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Dr. I.P. Pandey, Co-ordinatior
Universities' Journal of Phyto-Chemistry and Ayurvedic Heights,
1-Inder Road, Dehradun - 248001 (Uttarakhand) India
E-mail : sfarooq@satyam.net.in; editor@ujpah.in
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Editorial

India's emergence as major global player in Pharma sector is quite possible, If we just concentrate to produce herbal Pharma patented products of oncology which will give return in millions of dollars, though US is targeting India's patent law but hopefully we will not be harmed because there are complaints of countries as diverse as Brazil and Canada and the guidelines used by South American Nations are a carbon copy of India's section 3D. Anyhow, well researched patented herbal products of different ailments will change national economy as well as lead to more employment opportunities especially for millions of rural poor. But this would not happen unless the country enjoys major share in the international markets.

India's export in this sector have not shown much buoyancy and have not exceeded more than † 1000 Crore, whereas according to Task Force Planning Commission report (2000), the export of medicinal plants should have touched † 10,000 Crore plus by 2010. At present India's share is only about 13% of the global market while china's is just the double.

India ranks among the so called 12 mega diversity rich areas of the world and is considered one of the richest centres for plant genetic resources. Across the various ecosystems from Himalaya to Kanyakumari and from Rajasthan to North-East, about 6560 spices are estimated to be used for human and veterinary health care. Forests which contribute 90% of medicinal plants to industry are to-day under great pressure due to increasing demand and unsustainable excessive harvesting resulting into depletion of plants-diversity. Also, the analysis of various problem-areas clearly bring out that there are many other weakness and gaps in the management of the sector adversely impacting on sustainability of the sector.

To bring India top an enviable position in the list of international market, it is desired that a meaningful plan be drawn as a multi-stakeholder endeavour with a multipronged approach which looks for benefiting people, keeping environment intact and growing national economy through sustainable Development of Medicinal plant sectors.

My thanks and good wishes to all the members of the Editorial Board, Advisory Board and researchers for their valuable contributions to the release of this journal.

(Dr. S. Farooq)
Chief Editor

Antimicrobial Efficacy and Interaction of Plant Extracts With and Without Antibiotics Against Drug Resistant Bacteria

Javed Ahmad Khan[1], Mohd. Musheer Altaf[1], S. Farooq[2] and Iqbal Ahmad[1]*
[1]Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh-202002
[2]The Himalaya Drug Company, Dehradun- 248001
*Corresponding Author
Prof. Iqbal Ahmad, Department of Agricultural Microbiology, Faculty of agricultural Sciences, Aligarh Muslim University, Aligarh- 202002, India
Tel: +91-0571-2703516
E-mail: ahmadiqbal8@yahoo.co.in

Abstract- New sources of antimicrobial drugs need to be identified and improved strategy should be developed to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their synergistic action between extracts and with antibiotics to exploit it in modern phytomedicine and combinational therapy. In the present study, alcoholic extracts of fifteen medicinal plants were screened for their antimicrobial efficacy against a wide variety of drug resistant bacteria and yeast. The extracts of *Carum copticum, C. juncea, H. spicatum, Z. officinale, S. aromaticum, Camellia sinensis, T. foenum grae*cum, *Piper cubeba, C. longa* and *A. barbadensis* showed promising action against one or more drug resistant bacteria as well as against *Candida albicans* with MIC ranged from 0.5 mg/ml to 9.5 mg/ml. Many combinations of these extracts showed synergistic action. The extract of *Carum copticum* exhibited synergy with antibiotics, tetracycline, chloramphenicol, ampicillin and gentamicin against methicillin resistant *S. aureus* which has indicated their potential to be exploited in combination drug therapy after careful evaluation *in vivo* model.

Key words: Antimicrobial activity, MDR bacteria, MIC, synergistic activity antibiotics

Introduction

The use of herbal and other natural substances is part of the fabric of traditional medicine in different part of the world. Medicinal plants have been found good source of therapeutic and novel compounds. Targeted screening of a large diversity of medicinal plants is expected to yield novel biological activities including problematic group of multidrug resistant bacterial pathogens (Ahmad et al., 2008).

