media and antibiotics: Nutrient Agar was used for the culture of the bacteria. Ciprofloxacin was used as a standard antibiotic for bacteria.

Screening for anti-bacterial activity: Antibacterial activity of all the extracts was tested by well diffusion agar method. Culture plates were prepared by first preparing the stock bacterial culture by mixing a loop full of *S.aureus* in autoclaved nutrient agar. Stock culture was then incubated at 37^0C for 48 hours. 2 ml of stock culture was then added in autoclaved working culture at 40^0C to 60^0C. 20 ml of working culture was poured in sterile plates and kept aside for solidifying. After the media in the plated solidifies a sterile cork borer was used to make wells of 5 mm diameter in the centre of the plate. The wells were filled with sample extracts. The same procedure was used for E.coli. For positive control Ciprofloxacin antibiotic was used and negative controls were made with acetone, chloroform, ethanol and methanol instead of sample extracts. The anti-bacterial assay plates were then incubated at 37^0C for 24 hours. The diameter of the zone of inhibition around each well was taken as a measurement for antibacterial activity.

Results and Discussions

Results of Phytochemical Screening

The various Secondary metabolites that are present in the rhyzome of *Curcuma longa* are responsible for this therapeutic effect. Presences of phytochemicals were analyzed by the qualitative tests which are shown in the table-1. According to the tests conducted Acetone extract showed presence 8 phytochemicals (Alkaloids, Flavonoids, Saponins, Tannins, Steroid, Phenols, Carbohydrates, Reducing Sugars) Chloroform (Alkaloids, Flavonoids, Saponins, Phytosterols, Steroid, Phenols, Carbohydrates, Reducing Sugars), ethanol (Alkaloids, Flavonoids, Saponins, Tannins, Phytosterols, Steroid, Phenols, Reducing Sugars) and methanol(Alkaloids, Flavonoids, Saponins, Tannins, Steroid, Phenols, Carbohydrates, Reducing Sugars) extract showed presence of 9 phytochemicals in each of them.

Table-1Phytochemical Screening in Different extract of *Curcuma long*

Phytochemical	Acetone	Chloroform	Ethanol	Methanol
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	-	+	+
Phytosterols	-	+	+	-
Steroid	+	+	+	+
Phenols	+	+	+	+
Carbohydrates	-	+	-	+
Reducing Sugars	+	+	+	+

Results of Polyphenolic Content

Table-2Polyphenolic Content of *Curcuma longa* at 760nm

Sample	**Absorbance**	**% Polyphenolic content**
Curcuma longa	0.326	0.611%
Standard	0.331	

Results of HPLC- Curcuminoid Content

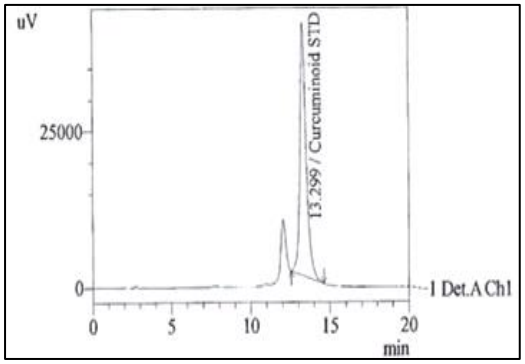

Figure-6 Curcuminoid Standard

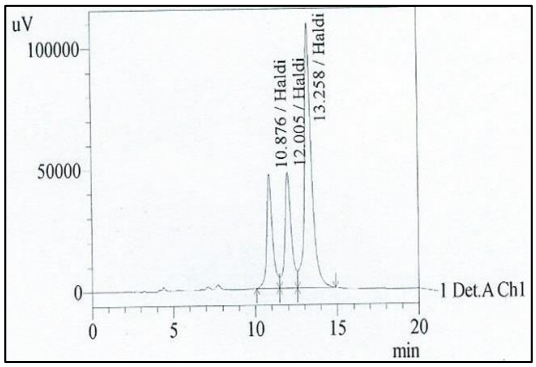

Figure-7*Curcuma longa* Sample

1st peak of Bisdemethoxycurcumin,
2nd of Demethoxycurcumin,
3rd of Curcumin

Table No-3HPTLC Results of Haldi (*Curcuma longa*)

Curcuminoid Name	Amount Present
Bisdemethoxycurcumin	0.9622 % (9622 ppm)
Demethoxycurcumin	1.0804 % (10804 ppm)
Curcumin	2.7705 % (27705 ppm)
Total Curcuminoid	4.8166 % (48166 ppm)

The most active component of turmeric is curcumin which makes up to 2-5% of the spice, and is responsible for most of the therapeutic effects. Turmeric contains a wide variety of phytochemical including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, eugenol.The characteristic yellow color of turmeric is due to Curcuminoid first isolated by Vogel in 1842[18].According to above studies out of the total curcuminoid content Beside methoxycurcumin is .9622 %, Demethoxycurcumin is 1.0804 % and Curcumin is 2.7705%.

Anti-Bacterial Assay

Table-4Anti- Bacterial Activity of *Curcuma longa*

Solvents	***Staphylococcus aureus***	***Escherichia coli***
Acetone	13 mm	12 mm
Chloroform	15 mm	13 mm
Ethanol	20 mm	17 mm
Methanol	25 mm	13 mm
Ciprofloxacin	30 mm	28 mm

The antibacterial activity of Acetone, chloroform,ethanol,and methanol extract of Curcuma longa determined against S.aureus and E.coli by well agar diffusion method. The growth inhibitory activities off all the extracts against the tested bacteria are summarized in Table-4.

The results of antibacterial activity assay clearly show that all the extracts have antibacterial activity against the S.aureus. Bacterial growth inhibition results shown in Figure-8 clearly indicate that Methanol extract has given the best anti-microbial activity against *S. aureus* (Gram +ve) and Ethanolic extract has given the best anti-

microbial activity against *E. coli* (Gram -ve). This antimicrobial activity of rhizome of Curcuma longa is due to the presence of phytochemical constituents , viz. alkaloids, terpenoids, flavanoids, phenolics, tannins,Saponins that are known for their antimicrobial properties.

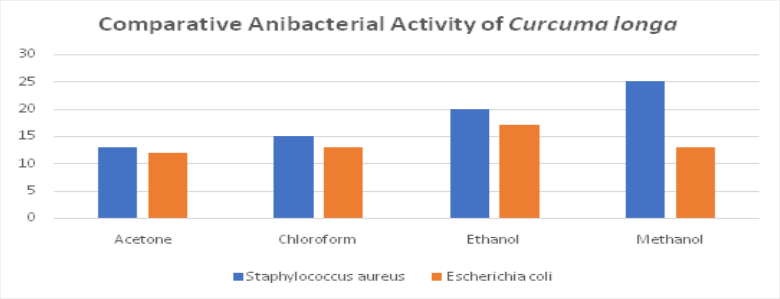

Figure-8 Different solvents in *Staphyllococcus aureus*

Figure-9 Different solvents in *Staphyllococcus aureus*

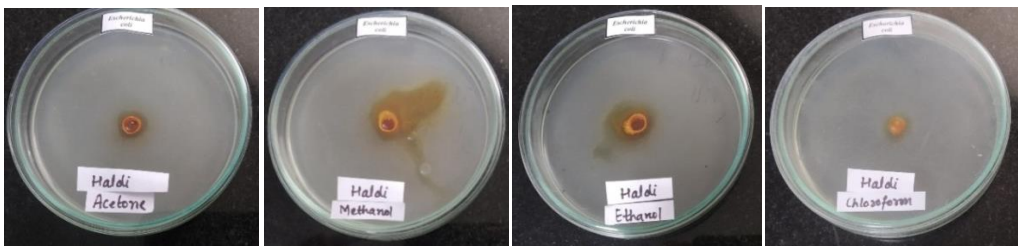

Figure-10 Different Solvents in *Escherichia col.*

Conclusion

In the light of above data ,it can be concluded that a simple chemical profiling and semi quantitative method for natural products using analytical method might be applied to diverse field related quality control of medicinal plants. It is also clear that turmeric has lots of potentials and has broad spectrum pharmacological actions and is beneficial for long term and daily usage. Turmeric is regarded as one of the best drug in many diseases like Diabetes, Skin diseases etc, which is in use since ages owing to its multiple pharmacological activities. Turmeric is enrichedwith manyuseful phytoconstituents which are responsible for its efficacy. Curcumin is one such phytoconstituents, a nutraceutical substance with numerous pharmacological activities. It can also be stated that many of these phytochemicals can be used safe substitute to many of the chemical drugs which are present in the market presently as these won't cause any side effect it taken for a longer time. These phytochemicals are also easy to obtain as they need no artificial synthesis.

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Photo-Catalytic Degradation Of Toxic Dyes By Silver Nano Particles Synthesized From Peels Extracts Of *Citrus medica*t Through Green Method.

[1]Sumit Ringwal[1], [1]Ankit S. Bartwal, [2]Asha Dobhal [2]and [*1]Satish C. Sati

[1]Department of Chemistry, H.N.B. Garhwal University (A Central University) Srinagar Garhwal, Uttarakhand, India-246174

[2] Department of Botany Govt. PG College New Tehri, Tehri Garhwal Uttarakhand India

[*]Email: sati_2009@rediff mail.com

Abstract-Dyes are chemical compounds which are used to convey colour to various materials, but during the processing and operation it produces toxics and hazardous side product which is very harmful to ecosystem and biodiversity. Researchers are developing various methods to degrade such toxic dyes and use of noble metal nano particle as a catalyst is emerging field of interest among scientific community. In this research article we had scanned our previously synthesized silver nanoparticle from *Citrus medica* peels extracts; Synthesis and characterization were previously reported by authors.Synthesized AgNPs has excellent photo-catalytic potential against various toxic dyes like Methylene blue, Rose bengal, Acridine Orange, Methyl Orange. Our nano-catalyst methylene blue nearly 52.15% in 5 hours while in absence of nano-catalyst dye degrades nearly32.85%. It is also capable of degrading acridine orange study shows it degrade Acridine orange dye 58.74% in just 4 hours and rose bengal dye 59.57 % followed by 4 hours of Continuous UV absorption. It also degrades methyl orange dye nearly 52% in 10 hours of solar irradiation. Synthesized AgNPs can be used as photo-catalyst for degrading toxic dyes.

Keywords- Photo-catalyst, Methylene Blue, Acridine Orange, Methyl Orange, Rose Bengal

Introduction

Water resources are limited and water requirement is increasing at very high rate in developing countries like India so there is great need of water recycling and water purification so it can be used for various propose like in agriculture and industry. Solar energy is quite suitable source for photo-catalytic detoxification of waste water[1-4]

Non bio-degradable and hazardous nature of organic toxic dyes is one of the major concerns for scientific community. Waste water generated during the processing and operation of dyes is one of the remarkable sources of water pollution which is harmful to aquatic creatures and human health[5, 6]. Most of the organic dyes are non bio-degradable by standard biological methods. Methods including adsorption on activated carbon, ultrafiltration, reverse osmosis, coagulation, ion exchange and oxidation with peroxide are usually applied efficiently. Nevertheless, they do not destruct the pollutant molecule[7–9].

Among Nobal metal nanoparticle, silver nanoparticle (AgNPs) receiving a lot of consideration because of their non-toxic nature and a variety of reimbursement, including as antibacterial agents, Optical property and excellent photo-catalytic activity[10-11].Several research has revealed that silver nano-particle can degrade 95% of methylene blue dye in within 72 hours and AgNPs are capable of degrading 90% of methylene orange dye within 6 hours, at pH 2[12]

In this research article we had scanned our previously synthesized silver nanoparticle from *Citrus medica* peels extracts, Synthesis and characterization were previously reported by authors.Synthesized AgNPs has excellent photo-catalytic potential against various toxic dyes like Methylene blue, rose bengal , Acradin Orange, Methyl Orange.

Material and Methods

We had synthesized silver nanoparticle from *Citrus Medica* Peels extract by green method.

Characterization of synthesized AgNPs was done by different spectroscopic technique like UV-Visible spectroscopy, X-ray diffraction method followed by SEM, EDAX and HR-TEM studies. The results of UV-Vis absorption study show a strong absorption bandof silver nanoparticles (AgNPs) at λ_{max}440 nm. The X-ray diffraction analysis confirmed that the

synthesized AgNPs are cubic crystal solid. The SEM analysis of AgNPs shows average sizeof 1.35 µm, while the EDAX analysis confirmed the significant presence of silver with carbon, oxygen, chlorine and Iron as other contaminants. This analysis is followed by TEM which shows that the water-soluble AgNPs, have approximate size 53nm, previously communicated by authors[13].

Assessment of photo-catalytic activity

The photo-catalytic activity of AgNPs was investigated using different dyes like Methylene blue, rose bengal, Acridin orange and Methyl orange. Before exposing the suspension to sunlight it was kept in dark condition until adsorption-desorption equilibrium was established. [14].

The experiment was performed with or without Ag NPs in solar irradiation and dark condition. The concentrations of the dyes for each prepared sample were analyzed by using UV–Vis spectroscopy technique, and the absorptions peaks of the dyes at characteristic wavelength were observed in all prepared sample. Calibration curves were used to calculate the concentrations of the dyes. The decolourization efficiencies of the dyes were estimated using the following relation.

Degradation (%) = $(C_o–C_t)/C_o×100$

where C_o and C_t represents the concentration of dye before illumination and dye after a certain irradiation at time *t* respectively.

Results and discussion

Photo-catalytic degradation of methylene blue dye by using synthesized AgNPs

Photo catalytic degradation of MB dye with the help of synthesized AgNPs was verified by UV-Visible spectrometer. The UV-Visible spectroscopy result shown decrease in the peak of MB at different interval of time. Initially, the absorption peak of MB was found at 664nm and at a high absorption value which decreased rapidly on exposing to sunlight, see **Figure-1(a).** The completion of the photo catalytic degradation of dye was confirmed when absorbance value for MB peak reach near to baseline. Amount of degradation of dye in percentage (%) of AgNPs was calculated 52.15% in 5 hrs, **Figure-1(b)** shows degradation on Methylene blue dye without nano catalyst amount of degradation of dye in percentage (%) was found32.85% in 5 hrs and UV-Visible spectroscopy results clearly reveals that dye degrade faster with synthesized nano-catalyst **Figure-1(c)** shows comparative study of dye degradation in different condition, our results also match with previous study[15]

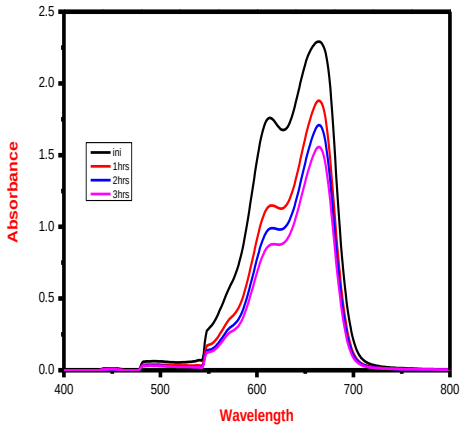

Figure-1(a) Degradation of MB in presence of AgNPs,

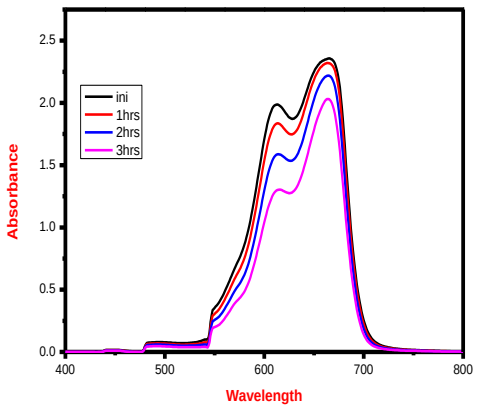

Figure-1(b) Degradation of MB without catalyst

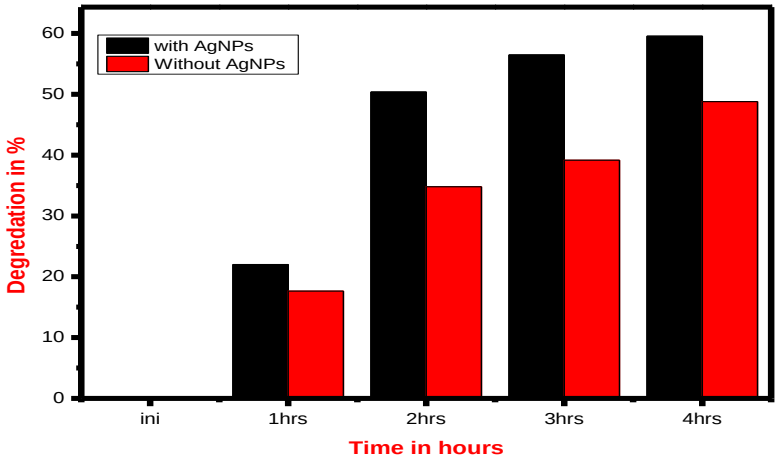

Figure 1(c) Comparative study of dye degradation

Photo-catalytic degradation of rose bengaldye by using synthesized AgNPs

Photo catalytic degradation of RB dye with the help of synthesized AgNPs was verified by UV-Visible spectrometer. The UV-Visible spectroscopy result shown decrease in the peak of RB at different interval of time. Initially, the absorption peak of RB was found at 543 nm and at a high absorption value which decreased rapidly on exposing to sunlight, see **Figure-2(a)**. The completion of the photocatalytic degradation of dye was confirmed when absorbance value for RB peak reach near to baseline. Amount of degradation of dye in percentage (%) of AgNPs was calculated 59.57% in 4 hrs, **Figure**-2(b) shows degradation on rose bengal dye without nano catalyst amount of degradation of dye in percentage (%) was found 48.88% in 4 hrs and UV-Visible spectroscopy results clearly reveals that dye degrade faster with synthesized nano-catalyst **Figure-**2(c) shows comparative study of dye degradation in different condition, our results also match with previous study[16].

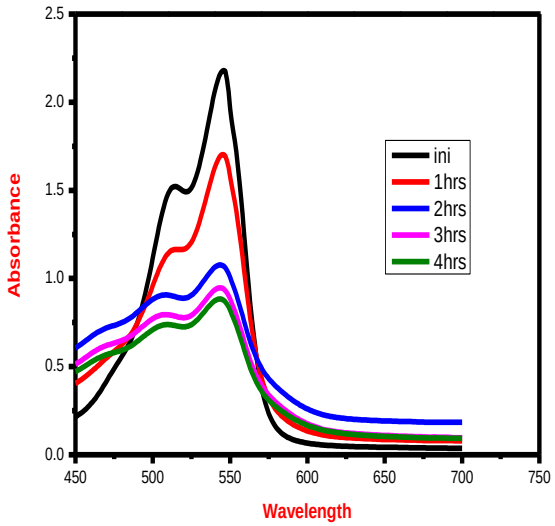

Figure-2(a) Degradation of RB in presence of AgNPs,

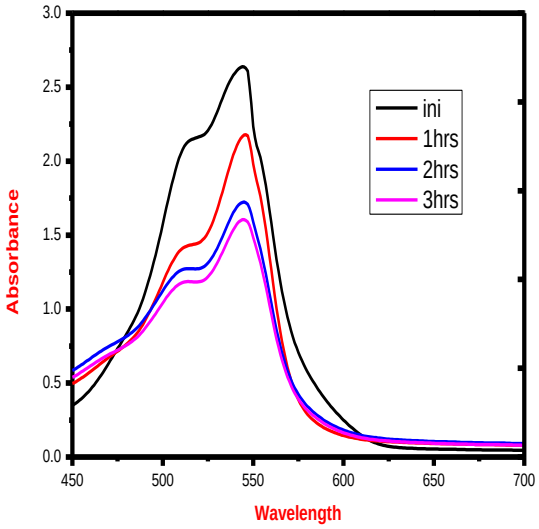

Figure-2 (b) Degradation of rose bangle without catalyst

Photo-catalytic degradation of acridin orange dye by using synthesized AgNPs

Photo catalytic degradation of AO dye with the help of synthesized AgNPs was verified by UV-Visible spectrometer. The UV-Visible spectroscopy result shown decrease in the peak of AO at different interval of time. Initially, the absorption peak of AO was found at 491nm and at a high absorption value which decreased rapidly on exposing to sunlight, see **Figure-3(a).** The completion of the photo catalytic degradation of dye was confirmed when absorbance value for AO peak reach near to baseline. Amount of degradation of dye in percentage (%) of AgNPs was calculated 58.74% in 4 hrs, **Figure-3(b)** shows degradation on acridin orange dye without nano catalyst amount of degradation of dye in percentage (%) was found 46.75% in 4 hrs and UV-Visible spectroscopy results clearly reveals that dye degrade faster with synthesized nano-catalyst **Figure -3(c)** shows comparative study of dye degradation in different condition, our results also match with previous study[17]

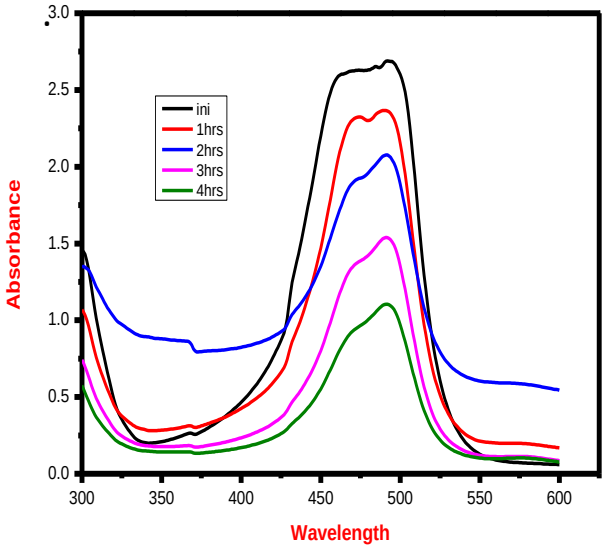

Figure-3(a) Degradation of AO in presence of AgNPs,

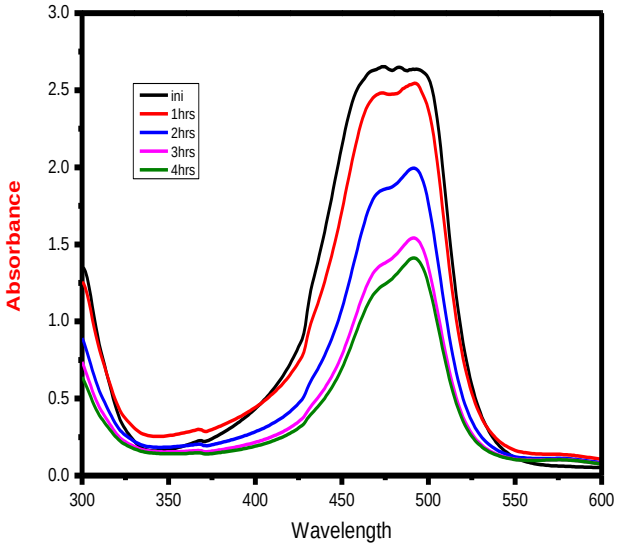

Figure- 3(b) Degradation of AO in absence of AgNPs

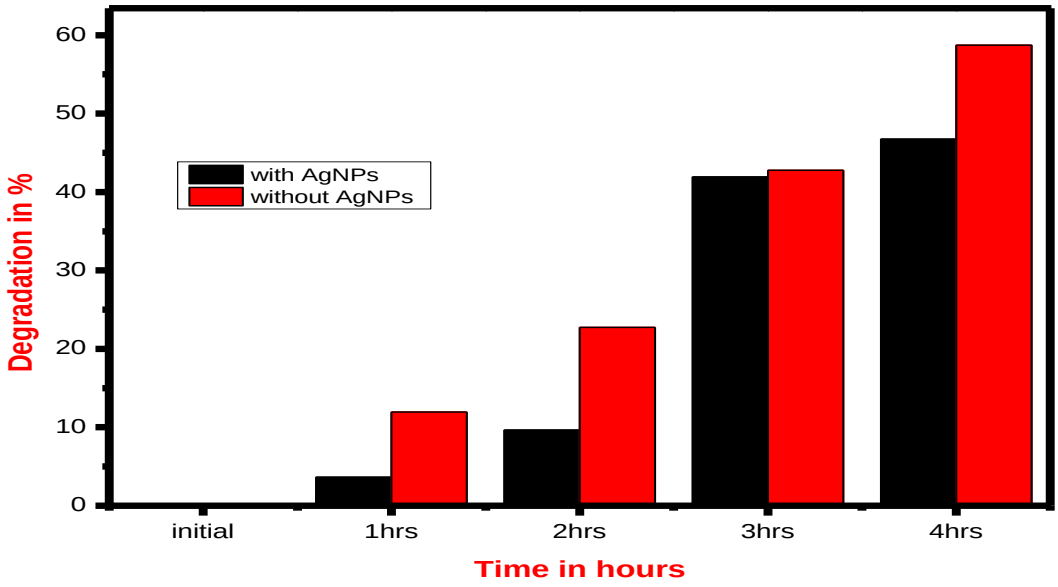

Figure- 3(c) comparative study of dye degradation

Photo-catalytic degradation of Methyl Orange dye by using synthesized AgNPs

Photo catalytic degradation of MO dye with the help of synthesized AgNPs was verified by UV-Visible spectrometer. The UV-Visible spectroscopy result shown decrease in the peak of MO at different interval of time. Initially, the absorption peak of MO was found at 507 nm and at a high absorption value which decreased rapidly on exposing to sunlight, see **Figure-**4(a). The completion of the photo catalytic degradation of dye was confirmed when absorbance value for MO peak reach near to baseline. Amount of degradation of dye in percentage (%) of AgNPs was calculated 52% in 10 hrs, **Figure-**4(b) shows degradation on methyl orange dye without nano catalyst amount of degradation of dye in percentage (%) was found35% in 10 hrs and UV-Visible spectroscopy results clearly reveals that dye degrade faster with synthesized nano-catalyst **. Figure-**4(c) shows comparative study of dye degradation in differentcondition, our results also match with previous study[18].