Bacteria have evolved numerous defenses against antimicrobial agents and drug resistant pathogens are on the rise and such bacteria have become a global health problem. Nearly twenty years ago over 90% *S. aureus* strains were reported b-lactamase positive. Strains of b-lacatam resistant *Staphylococcus aureus* including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000). The production of b-lactamase is recognized as one of the main mechanism of bacterial-resistance to b-lactamase antibiotics. Numerous compound have been included in the list of b-lactamase inhibitors and some of these have shown potential clinical usefulness based on their synergistic-effects when they are combined with b-lactamase-labile antibiotics. Many b-lactamase were found to be resistant to b-lactamase inhibitors. Similarly multidrug resistant problem is common in members of family Enterobacteriaceae specially *E.coli*, *Salmonella*, *Shigella* and several other humans and animal pathogen like *Haemophilus influenza*, *Campylobacter*, *Pseudomonas* aeruginosa, *Mycobacterium tuberculosis* both in developing and developed countries (Eldelstein *et al.,* 2001; Tonkic *et al.,* 2005; Ahmad et al., 2008).

India has one of the world's richest flora with about 120 families of plant comprising 1, 30,000 species. A large portion of the world population especially in the developing countries depends on the traditional-system of medicine for a variety of diseases. The world health organization (WHO) reported that 80% of the world's population rely chiefly on traditional medicines and major part of the traditional therapies in-

volve the use of plant extracts or their active constituents (WHO 1993).

According to an estimate about 119 secondary plant metabolites are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angiosperms have been investigated chemically and that 74% of pharmacologically active plant derived components were discovered after following upon ethanobotanical use of plants (Eloff, 1998). The plants are valuable in the three basic ways: (1) they are used as source of direct therapeutic agent. (2) As a source of new bioactive metabolites including antimicrobial, antihelminthic and antiprotozoan etc. (3) they serve as raw material base for elaboration of more complex semisynthetic chemical compounds.

According to a report published in the 'Journal of the American Medical Association', more than 630 million visits are made to alternative practitioners each year in U.S. also more than 15 million adults take herbal remedies while taking other medication (Hoffman, 2004).

Concerted efforts have been made all over the world to explore the various biological and specific pharmacological activities and their active compounds all over the world. The antibacterial and antifungal activities of Indian medicinal plants are widely known against a variety of pathogenic and opportunistic microorganisms (Aqil and Ahmad, 2007). However, targeted screening with improved strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy. It is expected that plant extract showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.,* 1998). In the recent years plants have been screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Salmonella paratyphi, Escherichia coli, Shigella dysentriae* and *Candida albicans*. The selection of medicinal plant was based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et al.,* 1999). The recent development in the phytopharmacology is development of multicombinational drug against multidrug resistant bacteria. This has been possible due to interaction among plant extracts (Phytocompounds) and with other chemotherapeutic agents that may be synergistic or additive in their interaction. The development of these drugs has grown a new future in the area of phytopharmacology and medical practices.

At present multi-drug therapy or combinational antibiotic therapy is in use. However its efficacy may be severely hindered against several MDR bacteria. Therefore, there is an increased request to develop novel drugs against multi drug resistant bacteria. One possible approach is to screen/unexplored Indian medicinal bioactive plant extracts for their potential to be used against multi drug resistant bacteria.

Considering the vast potential of Indian medicinal plants as an anti-infective agent, we have selected 15 plants on the basis of their traditional uses, ethanopharmocoloical data and local availability. The present screening programme has been planned to identify most effective plants with broad spectrum activity against drug resistant microbial pathogens and to assess synergy with antibiotics *in vitro.*

Material and Methods

Plants material

The authentic plant material was obtained from the Himalaya Drug Company, Dehradun and some are collected directly in the vicinity of Aligarh University campus. The identification of the samples was further confirmed by the plant taxonomist in the Department of Botany, Aligarh Muslim University, Aligarh. The voucher specimen has been deposited in the Department of Agricultural Microbiology, Faculty of Agricultural Sciences, AMU, Aligarh as shown in Table- 2.

Drug resistant and sensitive bacterial strains used in the screening programme

The Standard strains were obtained from different National and International Culture Collection Centers/ Collection of individual scientist and clinical isolates were collected from Department of Microbiology, J N Medical College, AMU, Aligarh. Multidrug resistant bacteria include the strains of *Shigella, Salmonella typhi*, *Staphylococci* including methicillin resistant *Staphylococcus aureus* (MRSA), *Psuedomonas aeruginosa* and R-plasmid harbouring strains of *E. coli.* MRSA and

some other Gram positive and Gram negative bacteria were also used in our laboratory. The details of the test strains and their relevant characteristics are mentioned in Table- 1.