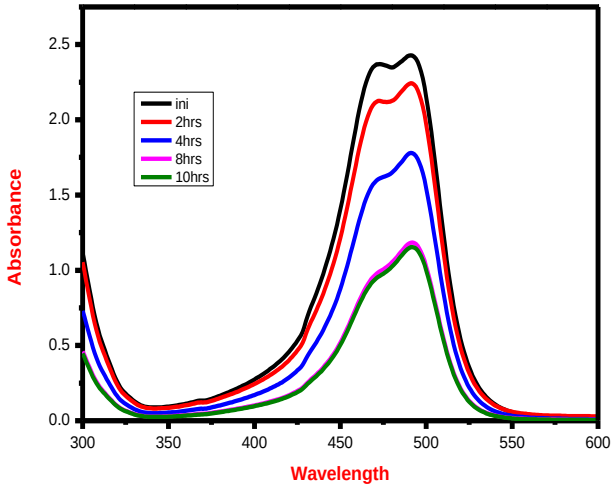

Fig 4(a) Degradation of MO in presence of AgNPs,

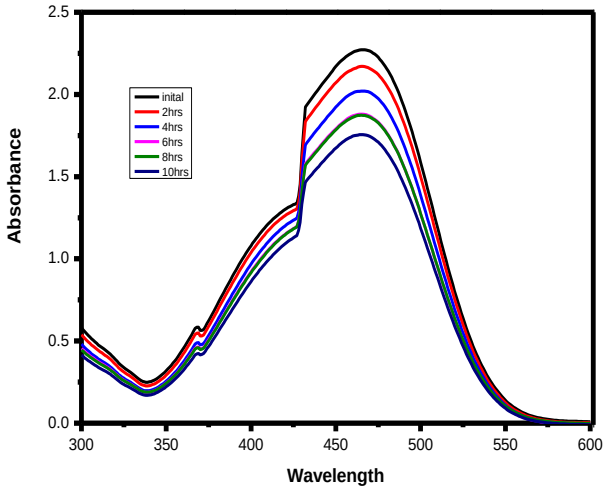

Figure-4(b) Degradation of MO in absence of AgNPs

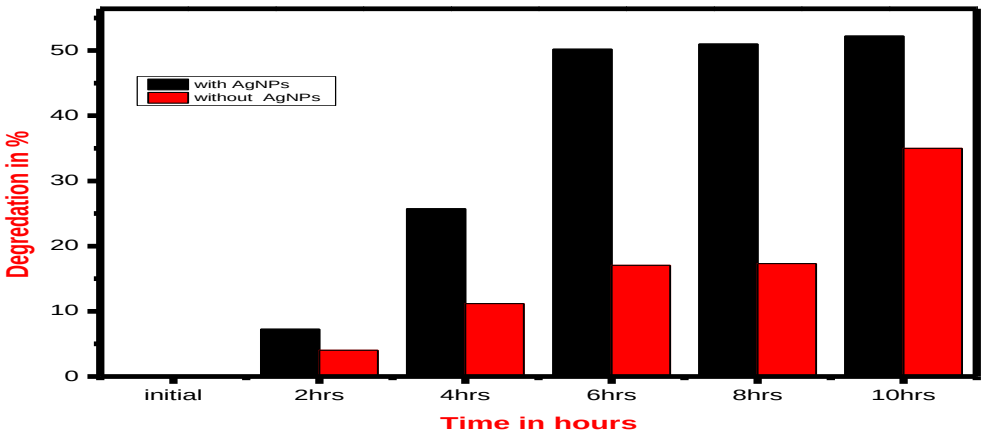

Figure- 4(c) Comparative study of dye degradation

Conclusion

1. Dyes are chemical compounds which are used to convey colour to various materials, but during the processing and operation it produces toxics and hazardous side products which is very harmful to ecosystem and biodiversity. Researchers are

developing various methods to degrade such toxic dyes and use of noble metal nano particle as a catalyst is emerging field of interest among scientific community. Synthesized AgNPs has excellent photo-catalytic potential against various toxic dyes like Methylene blue, Rose bengal, Acridin Orange, Methyl orange. Our nano-catalyst depredates methylene blue nearly 52.15% in 5 hours while in absence of nano-catalyst dye degrades nearly32.85%. It is also capable of degrading acridine orange. Study shows that it degrades acridine orange dye 58.74% in just 4 hours and rose bengal dye 59.57 % followed by 4 hours of continuous UV absorption. It also degrades methyl orange dye nearly 52% in 10 hours of solar irradiation. Synthesized AgNPs can be used as photo-catalyst for degrading toxic dyes.

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Evaluation Of Heavy Metal Tolerance And Multi Drug Resistance Of Microbial Species Isolated From River Yamuna, Delhi, India

[1]Amita Gaurav Dimri, [1]Dushyant Singh and [2]Abhishek Chauhan
[1]Shriram Institute for Industrial Research, 19, University Road, Delhi-110007, India.
[2]Amity Institute of Environmental Toxicology, Safety and Management,
Amity University, Sector-125, Noida, India.

Abstract-The present study was investigated for the contamination level in river Yamuna, Delhi. A total 10 samples of Yamuna river water from different locations specifically under the territory of Delhi were collected and then analysed quantitatively for Total Bacterial Count (TBC), Total Coliform Count (TCC) as well as Total Yeast & Mold Count. Simulataneously, qualitative analysis for *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was also made in order to confirm the pathogenicity of Yamuna water. The isolates isolated also showed potential heavy metal tolerance study because they show complete resistance against 1000ppm concentration of iron. Hence, they can be used for bioremediation purposes for the removal of heavy metals.

Keyword-River Yamuna, Microbial Contamination, Heavy metals.

Introduction

Human health depends upon safe water more than any other factor. Basically,there is a direct correlation between safe consumable water and human health. Most of the problems in developing countries are mainly due to the lack of safe drinking water (Parson and Jefferson, 2006). Safe water supply is one of the main requirements in any community. It is apparent that health of individuals depends on safe drinking water[1].

The growing population of the world has resulted in the increased the water needfrom sources. Another cause for concern isthe future trends in water use, where it hasbecome somewhat difficult to evaluate the way in which water resources will deplete. Amongst them, pollution of water sources is very important point for health and human safety and considerable attention is necessary for it. Thus, it is necessary to monitor water quality to achieve safe water[2,3].

Rivers of India play an important role in the survival of Indian people. But with the rapid increase in human population, urbanization and economic activities a lot of pressure is created on riverine water resources which has become a serious issue and requires lot of attention. Industrial, urban and agricultural waste are entering water bodies contaminating the aquatic environment and increasing the biological oxygen demand.Also scared rituals which are performed in these rivers during festive season contributes to lot of pollution in the water bodies. The microorganism which are introduced into these water bodies due tolincrement in organic and contaminated decomposed material utilizes the great amount of dissolved oxygen.This situation is leading to decreased oxygen content in the water which disrupts the aquatic life.Thereby posing a serious threat to water resources and nature too.In the national capital, river Yamuna is the most polluted source of water is the principal stream of water reaching households. The Yamuna is the largest tributary of river Ganga which originates from Yamunotri glacier with a total length of 1,376 kilometers. Central location of Yamuna River is $28^0$36'N and longitude $77^0$12'E, at an altitude of 216m above the mean sea level[4,5,6]. It goes through Uttarakhand, Haryana, Delhi, and Uttar Pradesh. It converges with the Ganga at Allahabad in Uttar Pradesh[4]. Yamuna Riveris also called mailee (dirty) river and river of sorrow to Delhi, Mathura and Agra. The river water is extremely black, it appears like an industrial drain in Delhi, as majority of the industries are on its bank and used to dump the untreated effluents into the river. The water in the Yamuna remains stagnant for approximately nine months in a year. There are unlimited numbers of industrial units,

draining immense amount of untreated water in Yamuna existing in Delhi, Faridabad, Mathura and Agra. Central Pollution Control Board (CPCB) had estimated that there wereapproximately 359 industrial units, which directly or indirectly discharge their effluents in Yamuna. A report of CPCB indicates that there were about 42industrial units in Delhi directly polluting the Yamuna[5,6].According to the Centre for Science and Environment, approximately 75 to 80 percent of the river's pollution is the result of raw sewage, industrial runoff and the garbage thrown into the river and it totals over 3 billion liters of waste per day. About 20 billion rupees, or almost US $500 million, has been spent on various cleanup efforts.According to a Central Pollution Control Board (CPCB) survey, Delhi contributes 23 percent of the total wastewater generated by Class I cities (cities with more than 100,000 people)[7,8,9]. More shockingly, this is 47 per cent of the waste generated by 101 Class I cities and 122 Class II cities (Population: 50,000-99,999) in the Ganga basin. The water becomes untreatable when the ammonia concentration in Yamuna River reaches to 0.4 mg/L or more. In Delhi often ammonia in Yamuna River has been found more than 0.4 mg/L especially during summer. The river has turned grossly polluted due to continuous discharge of domestic wastewater from Palla to Etawah.As per the report of Yamuna Action Plan the content of suspended solids in Yamuna is 1000-10,000 mg/L and the permissible content of suspended solids is 100 mg/L. Efforts will be made to resort to a bottom-up approach rather than a top-down one to help this highly polluted river, which is the major life-supporting artery of Delhi, Mathura, Agra and Etawah and many other cities in India[10]. To apply the strategies effectively, we need to develop awareness among masses, education, and improved watershed management that will improve the water quality of this holy river.

Figure-1 Current status of River Yamuna from two different location

Table-1 Some Bacterial Diseases Transmitted Through Drinking Water[11,12]

Disease	**Causal bacterial agent**
Cholera	*Vibrio cholerae*, serovars O1 and O139
Gastroenteritis caused by vibrios	*Vibrio parahaemolyticus*
Typhoid fever and other serious salmonellosis	*Salmonella enterica* subsp. *enterica*serovarParatyphi *Salmonella enterica* subsp. *enterica* serovar Typhi *Salmonella enterica* subsp. *enterica* serovar Typhimurium
Bacillary dysentery or shigellosis	*Shigelladysenteriae*, *Shigellaflexneri*, *Shigellaboydii*, *Shigellasonnei*
Acute diarrheas and gastroenteritis	*Escherichia coli*, particularly serotypes such as O148, O157 and O124

Material and Methods

Sample collection and storage

All these 10 samples were collected from different locations in sterile autoclaved bottles along with the check over temperature of water at the site collection and kept straight in ice box until brought to laboratory (within 6 hours). Samples were collected early in the morning and were subjected to microbial analysis on the same day. Total Coliform count, Total Bacterial count, Yeast and Mould count, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were the microbiological parameters taken in the designed study.

Table-2 Representation of collected water Samples and Location:

S.No.	Sample No.	Site Name	Location	Temp.
1.	S-1	Wazirabad, Old bridge	N 28°42'40.1"; E 77°13'56.6"	19.3°C
2.	S-2	Vikas Marg, Yamuna bank	N 28°39'38.86559"; E77°15'27.66583"	19.9°C
3.	S-3	Old iron bridge, Gandhi Nagar, Seelampur	N 28°39'45.80899"; E 77°14'45.5928"	20.1°C
4.	S-4	GT Road Metro Vihar, Shastri park	N 28°40'22.5141"; E 77°14'0.81778"	19.4°C
5.	S-5	Outer ring road. MajnukaTila	N 28°41'43.64473"; E 77°13'47.53481"	19.5°C
6.	S-6	Jagatpur bund road, Wazirabad	N 28°44'21.06157"; E 77°13'51.087"	19.0°C
7.	S-7	Geeta colony bridge	N 28°39'9.15009"; E 77°15'44.58668"	19.8°C
8.	S-8	Laxmi Nagar	N 28°38'34.03313"; E 77°15'50.04833"	19.6°C
9.	S-9	Noida, Delhi (NCR)	N 28°32'16.95355"; E 77°19'26.09423"	19.7°C
10.	S-10	Okhla bird sanctuary, sec.95 Noida	N 28°33'47.9117"; E 77°17'56.89375"	19.9°C

Multi drug resistance test using antibiotics:Antibiotics used:The antibiotics used were Azithromycin, Cefixime, Ceftriaxone and Kanamycin.

Inoculum Preparation:Allisolated bacterial strain culture were sub cultured on non-selective nutrient agar slants. The bacterial cultures were incubated overnight at 37°C. 0.5 McFarland density of bacterial isolates was adjusted using normal saline (0.85% NaCl) using densitometer to get bacterial population of 1.0 x 10^8 cfu /ml. The working solution of antibiotics is 5mg/ml.

Agar well diffusion assay (zone of inhibition evaluation):Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 200µl of each of the adjusted cultures were mixed into separate 200 ml of sterile, molten, cool media, mixed well and poured into sterile petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 10µl antibiotic solutions were pipette out into the well in assay plates. Plates were incubated overnight at 37^{o}C. Following incubation, petri-plates were observed for the inhibition zones, diameters of which were measured by using Vernier Caliper.

Preparation of antibiotic solution:Stock solution of antibiotic was prepared by taking average weight of tablets of antibiotic drug by dissolving 50 mg antibiotic in 50 ml of solubilizing agent and then 1 ml from it, to the 100 ml of solubilizing agent into another volumetric flask.

Finally working solution of 10µg per ml was prepared from stock solution in a volumetric flask.

Observations

Table-3 Quantitative and qualitative analysis of organisms isolated.

Sample Code	Total Bacterial Count, cfu/ml	Total Coliform Count	Yeast & Mould Count, cfu/ml	Pathogen Isolates (Initial)		
				E.coli	*P. aeruginosa*	*S. aureus*
S-1	1.7×10^5	9.0×10^4	1.21×10^3	Present	Present	Absent
S-2	1.41×10^5	7.18×10^4	1.1×10^3	Present	Present	Present
S-3	1.41×10^6	1.6×10^5	4.4×10^2	Present	Present	Present
S-4	1.5×10^7	3.5×10^6	4.6×10^2	Present	Present	Present
S-5	7.5×10^6	7.0×10^5	7.7×10^2	Present	Present	Present
S-6	2.1×10^4	3.5×10^3	No colony observed	Present	Present	Present
S-7	2.1×10^6	1.6×10^6	2.1×10^2	Present	Present	Absent
S-8	2.2×10^6	5.4×10^5	2.5×10^2	Present	Present	Present
S-9	1.0×10^6	3.5×10^5	9.0×10^3	Present	Present	Absent
S-10	8.1×10^5	7.0×10^4	1.0×10^2	Present	Present	Absent

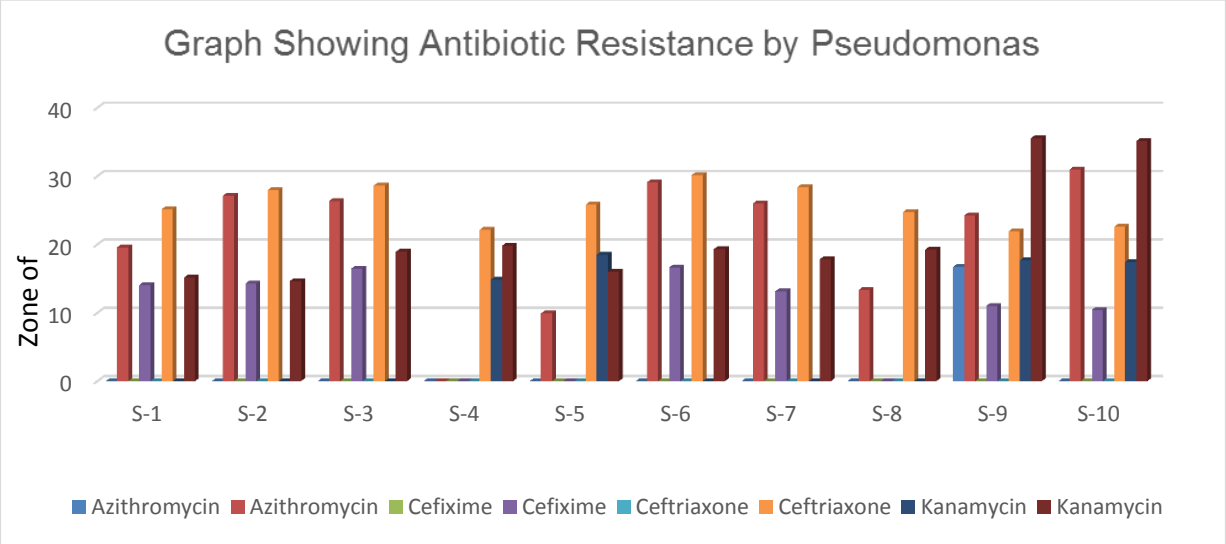
Results

Analysis of Antibiotics resistance from isolates.

Table-4Antibiotic resistance patterns of *Pseudomonas aeruginosa* (Gram negative)

Sample	Azithromycin		Cefixime		Ceftriaxone		Kanamycin	
	10µl	100 µl	10 µl	100 µl	10 µl	100 µl	10 µl	100 µl
S-1	NZI	19.57	NZI	14.07	NZI	25.14	NZI	15.14
S-2	NZI	27.13	NZI	14.26	NZI	27.93	NZI	14.65
S-3	NZI	26.33	NZI	16.46	NZI	28.59	NZI	18.92
S-4	NZI	NZI	NZI	NZI	NZI	22.13	14.82	19.79
S-5	NZI	9.90	NZI	NZI	NZI	25.80	18.48	15.99
S-6	NZI	29.07	NZI	16.63	NZI	30.13	NZI	19.31
S-7	NZI	25.98	NZI	13.13	NZI	28.38	NZI	17.82
S-8	NZI	13.33	NZI	NZI	NZI	24.74	NZI	19.25
S-9	16.71	24.22	NZI	11.01	NZI	21.91	17.64	35.51
S-10	NZI	30.96	NZI	10.42	NZI	22.56	17.43	35.12

*Zone of inhibition in mm. Diameter including well diameter of 6.0 mm. Results from 2x plates. NZI- No Zone of Inhibition



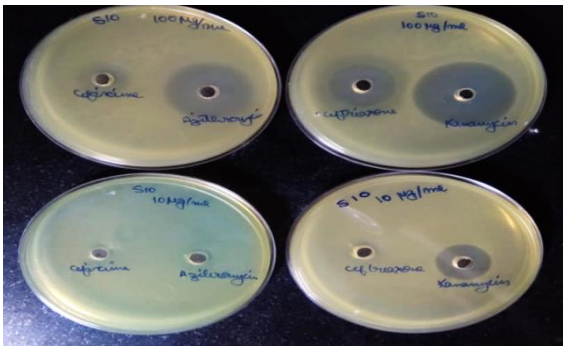
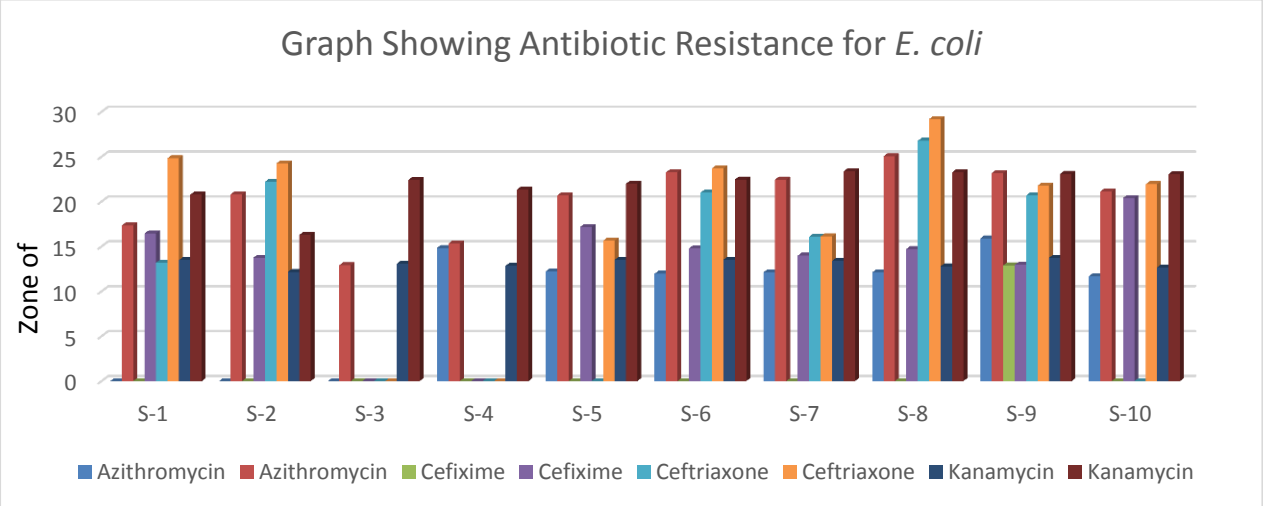


Figure-2 Test results for antibiotic assay for *Pseudomonas*

Table-5Antibiotic resistance patterns of *Escherichia coli* (Gram negative)

Sample	Azithromycin		Cefixime		Ceftriaxone		Kanamycin	
	10µl	100 µl	10 µl	100 µl	10 µl	100 µl	10 µl	100 µl
S-1	NZI	17.325	NZI	16.45	13.19	24.83	13.47	20.78
S-2	NZI	20.80	NZI	13.75	22.20	24.25	12.16	16.29
S-3	NZI	12.92	NZI	NZI	NZI	NZI	13.07	22.39
S-4	14.83	15.31	NZI	NZI	NZI	NZI	12.84	21.34
S-5	12.22	20.68	NZI	17.17	NZI	15.63	13.50	21.99
S-6	11.96	23.26	NZI	14.78	20.99	23.70	13.52	22.45
S-7	12.12	22.45	NZI	13.99	16.10	16.13	13.40	23.37
S-8	12.11	25.05	NZI	14.73	26.77	29.2	12.79	23.25
S-9	15.91	23.19	12.90	12.94	20.68	21.76	13.73	23.095
S-10	11.68	21.11	NZI	20.39	NZI	21.94	12.68	23.07

*Zone of inhibition in mm. Diameter including well diameter of 6.0 mm. Results from 2x plates. NZI- No Zone of Inhibition.



Analysis of Heavy Metal Tolerance from isolates.

Table-6Heavy Metal Tolerance patterns of *Pseudomonas aeruginosa* (Gram negative)

Sample	Mercury			Cadmium			Iron		
	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
S-1	11.83	16.27	29.62	NZI	NZI	12.35	NZI	NZI	NZI
S-2	12.92	18.06	27.60	NZI	17.75	28.77	NZI	NZI	NZI
S-3	NZI	14.18	25.95	NZI	14.27	24.20	NZI	NZI	NZI
S-4	NZI	10.91	24.15	NZI	13.30	24.49	NZI	NZI	NZI
S-5	NZI	12.31	25.31	NZI	16.54	25.31	NZI	NZI	NZI
S-6	NZI	11.90	26.44	NZI	NZI	11.32	NZI	NZI	NZI
S-7	NZI	14.36	28.33	NZI	NZI	10.58	NZI	NZI	NZI
S-8	10.95	14.43	26.14	NZI	9.91	23.03	NZI	NZI	NZI
S-9	NZI	13.94	26.79	NZI	15.36	21.60	NZI	NZI	NZI
S-10	11.42	13.10	27.61	10.70	20.39	34.40	NZI	NZI	NZI

*Zone of inhibition in mm. Diameter including well diameter of 6.0 mm. Results from 2x plates. NZI- No Zone of Inhibition.

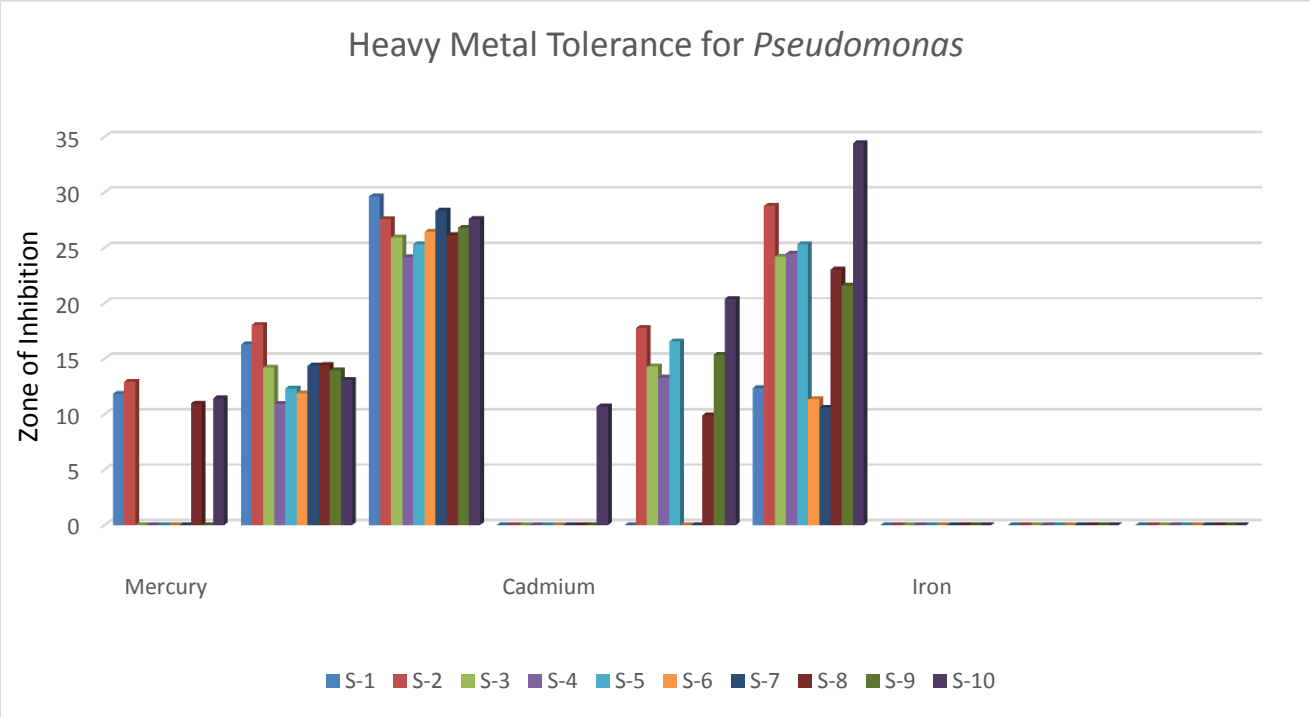
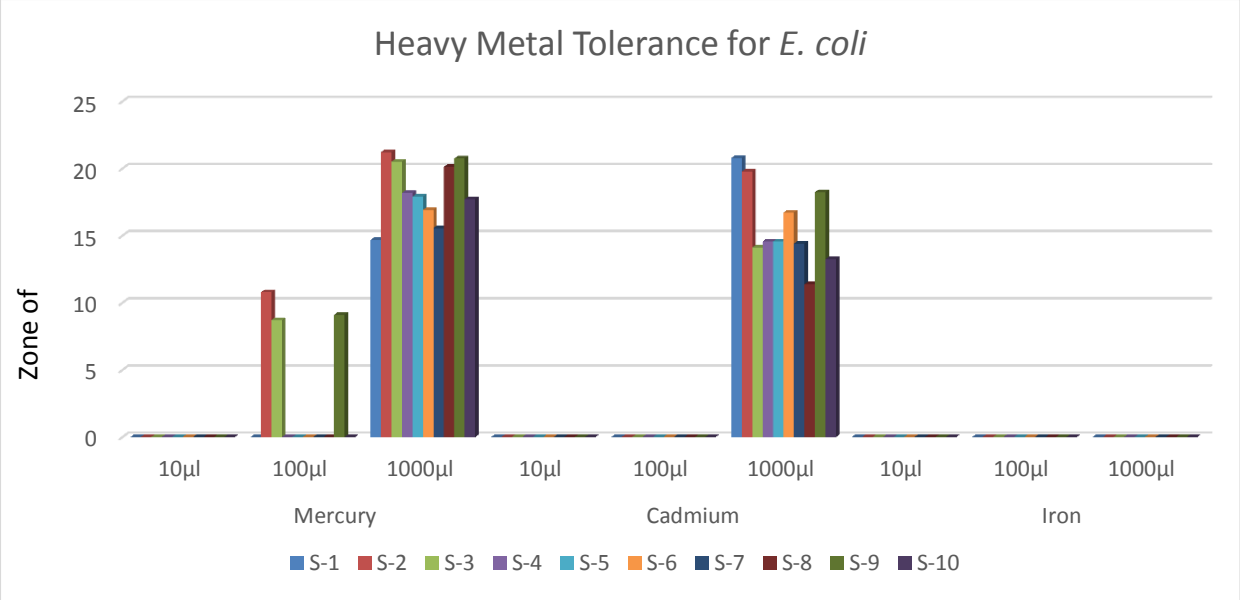


Figure-3 Test results for Heavy Metal Tolerance for *Pseudomonas*

Table-7Heavy Metal Tolerance patterns of *E. coli* (Gram negative)

Sample	Mercury			Cadmium			Iron		
	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
S-1	NZI	NZI	14.69	NZI	NZI	20.79	NZI	NZI	NZI
S-2	NZI	10.79	21.23	NZI	NZI	19.78	NZI	NZI	NZI
S-3	NZI	8.71	20.52	NZI	NZI	14.12	NZI	NZI	NZI
S-4	NZI	NZI	18.18	NZI	NZI	14.58	NZI	NZI	NZI
S-5	NZI	NZI	17.91	NZI	NZI	14.58	NZI	NZI	NZI
S-6	NZI	NZI	16.92	NZI	NZI	16.73	NZI	NZI	NZI
S-7	NZI	NZI	15.57	NZI	NZI	14.42	NZI	NZI	NZI
S-8	NZI	NZI	20.13	NZI	NZI	11.38	NZI	NZI	NZI
S-9	NZI	9.11	20.75	NZI	NZI	18.24	NZI	NZI	NZI
S-10	NZI	NZI	17.73	NZI	NZI	13.25	NZI	NZI	NZI

*Zone of inhibition in mm. Diameter including well diameter of 6.0 mm. Results from 2x plates. NZI- No Zone of Inhibition.



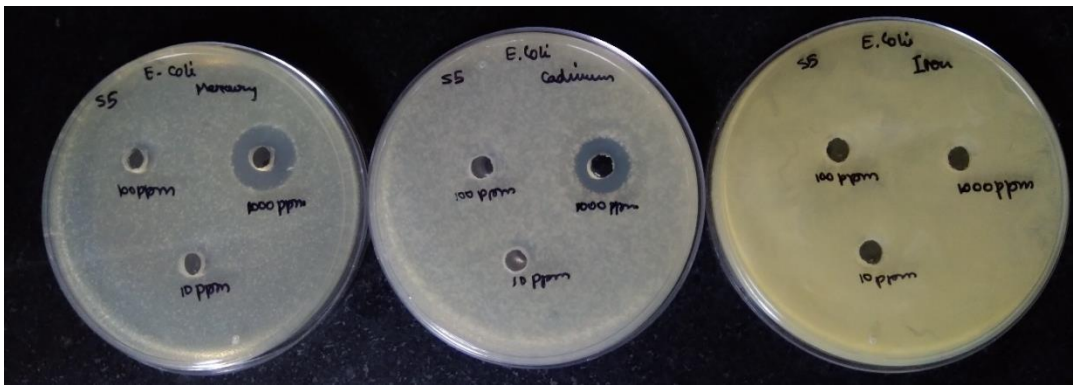


Figure-4 Test results for Heavy Metal Tolerance for *E. col*

Discussion & Conclusion

After enumeration, isolation and identification of the isolates from Yamuna River depicts the extreme level of contamination. As per the study, total 10 samples of yamuna water from different locations specifically under the territory of Delhi were collected and then analysed quantitatively for Total Bacterial Count (TBC), Total Coliform Count (TCC) as well as Total Yeast & Mold Count. Simulataneously, qualitative analysis for *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was also made in order to confirm the pathogenicity of Yamuna water.

As per the data attained, TBC confirmed the total count of bacterial presence which included coliforms as well as all pathogens. That's why, TBC always appeared more than the coliform count . In reference with the data, maximum TBC obtained was in sample 4 which was 1.5 x 10^7 cfu/ ml and TCC was 3.5 x 10^6 cfu/ ml. On the other hand, in sample 9 Yeast & Mold Count appeared maximum i.e., 9.0 x 10^3 cfu/ml but the bacterial count wasn't the maximum in the same sample (S-9) i.e., 1.0 x 10^6 cfu/ml which confirmed that No relation existed between these two entities for the survival. Descriptively, Y&M Count only gave information about the presence of Yeast Colonies (shiny bulged appearance) and Fungal Colonies (profused growth of hyphae), which itself depicted the presence of dead &

deacying matter in water whose explaination could be the emergence of drains from all over the province.

Pathogen's presence was also detected in the water samples i.e., presence of *E.coli* was approved in every sample and reason behind this could be the emergence of drains from all over Delhi into the Yamuna River, which ensured the faecal contamination in water. *Pseudomonas aruginosa* was also obtained from every sample because of the high contamination level of water. But the presence of *Staphylococcus aureus* was found to be exclusive among all pathogens. In reference with the data obtained, *S.aureus* was isolated from only 5 samples and the reason could be its origin because it is an airborne microbe and water doesn't approve its survival.

This is how, quantitative and qualitative analysis of Yamuna water samples explained "the extreme level of contamination".

Afterwards, biochemical tests were performed to confirm the pathogenicity with respect to the mentioned pathogens. For *E.coli* ,4 major tests were performed to confirm its presence which were Gram staining, Indole test, Methyl-red test and Voges Proskuer test. Gram staining , being itself a differential staining confirmed the *E.coli* presence by showing "pink stained rods" over the slide under microscope which ensured the microbe as gram negative. The acquaintance of pink stain which was of safranin was because of the elemination or washing off the lipopolysaccharide layer around the microbe by the action of alcoholic de-coloriser. That's why, stain of Crystal-Violet washed off, too. Indole test confirmed the presence of *E.coli* by the production of red colored ring "Rasoindole" which was formed from the interaction of indole and Kovac's reagent. This test was performed to check the production of indole from the Enterobacteriaceae species which was done by the hydrolysis of Tryptophan amino acid with the end products such as indole, pyruvic acid and ammonium ion. Methyl-red test ensured *E.coli* presence by confirming the production of mixed acids (pyruvic acid) from the fermentaion of glucose (Embden Mayerhoff glycolytic pathway) with the help of methyl-red indicator which turned the entire media "red". VP test confirmed *E.coli* presence by showing negative results which means no red colored dye production took place because Enterobacteriaceae species doesn't show Butylene Glycol pathway for Acetoin production, which is responsible for red color appearance in VP test.

For *Pseudomonas aeruginosa,* 5 major test were performed to confirm its presence which were Gram staining, Catalase test, oxidase test, Hugh-Leifson test and Skimmed milk agar test. Gram staining, being itself a differential staining confirmed the *Pseudomonas aeruginosa* presence by showing "pink stained rods" over the slide under microscope which ensured the microbe as gram negative. Catalase test confirmed its presence by showing oxygen bubbles which were because of the hydrolysis of hydrogen peroxide into water molecule and oxygen. Oxidase test confirmed its presence by showing blue colored compound (indophenol) from tetramethyl (p)-phenylenediamine under the effect of cytochrome c oxidase enzyme, which is supposed to be used by bacterial species in their electron transport chain system. Hugh-Leifson test confirmed their presence with the change in its color from green to yellow because of the production of mixed acids from glucose breakdown either oxidatively or fermentively. In the case of *Pseudomonas aeruginosa* (facultative anaerobe), growth appeared in both aerobic and anaerobic conditions i.e., oxidation and fermentation took place, respectively. SMA test confirmed its presence with the "zone of hydrolysis" on SMA plates around the culture, which was because of casein hydrolysis by the action of *Pseudomonas aeruginosa.*

For *S.aureus,* 3 major tests were performed. Gram staining confirmed its absence. Catalase test ensured its presence with oxygen bubbles. In coagulase test, agglutination confirmed the absence of *S.aureus.* Agglutination occurs because of the conversion of fibrinogen (soluble) into fibrin (insoluble).

Later, tests were performed to check the antibiotic resistance pattern. Resistancy was

calculated with the size of the zone of inhibition in an "inverse proportional manner". The resistance of an organism against an antibiotic can be determined by the zone of inhibition that is seen. The formation of an inhibitory zone shows that the antibiotic is successful in killing all of the microorgaisms present.

Four antibiotics were chosen for this purpose, which were Azithromycin, Cefixime, Ceftriaxone and Kanamycin. Two different concentrations of 10µg/ml and 100µg/ml were chosen for each. For *Pseudomonas*, Ceftriaxone is the most efective antibiotic as it shows an overall similar result against different samples of *Pseudomonas*. It is followed by Azithromycin and Kanamycin. Kanamycin shows a smaller zone of inhibition but still shows inhibition in concentration as less as 10µg/ml. While cefixime is the least effective antibiotic among the four.

For *E. coli* the results were different with Kanamycin being the most effective antibiotic showing inhibition in both high and low concentration. It was followed by azithromycin then ceftriaxone and ultimately cefixime.

This shows us that slowly and steadily the organisms have started to show resistance against the antibiotics present in the market today.

As for the resistance against heavy metals the metals chosen were Mercury, Cadmium and Iron. The concentrations chosen were of 10ppm, 100ppm and 1000ppm. Both *Pseudomonas* and *E. coli* were completely tolerant against iron in all concentrations.

In *Pseudomonas* most samples can tolerate cadmium for a minimum of 10ppm and a few samples show tolerance even for 100ppm. In mercury, 10ppm seems to be the limit of their tolerance as inhibition is seen in all samples for 100ppm.

In *E. coli*, tolerance of upto 100ppm is seen for Mercury and Cadmium in all samples.

Therefore, all of these samples can be used potentially for heavy metal tolerance study because they show complete resistance against 1000ppm concentration of iron while show very positive results in sustaining themselves of upto a concentration of 100ppm. Hence, they can be used for bioremediation purposes for the removal of heavy metals.

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In Vitro Antibacterial Efficacy Of Some Ayurvedic Plants Against Biofilm Forming MDR Enteric Bacteria And GC-MS Analysis Of The Active Extract

[1]Meenu Maheshwari, [1,2]Malika Ait-Sidi-Brahim, [1]Faizan AbulQais, [3]Zafar Mehmood, [*1]Iqbal Ahmad, and [3]S.Farooq

[1]Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002, UP, India.

[2] Laboratory of Biotechnology, Protection and Valorization of Plant Resources; Phytochemistry and Pharmacology of Medicinal Plants Unit, (URAC35 Association Unit) Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco.

[3] Himalaya Drug Company, Dehradun, Uttarakhand, 248001. India

***E-mail: ahmadiqbal8@yahoo.co.in**

Abstract-Infectious diseases are still one of the leading causes of human mortality and morbidity across the globe. Among bacteria, emergence and spread of antimicrobial resistance (AMR) globally has threatens the successful treatment of infectious diseases. The continuous emergence of new types of resistant bacterial pathogens have decreased the efficacy of available antibacterial drugs. Evaluation of medicinal plants known for their biological activity against MDR bacteria is needed to explore and exploit the rich diversity of bioactive extracts of medicinal plants as an alternative antibacterial agent in combating AMR. In this study, methanolic extracts 10 medicinal plants were screened for antibacterial activity. The extracts were preliminary screened against strong biofilm forming MDR bacteria by agar well diffusion method at the concentration of 1.0 mg/mL of each plant extract. The zone of inhibition was measured for the comparative analysis of their antibacterial activity. Among tested plants, *Acoruscalamus* exhibited considerable antibacterial activity with mean of zone of inhibitions 15.7 mm against test isolates and highest zone of inhibition was found to be against *E. coli* isolates ECM4 (17.6±0.57). *Holarrhena antidysentrica* and *Hemidesmusindicus* showed moderate antibacterial activity. *Terminaliachebula*, *Punicagranatum* and *Plumbagozeylanica* showed relatively low activity against test isolates with mean of zone of inhibitions less than 12.66 mm. The MIC of extracts ranged from 0.125 mg/mL to 4 mg/mL against test bacteria. It was found that methanolic extract of *A. calamus* showed considerable antibacterial activity with lowest MIC 0.125 mg/mL. MIC against ESβL producing bacterial isolates ranged from 0.125 mg/mL to 2.0 mg/mL. The findings indicated that bioactive extracts might be effective in treating infection caused by MDR bacteria.

Keywords: Antimicrobial Resistance; Plant Extracts; Indian Medicinal Plants; Esβl; *Acorus calamus*.

Introduction

Infectious diseases are still one of the leading causes of human mortality and morbidity across the globe. Annual deaths worldwide due to AMR continue to climb to around 750,000 and are projected to reach as high as 10 million by the year 2050 (O'Neill, 2016). Emergence and spread of antimicrobial resistance (AMR) globally have threatens the successful treatment of infectious diseases (McEwen and Collignon, 2018). Global efforts are going on to understand drivers of AMR and to develop strategies to tackle AMR problem.Manychallenges both scientific and economic in antimicrobial development has caused slow development of new drugs (WHO, 2019a; WHO, 2019b). Various measures have been suggested to prevent rise and spared of AMR and to including use of alternative antibiotics in poultry and agriculture and possibly in combinational therapy. Medicinal plants traditionally used in Indian system of medicine (Ayurveda, Unani, and Siddha) are rich source of bioactive compounds and antimicrobial secondary metabolites.

Plant secondary metabolites are important for the adaptation in plants to environment they also ser serve as surveillance system. Secondary metabolites are the byproducts of primary metabolic pathways, and these are also responsible for specific tastes, odors, and colors of plant tissues. Such metabolites assist plant to cope with abiotic stresses. They are important for proper development and growth (Kessler and Kalske, 2018; Zaynab et al., 2018; Wink, 2020). Plant secondary metabolites commonly fall in one of these chemical classes, *viz.*terpenoids, phenolics and alkaloids. Terpenoids are the most diverse secondary metabolite groups, and it comprises of >50,000 known phytocompounds (Chassagne et al., 2019; Belcher et al., 2020).

Due to continuous emergence of new types of resistant bacterial pathogens the efficacy of available antibacterial drug is decreasing. On the other hand, plant-based screening studies have mainly utilized sensitive or less commonly resistant bacteria in test system. Such information become less relevant when new resistant strains develop. WHO has classifies these resistant bacterial pathogens in to different categories based on their pathogenicity and problematic nature (WHO, 2016).Extended spectrum β-lactamases (ESβL) enteric bacteria including *E. coli*, *Klebsiella*, Enterobacter etc. have been identified as one of the major problematic MDR bacteria (Adler et al., 2020). Evaluation of medicinal plants known for their biological activity and safe use in traditional medicine against such MDR bacteria is needed to explore and exploit the rich diversity of bioactive extracts of medicinal plants as alternative antibacterial agent in combating AMR. In the present study, Indian plants were evaluated against MDR bacterial strains and the most active extract of *A. calamus* was subjected to GC MS analysis to identify major compounds.

Material and Methods

Collection of plant material

The authentic plant samples were provided as gift from the Himalaya Drug Company, Dehradun, India. Some of the plant materials were purchased from local authorized supplier, Aligarh UP, India in January-March 2015. The taxonomic identification of the plant material was confirmed by Professor S. Hayat, Department of Botany, AMU, Aligarh (India). The voucher specimen has been submitted in the Department of Agricultural Microbiology, Faculty of Agricultural Sciences, AMU, Aligarh.

Preparation of methanolic plant extracts

The methanolic plant extracts were prepared by using method as described earlier by (Aqil et al., 2005). The plant materials were shade dried and powdered. Five hundred grams (500 g) of dried and powdered plant material was soaked in 2.5 liter of methanol (purity ≥ 99.8%) for 5 days with intermittent shaking. The extract was filtered through Whatman filter paper No.1 and concentrated to dryness under reduced pressure on rotatory evaporator (RE-2000A, Associated Scientific Technologies, Delhi, India) at 40°C and stored at 4°C for further study. The yield of crude methanolic plant extracts was obtained and calculated in percentage of total dry weight of starting material. The dried crude extracts were reconstituted in DMSO (<1%) to prepare stock/working solutions of desired concentrations needed for experiments.

Determination of antibacterial activity

The antibacterial activity of plant extracts was determined by agar well diffusion method described previously (Perez et al., 1990) with little modification as adopted earlier (Aqil et al., 2005). Briefly, 0.1 mL of freshly grown diluted inoculum (10^5 CFU/mL) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with plant extract of final concentration of 1.0 mg/mL and solvent blank (<1% DMSO) separately. The plates were incubated for overnight at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition against test organism. Antibiotic to which organism was sensitive was used as control.

Determination of MIC of plant extracts

The minimal inhibitory concentration (MIC) of plant extracts against bacterial strains was determined by broth microdilution susceptibility testing method as described

by Eloff et al., (1998) using 2,3,5-triphenyltetrazohum chloride [TTC, tetrazolium red, purity min. 99%) as a growth indicator dye. The concentrations of plant extracts were two-fold serially diluted ranged from 0.0125 to 4 mg/mL in a sterile 96-well polystyrene microtiter plates 100µl of bacterial inoculum (≈1×10^6 CFU/mL) were added to each well. The covered microtiter plates were incubated for 18 h at 37°C. To indicate bacterial growth, 40 µl of TTC dissolved in water (2 mg/mL w/v) were added to each well and incubated at 37°C for 30 min. The wells were examined for the color change to red, indicated the actively growing cells while no change in color indicate lack of bacterial growth. MIC was defined as the minimum concentration of plant extracts which inhibited the visible growth of test strains.

Phytochemical analysis of plant extracts by color test

Selected plant extracts were screened for the presence of major phytocompounds using standard methods as described below:

Alkaloids: Two mL of extract was taken in 5 mL distilled water and mixed with hydrochloric acid (2.0 M). Once the acid reaction stopped, 1 mL of Drangendorff's reagent was added. A red or orange precipitate indicates the presence of alkaloids (Kapoor et al., 1969; Wagner and Bladt, 1996).

Flavonoids: The method described by Kapoor et al., (1969), was used for detection of flavonoids. Hundred microliter extract (100 mg/mL) was dried over a water bath followed by the addition of 5-10 drops of concentrated hydrochloric acid. Thereafter, a pinch of Zn powder in reaction mixture was added. A pink or brown color indicates the presence of flavonoids.

Glycosides: Hundred microliter extract (100 mg/mL) was dissolved in distilled water and 1 mL of Sodium hydroxide solution (1%) was added. The development of yellow color indicates the presence of Glycosides (Odebiyi and Sofowora, 1978).

Phenols: Hundred microliter extract (100 mg/mL) was dissolved in distilled water and few drops of 10% aqueous ferric chloride solution were added. The presence of phenolic compounds will be indicated by development of blue green color (Fadeyi and Akpan, 1989).

Tannins: a few drops of 5% aqueous ferric chloride were added into 1 mL of extract followed by the addition of dilute sulphuric acid to the reaction mixture. On acidification, the development of blue black or green browncolor indicates the presence of tannins (Segelman and Farnsworth, 1969).

Saponin: Hundred microliter extract (100 mg/mL) was taken and few drops of 1% sodium carbonate solution was added. Mixture was shaken vigorously and left stand still for 3 minutes. A honeycomb like froth indicated the presence of saponins (El-Tawil, 1983).

Gas chromatography - mass spectrometry (GC-MS) of *A. calamus*

Methanolic extract of *A. calamus*was analyzed by Gas chromatography-mass spectrometer (Instrument model GCD 1800A, Hewlett Packard). The sample was injected into a split inlet at 260°C, with a split ratio 1:10. Helium (purity 99.999%) was used as a carrier, with a constant flow of 1.21 mL/min. The separation was achieved on HP-1 column (Thermo scientific), using the following temperature program: start at 700C and hold for 2 min, 5°C/min to 250°C and hold for 2 min, 10°C/min to 280°C and hold for 17.0 min (total run time 50 min). Elute was delivered to the mass spectrometer with Ion source temperature 230°C and interface temperature 270°C Data was acquired in Scan mode (m/z range 40–650). The compounds were identified by mass spectra comparison with libraries (Wiley Registry of Mass Spectral Data 7th ed. (McLafferty, 2005), and NIST/EPA/NIH Mass Spectral Library 05 (NIST/EPA/NIH, 2005). Relative amounts of components, expressed in percentages, were calculated by normalization measurement according to peak area in total chromatogram.

Results

Antibacterial activity of methanolic extracts of medicinal plants

A total of 10 medicinal plants extracts were collected for this study to evaluate the antibacterial activity. Methanolic extracts of all selected plants were primarily screened against MDR bacteria by agar well diffusion method at the concentration of 1.0 mg/mL of each plant extract. The zone of inhibition was measured, and data recorded is summarized in **Table-1**. *Acoruscalamus* exhibited considerable antibacterial activity with mean of zone of inhibitions 15.7 mm against test isolates and highest zone of inhibition was found to be against *E. coli* isolates ECM4 (17.6±0.57). *Holarrhena antidysentrica* and *Hemidesmusindicus* showed moderate antibacterial activity with mean of zone of inhibitions 14.96 mm and 13.49 mm against test isolates respectively and maximum zone of inhibition was found against KPMA19 (16.6±0.57mm) and ECMA2 isolates (15.3±1.52) respectively. *Terminaliachebula*, *Punicagranatum* and *Plumbagozeylanica* showed relatively low activity against test isolates with mean of zone of inhibitions less than 12.66 mm. Four methanolic plants extracts namely *Cuminumcyminum*, *Elettaria cardamomum*, *Foeniculum vulgare* and *Psidiumguajava* showed no significant activity against bacterial isolates at tested concentration. On comparing mean of zone of inhibitions, the activity of above plants extracts was found highest in *A. calamus*followed by *H. antidysentrica>H. indicus>T. chebula>P. zeylanica>P. granatum*.

The methanolic plant extracts of *A. calamus*, *H. antidysentrica* and *H. indicus* demonstrated relatively high antibacterial activity by agar well diffusion assay in comparison with other plant extracts. These plant extracts were further investigated to evaluate their antimicrobial efficacy against all ESβL producing enteric bacteria in terms of MIC. The data presented in **Table-2** ranged from 0.125 mg/mL to 4 mg/mL against test bacteria. It was found that methanolic extract of *A. calamus* showed considerable antibacterial activity with lowest MIC 0.125 mg/mL. MIC against ESβL producing bacterial isolates ranged from 0.125 mg/mL to 2.0 mg/mL. On the other hand, *H. antidysentrica* and *H. indicus* exhibited moderate antibacterial activity. MIC ranged from 1 to ≥4 mg/mL against test isolates with lowest MIC 1.0 mg/mL. To determine comparative efficacy of bioactive plant extracts against ESβL producing enteric bacterial isolates, MIC_{50} and MIC_{90} were calculated using Probit regression analysis. **Table 3** showed thatmethanolic extracts of *A. calamus* showed considerable antibacterial potency against ESβL producing bacterial isolates with $MIC_{50}$and MIC_{90} (0.517 and 1.280 mg/mL) against *E. coli* isolates and for *Klebsiella* isolates, MIC_{50} and MIC_{90} values were 0.542 and 1.158 mg/mL respectively. The MIC_{50} and MIC_{90} for *H. antidysentrica* were found 1.248 and 2.268 mg/mL against *E. coli* isolates and 1.166 and 2.214 mg/mL against *Klebsiella* isolates, indicating moderate antibacterial activity of this plant extract. The methanolic plant extract of *H. indicus* was found least active against ESβL producing enteric bacteria with MIC_{50} and MIC_{90} values 2.052 and 3.464 mg/mL against *E. coli* isolates while, 2.0 and 3.752 mg/mL against *Klebsiella* isolates.

Comparative antibacterial efficacy of bioactive plant extracts was also determined by evaluating MIC range in terms of per cent of test isolates inhibited. *A. calamus* exhibited considerable MIC range in which 5.4% isolates inhibited at MIC as 0.25 mg/mL and 70.27% isolates exhibited MIC range of 0.5-1.0 mg/mL. 24.3% isolates showed MIC range 1.0-2.0 mg/mLas indicated in **Figure-1**. In *H. antidysentrica*, 29.72% isolates exhibited MIC range 0.5-1.0 mg/mL and 70.27% isolates showed MIC range 2.0-4.0 mg/mL. This is attributed to moderate MIC range of *H. antidysentrica* against ESβL producing enteric bacteria.*H. indicus* displayed highest MIC range in which most of the isolates (91.89%) exhibited MIC range 2.0-4.0 mg/mL and 2.7% isolates showed MIC even greater than 4 mg/mL. Based on lowest to highest MIC range, the antibacterial efficacy of selected bioactive plant extracts was found in order of *A. calamus>H. antidysentrica>H. indicus*.

Phytochemical analysis of plant extracts

Phytochemical analysis of all four methanolic plant extracts by color test is presented in **Table 4**. All extracts were found positive for the presence of flavonoids, phenols, tannins and saponins except *H. indicus*. Similarly, alkaloids were detected in all plant extracts. Glycosides were detected in *H. indicus*.

To detect the presence of potentially active phytocompound in methanolic extracts of *A. calamus*, GC-MS analysis was performed. The GC-MS analysis of *A. calamus* rhizome extract revealed the presence of 13 major components (**Table-5**). The compounds identified with % area of peak were α-asarone (66.23%), cis-linoleic acid (5.68%), lupeol acetate (2.26%), γ-asarone (2.12%), β-asarone (1.43%) etc. GC-MS chromatogram of methanolic extract of *A. calamus* is presented in **Figure-2**. Many other small peaks represent other phytocompounds in trace amount.

Table-1 Antibacterial activity of selected medicinal plants extracts against biofilm forming MDR bacteria by agar well diffusion assay.

Bacteria	**Antibacterial activity of plants extracts (*diameter of zone of inhibition in mm)**					
	Acoruscalamus	***Hemidesmusindicus***	***Holarrhenaantidysenterica***	***Plumbagozeylanica***	***Punicagranatum***	***Terminaliachebula***
ECMA2	14.33±0.57	15.33±1.15	15.33±0.57	12.66±1.15	13.0±1.73	10.0±0.57
ECM4	17.66±0.57	15.33±0.57	15.33±1.52	14.33±0.57	10.66±0.28	12.33±1.15
ECMW9	12.33±0.57	12.33±0.76	14.66±0.57	10.33±0.57	11.66±1.15	14.33±0.57
ECUA1	16.0±0.86	15.33±0.57	16.33±1.15	12.0±0.86	10.33±0.57	10.33±0.57
ECUA2	15.33±0.57	10.66±0.57	16.33±1.52	11.66±0.57	13.33±0.57	14.0±1.0
ECM49	17.33±0.57	14.33±1.15	16.33±0.57	11.33±0.57	10.66±0.57	12.66±0.57
ECG7	17.66±1.15	10.0±0.86	14.33±0.57	11.0±0.86	13.0±1.0	11.0±0.86
KPMA19	16.66±1.44	14.66±0.57	16.66±0.57	14.66±0.57	8.66±0.28	13.33±1.15
KCUA12	14.66±0.57	13.33±1.15	10.0±1.0	12.33±0.57	10.66±0.28	12.0±1.0
ENM32	15.0±0.86	12.33±0.57	15.66±0.57	10.66±0.28	9.33±0.57	13.66±0.57
ENM36	17.33±1.15	14.66±0.57	16.0±0.86	8.33±0.57	10.66±1.15	14.0±0.86
***E. coli* 25922**	15.0±0.86	14.66±0.57	16.0±0.57	12.0±0.86	12.33±0.57	14.0±1.0
Mean**	15.77^{b}	13.49^{c}	14.96^{b}	$11.74^{d,e}$	11.12^{e}	$12.6^{c,d}$

Note: *including well size; each well contains 1.0 mg/mL of plant extract and 0.1 mg/mL of active compound, -; indicates no zone of inhibition; No antibacterial activity was recorded in the crude methanolic extracts of *Cuminumcyminum* (Zeera), *Elettariacardamomum* (Elaichi), *Foeniculumvulgare* (Saunf) and *Psidiumguajava* (Amrud). **Mean values were compared using Duncan's multiple range test and letters indicate different significant groups at $p \leq 0.05$.

Table 2. MIC of selected methanolic plant extracts against ESβL producing enteric bacterial isolates.

Bacterial isolates	**Minimum inhibitory concentration (MIC) mg/mL**		
	A. calamus	***H. antidysenterica***	***H. indicus***
E. coli			
ECMA2	2	1	2
ECMA20	0.5	2	2
ECM 4	1	2	2
ECM8	2	4	>4
ECM16	0.5	2	4
ECM18	1	2	4
ECM49	2	2	2
ECMW5	1	2	2
ECMW6	0.5	1	2
ECMW9	2	2	4
ECMW21	2	4	4
ECMW30	1	2	4
ECM W31	1	2	4
ECMW41	1	2	2
ECUA1	1	2	4
ECUA2	2	2	2
ECUA8	0.5	2	4
ECGA4	0.125	1	1
ECGA7	1	2	4
ECUJ1	0.5	1	4
ECUJ2	0.5	1	2
ECUJ9	1	2	4
E. coli 25922	0.5	1	2
Klebsiella			
KPMA19	0.5	2	2
KPM27	0.25	1	1
KPM3	1	2	4
KPMA9	0.5	1	2
KPM S1	0.5	1	2
KPMA14	0.5	2	4
KPMA17	0.5	2	2
KPMEA17	2	2	4
KUJ12	2	4	4
KUJ13	0.5	1	2
KUJ16	1	2	4
Kp700603	2	2	4
E. cloacae			
ENM32	2	2	4
ENM36	1	1	2

Table 3. MIC_{50} and MIC_{90} of bioactive plant extracts against ESβL producing enteric bacterial isolates.

Bacterial isolates	Minimum inhibitory concentration (MIC) mg/mL			
	MIC_{50}	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
A. calamus	0.517	1.280	0.542	1.158
H. antidysenterica	1.248	2.268	1.166	2.214
H. indicus	2.052	3.464	2.000	3.752

Table 4. Major groups of phytocompounds of selected methanolic plant extracts.

Name of plant	**Alkaloids**	**Flavonoids**	**Glycosides**	**Phenols**	**Tannins**	**Saponins**
A. calamus	+	+	-	+	+ (CT)	+
H. antidysenterica	+	+	-	+	+ (CT)	+
H. indicus	+	+	+	+	+ (CT)	-

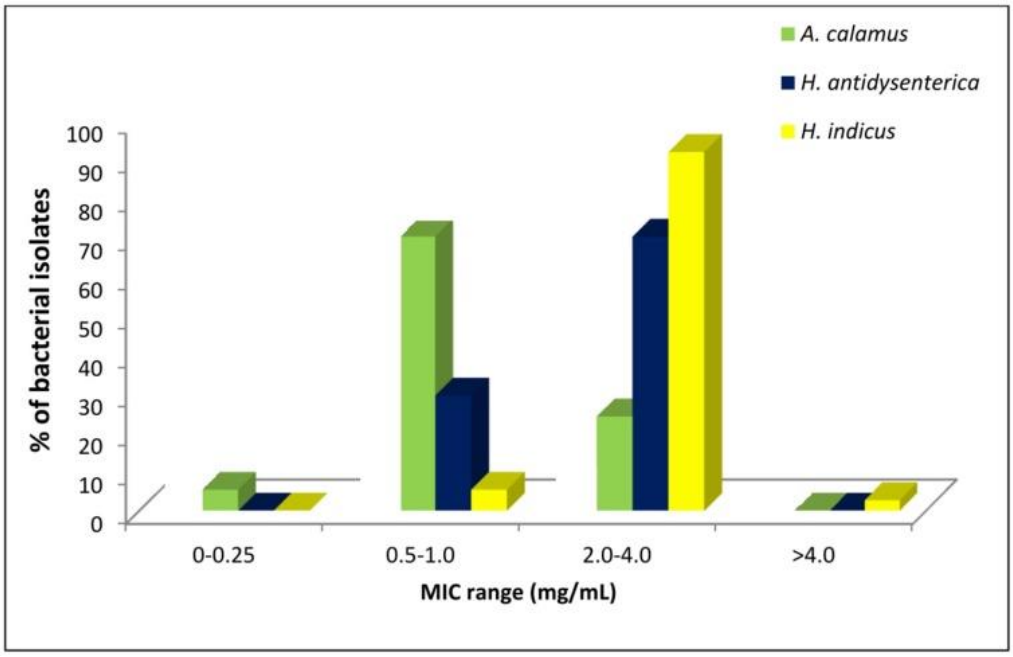
Note: CT represents catechin tannins

Table 5. Major components of methanolic extracts of A. calamus as identified by Gas chromatography–mass spectrometry.

Peak no.	**Retention time**	**Area %**	**Components**	**Chemical class**
12	17.43	66.23	α-Asarone	Phenylpropanoid
28	23.86	5.68	Cis-linoleic acid	Fatty acid
24	21.80	3.76	Tetra decanoic acid	Fatty acid
35	38.54	2.26	Lupeol acetate	Triterpene
10	16.33	2.12	γ-Asarone	Phenylpropanoid
25	23.29	1.85	Cis-linoleic acid methyl ester	Fatty acid methyl ester
32	35.84	1.67	γ-sitosterol	Phytosterol
21	19.16	1.47	Oplopanonyl acetate	Terpene
15	17.98	1.43	β-Asarone	Phenylpropanoid
3	14.61	1.39	Cis methyl isoeugenol	Phenol ether
5	15.61	1.42	Epishyobunone	Monoterpene
23	21.26	1.21	Hexadecanoic acid methyl ester	Fatty acid methyl ester
17	18.43	1.09	Tri-methyl benzyl alcohol	Phenol

Figures

Figure-1 MIC range of selected bioactive plant extracts against ESβL producing enteric bacterial isolates.



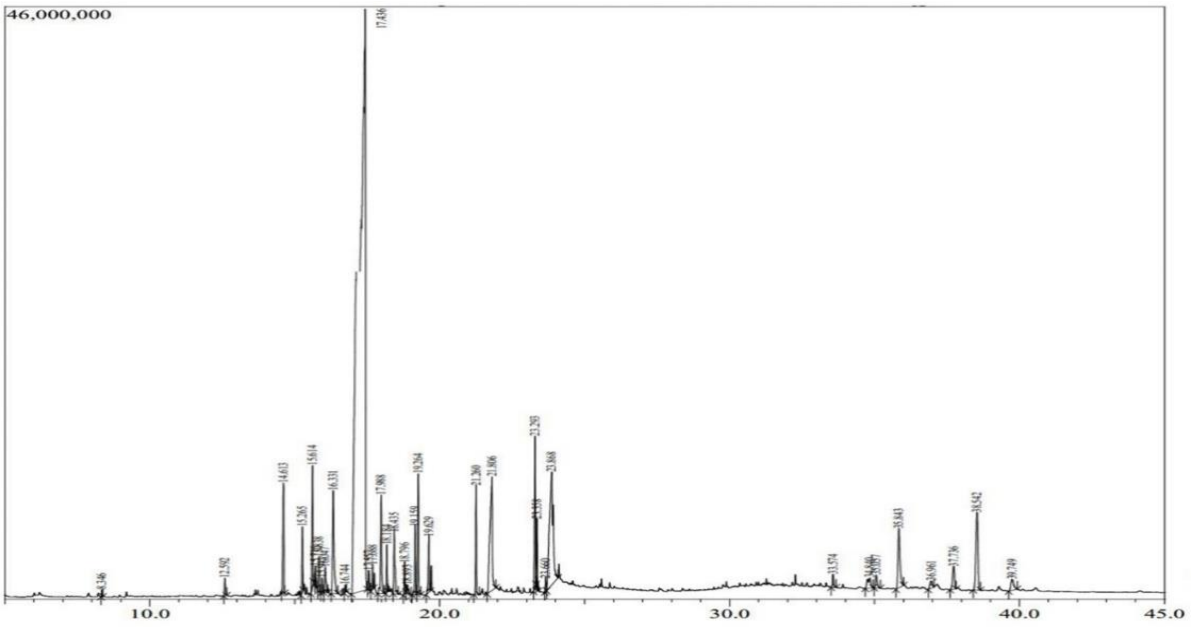


Figure-2 Gas chromatography–mass spectrometry (GC-MS) chromatogram of methanolic extract of A. calamus; Numbers above the peak refer to retention time.

Discussion

The emerging global scenario of multidrug resistance among pathogenic bacteria and poor discovery of new antibiotics with novel mode of action has led to the investigation of new combinations and possible alternative treatment strategies against MDR bacteria. Indian medicinal plants have been considered as a potential source of new antimicrobial agents. Since ancient times, medicinal plants have made significant contribution to human health. Many herbal formulations and decoctions have been used to cure various ailments and diseases. These herbal preparations have a diverse range of bioactive phytochemicals that can be explored for

various biological activities such as antimicrobial efficacy, antiviral activity, antibiofilm activities, combinational effect with antimicrobials, antidiabetic etc. (Cowan et al., 1999; Dahanukar et al., 2000; Mukherjee et al., 2006; Borges et al., 2016). Plant-derived medicines are considered relatively safer than chemical antimicrobial (Gogtay et al., 2002).

In the present study, methanolic extracts of eleven plants, traditionally used in Indian medicine were screened for antibacterial efficacy against biofilm forming ESβL producing enteric bacteria. Of these, seven plant extracts exhibited varying level of inhibition against test isolates. The mean values of zone of inhibition were compared using Duncan's multiple range test. Overall activity of tested plant extracts is in order of *A. calamus>H. antidysentrica>H. indicus>T. chebula>P. zeylanica>P. granatum*. Moreover, higher activity was found mainly into methanolic extracts of *A. calamus*, *H. antidysentrica* and *H. indicus* while lower activity was observed in *T. chebula*, *P. zeylanica* and *P. granatum*.

In vitro efficacy of four methanolic plants extracts including *A. calamus*, *H. antidysentrica* and *H. indicus* were assessed in terms of MIC against ESβL producing enteric bacteria. The MIC of the methanolic extracts of individual plants varies against different test isolates. Comparative antibacterial efficacy of selectedplantsextractswas determinedbycalculatingMIC_{50}andMIC_{90}.LowerMIC_{50}andMIC_{90}indicatehigherantibacterialactivity.The$MIC_{50}$and $MIC_{90}$of *A. calamus*, *H. antidysentrica* and *H.indicus* were ranged from 0.517 to 2.052mg/mL and 1.280 to 3.464mg/mL respectively against*E. coli* isolates.*A. calamus*exhibited lowest MIC_{50} 0.542 mg/mL and MIC_{90} 1.158 mg/mL against *Klebsiella* spp. isolates followed by*H. Anti dysentrica* (1.166 mg/mL and 2.214 mg/mL), and *H. indicus* (2 mg/mL and 3.752 mg/mL).

Antibacterial efficacy of bioactive methanolic plant extracts was also evaluated by comparative study on MIC range interms of percent of test isolates inhibited. *A. calamus* extract showed lowest MIC range against test bacterial isolates followed by *H.antidysentrica*. While *H.indicus* extractexhibitedhighest MIC range 2.0-4.0 mg/mL for 91.89% isolates.

Overall, antibacterial efficacy of selected methanolic plants extracts in terms ofMIC,(highestpotencyandlowestMICvalues)against ESβLproducingenteric bacteria are in order of *A. calamus>H. antidysentrica>H. indicus*.

These methanolic plants extracts exhibited antibacterial activity irrespective of the drug resistance pattern of the test bacteria. This reveals antibiotic resistance does not restrict antimicrobial potential of active plant extracts. The active compounds present in plant extracts might have different modes of actions with multiple target sites on test organisms (Omojate et al., 2014).

The results of the present study depicted that active methanolic plant extracts of *A. calamus*, *H. antidysentrica* and *H. indicus* exhibited a broad range of antimicrobial activity against multidrug resistant ESβL producing enteric bacteria. Ourfindingsagreewithpreviousstudieshighlightingthepotent antimicrobial activity of traditional Indian medicinal plants including *A. calamus*, *H. antidysentrica* and *H. indicus* against ESβL producing and MRSA strains (Ahmad et al., 2001; Ahmad and Aqil. 2007). Other studies have also been described the antibacterial activity of crude methanolic extracts of medicinal plants against multidrug resistant bacteria associated with diseases in human(Bisi-Johnsonetal., 2017; Mishra et al., 2017). These findings suggest that potentially effective medicinal plant extracts could be used in treatment strategies against infectious diseases caused by drug resistant bacteria.

The active components of plant extracts such as alkaloids, flavonoids, terpenoides, glycosides and phenolic compounds interact with enzymes and proteins of the cell membrane causing efflux of protons toward cell exterior which finally disrupt the membrane accompanied by cell death. These phytoconstituents may also inhibit enzymes necessary for amino acids biosynthesis (Barbieri et al., 2017).Phytochemical investigation of bioactive plant extracts and their major active components were explored

by GC-MS analysis. Incase of *A.calamus* GC-MS analysis predicted α-Asarone as major active constituents together with γ-Asarone, β-Asarone and terpenes. Consistent with this report, α-as a rone was found to be the major constituents of *A.calamus* rhizome that possess substantial antibacterial activity (Li and Wah, 2017).Moreover, this study is supported by the reports of other workers who have shown the antibacterial properties of *A. calamus* rhizome extracts (Kumar et al., 2014; Rawat et al., 2016).

The study clearly demonstrated that plant extracts, especially *A. calamus*, are potent against drug resistant bacterial pathogens which could be exploited to tackle AMR problem after careful *in vivo* investigation.

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About Flowers (Shown on the Cover Page)

Haldi(*Curcuma longa*)

Scientific classification

Kingdom :Plantae
Clade :Tracheophytes
Clade :Angiosperms
Clade :Monocots
Clade :Commelinids
Order :Zingiberales
Family :Zingiberaceae
Genus :*Curcuma*
Species : ***C. longa***

Curcuma is a perennial shrub found throughout India. The rhizome of curcuma is the most extensively used part and is an integral ingredient of Indian cooking and medicines. Regular intake of turmeric is shown to impart significant amelioration of morning stiffness, improve walking time, and reduce joint swelling (Rathore et al., 2007). Curcumin is the main active component of the rhizome and is a pleotrophic molecule with diverse medicinal properties attributed to it. Curcumin has been reported by several researchers to possess antiarthritic properties in both preclinical and human studies.

Animal studies have shown that turmeric inhibits the activation of nuclear factor kappaB (NF-κB) and key inflammatory genes influenced by NF-κB in the vascular endothelium and synovial cells in RA joints (Tsao et al., 1997). Additionally, curcumin is also shown to downregulate the expression of cyclooxygenase-2 (COX-2) and matrix metalloproteinase-9 (MMP-9) in human articular chondrocytes via suppression of NF-κB. Curcumin also inhibits the upregulation of neutral matrix MMPs and suppresses the expression of TNF-α-induced MMP-13 in primary chondrocytes. Various researches have observed that oral administration of curcumin to arthritic rats decreased elevated levels of the glycoprotein GpA72 and considerably lowered inflammation.

Kali Mirch (*Piper nigrum*)

Scientific classification

Kingdom :Plantae
Clade :Tracheophytes
Clade :Angiosperms
Clade :Magnoliids
Order :Piperales
Family :Piperaceae
Genus :Piper
Species : P.nigrum

Piperaceae, also known as the pepper family, is a large family of flowering plants that may be small trees, shrubs, or herbs. The most well-known species is *Piper nigrum*, which yields most peppercorns that are used as spices, including black pepper, although its relatives in the family include many other spices *P. nigrum* is native to southern India and is extensively cultivated there as well as elsewhere in tropical regions, including Africa. The plant is believed to cure illnesses such as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches . The alkaloid piperine has been reported to be the major cytotoxic constituent of *P. nigrum*.

Ashwagandha(*Withania somnifera*)

Scientific Classification

Kingdom :Plantae
Clade :Tracheophytes
Clade :Angiosperms
Clade :Eudicots
Clade :Asterids
Order :Solanales
Family :Solanaceae
Genus :Withania
Species :W. somnifera

Withania somnifera, also known as ashwagandha, is one of the prominent medicinal plants in Indian systems of medicine. Since antiquity, it is used against myriad of clinical conditions and, in fact, its history of use as a medicine dates back to AD 6000. The plant contains a range of different classes of chemical constituents such as alkaloids, steroidal lactones, and flavonoids. These chemical moieties are responsible for various biological activities of the plant. Laboratory studies demonstrated the plant to be antiinflammatory, antitumor, neuroprotective, antimicrobial, antistress, antidiabetic, and cardioprotective. These pharmacologic activities are in part due to the capability of *W. somnifera* to reduce reactive oxygen species, modulate mitochondrial function, regulate apoptosis, reduce inflammation, and enhance endothelial function. Additionally, it has been used singly or in combination against various diseases of humans. Here, we recapitulate ethnobotanical and pharmacologic characteristics and therapeutic applications of the plant and its active constituent.

Shatavari(*Asparagus racemosus*)

Scientific classification

Kingdom :Plantae
Clade :Tracheophytes
Clade :Angiosperms
Clade :Monocots
Order :Asparagales
Family :Asparagaceae
Subfamily :Asparagoideae
Genus :*Asparagus*
Species : ***A. racemosus***

Asparagus racemosus (*A. racemosus*) belongs to family Liliaceae and commonly known as Satawar, Satamuli, Satavari found at low altitudes throughout India. The dried roots of the plant are used as drug. The roots are said to be tonic and diuretic and galactgogue, the drug has ulcer healing effect probably via strenthening the mucosal resistance or cytoprotection. It has also been identified as one of the drugs to control the symptoms of AIDS. *A. racemosus* has also been successfully by some Ayurvedic practitioner for nervous disorder, inflammation and certain infectious disease. However, no scintific proof justify aborementioned uses of root extract of *A. racemosus* is available so far. Recently few reports are available demonstrating beneficial effects of alcoholic and water extract of the roots of *A. racemosus* in some clinical conditions and experimentally indused disease *e.g.* galactogougue affects, antihepatotoxic, immunomodulatory effects, immune adjuvant effect, antilithiatic effect and teratogenicity of *A. racemosus*. The present artical includes the detailed exploration of pharmacological properties of the root extract of *A. racemosus* reported so far.

Forth Coming Events

1200th International Conference on Chemical and Biochemical Engineering (ICCBE)
Dubai, United Arab Emirates
Conference Website :http://researchworld.org/Conference2022/UAE/1/ICCBE/
Date :Jan 01,2022

International Conference on Environmental Science and Biotechnology (ICESB)
Gulbarga, Karnataka, India
Conference Website :http://scienceplus.us/Conference/19176/ICESB/
Date :Jan 01,2022

International Conference on Medicinal and Aromatic Plant Sciences
Website: https://waset.org/medicinal-and-aromatic-plant-sciences-conference-in-january-2022-in-singapore
Date: Jan 11,12, 2022

International Conference on Environmental Science and Biotechnology (ICESB)
Bengaluru, Karnataka, India
Conference Website :http://scienceplus.us/Conference/19184/ICESB/
Date :Feb 02, 2022

International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB)
Chennai, Tamil Nadu, India
Conference Website :http://issrd.org/Conference2022/2/Chennai/ICMPB/
Date :Feb 02, 2022

International Conference on Traditional Medicine, Phytochemistry and Medicinal Plants
Website URL:https://traditionalmedicine.unitedscientificgroup.org/
Date: **February 07,09, 2022**

9th Asian Herbal and Traditional Medicine Paris, France
Website: https://traditionalmedicine.healthconferences.org/
Date:February 21,22, 2022

International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB)
Khandala, Maharashtra, India
Conference Website :http://issrd.org/Conference2022/2/Khandala/ICMPB/
Date :Feb 28, 2022

International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB)
Chennai, Tamil Nadu, India
Conference Website :http://issrd.org/Conference2022/3/Chennai/ICMPB/
Date :Mar 03, 2022

Ayurveda Medicinal Plants Conferences - Washington, United States
Website URL: https://medicinalplants.heraldmeetings.com/
Date:**March 04,05, 2022**

International Conference on Environmental Science and Biotechnology (ICESB)
Bhubaneswar, Odisha, India
Conference Website :http://scienceplus.us/Conference/19911/ICESB/
Date : May 01, 2022

International Conference on Organic Chemistry in Singapore, Singapore
Website URL: https://waset.org/organic-chemistry-and-chemical-engineering-conference-in-may-2022-in-singapore
Date:May 03,04, 2022

International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB)
Ratlam, Madhya Pradesh, India
Conference Website :http://issrd.org/Conference2022/5/Ratlam/ICMPB/
Date :May 06, 2022

International Conference on Environment, Agriculture and Biotechnology (ICEABT)
Jakarta, Indonesia
Conference Website :http://academicsconference.com/Conference/18641/ICEABT/
Date : Jun 01,2022

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