Chemicals and Antibiotics

All the antibiotic discs were purchased from Hi-Media Lab Pvt Ltd, Mumbai, India. The indicator dye p-iodonitro tetrazolium violet were purchased from Sigma Chemical Co., USA. MMS and Sodium azide were purchased from Sisco Reseach Laboratory, India. All the other media/chemicals used were of analytical grade.

Bacterial cultures

Bacterial isolates were obtained from different sources (Table-1) and were subjected to antibiotic sensitivity by disc diffusion method (Bauer et al., 1966).

ß-lactamase production

The method described earlier (Ahmad et al., 2008) was used for detection of proudtcion of ß-lactamase.

Culture Media and Inoculum preparation

Nutrient broth/ Agar and Muller–Hinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 °C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 –10^6 CFU/ml.

Preparation of plant extracts and its fractionation

Plant extract was prepared as described earlier (Ahmad and Beg 2001) with a little modification. 800 gram of dry plant powder was soaked in 2.5 liter of 70% ethanol for 8–10 days and stirred after every 10 hrs using a sterilised glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). This alcoholic filtrate was concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO, to have a stock solution of 100 mg/ml concentration.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Beg 2001) was used. 0.1 ml of 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 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

Minimum inhibitory concentration of plant extracts

Minimum inhibitory concentration of plant extracts against test bacterial strains was determined by tube broth dilution method, using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth (Eloff 1998). 2 ml of the plant extract was mixed with 2 ml of Muller-Hinton broth (Hi-Media Ltd., Mumbai, India) and serially diluted into the next tube and so on. 2 ml of an actively growing culture of different test strains was added before incubating for over night, at 37 °C. After examining turbidity visually, 0.8 ml of 0.02 mg/ml indicator dye (p-iodonitro tetrazolium violet) was added to each tube and incubated at 37 °C. The tubes were examined for the colour development after 30 min. Absence of growth was also confirmed by spreading 0.1 ml of broth from such test tube on normal nutrient agar plate.

Synergistic interaction of plant extracts with antibiotics

Synergistic interaction between antibiotics like ampicillin, tetracycline and chloramphenicol with crude plant extracts was studied by agar well diffusion method. For determining the synergistic effects of plant extract with antibiotic, the wells were punched at a predetermined distances so that their inhibitory circles touch each other only tangentially without influencing each other as recommended by Ahmad et al. (2008). The wells were inoculated with plant extract and antibiotic separately. Plates were then incubated at 37 °C, for 18 hrs. Enlargement of inhibition zones indicates a positive interaction (synergism).

Phytochemical analysis of plant extracts

Major phytocompounds in the crude extracts of plants, were detected by standard colour tests and thin layer chromatography, as described elsewhere (Ahmad and Beg 2001).

Results and Discussion

Antimicrobial activity of plant extracts against drug resistance pathogenic bacteria

Multiple drug resistance in pathogenic bacteria has emerged as important problem in many countries of the world. There are now increasing case reports documenting the development of clinical resistance to newer and broad spectrum antibacterial drugs like fluroquinolone (norfloxacin, ciprofloxacin, oflaxacin etc.) in many pathogenic bacteria. In the present study, clinical isolates of *S. aureus P. aeruginosa, Shigella spp. E. coli, Citrobacter spp., B. subtilis* and *Candida albicans* were used. These microbial strains are found to be resistant to one or more antibiotics, showing the common occurrence of drug resistance. These finding are in agreement with the reports of previous workers as these strains have been previously tested for their sensitivity to antibiotics (Ahmad and Arina, 2001; Aqil et al., 2005, Aqil and Ahmad, 2007; Jafri et al., 2014). Further, these test isolates of bacteria were also tested for the production of b-lactamases (Table- 1).

In the present study, 15 medicinal plants were selected on the basis of their traditional uses in treatment of different disease in India and worldwide. Only alcoholic extracts of plant material have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff,1998;Ahmad et al.,1998).

The details of collected plant materials, their ethanobotanical data and parts used have been given in the Table- 2. Antibacterial activity of crude extracts of the 15 medicinal plants against Gram positive bacteria (7 distinct isolates of *S. aureus* and *B. subtilis*) and Gram- negative bacteria (*E.coli*, *P. aeruginosa, Citrobacter* and *Shigella spp.*) and a yeast (*C. albicans*) is presented in Table- 3 and 4. Activity of ethanolic curde extracts against Gram positive bacteria showed broad spectrum by *A. barbadensis*, *C. copticum*, *C. juncea, C. sinensis, H. spicatum, P. guajava, S. aromaticum* and *Z. officinale* (Table- 3). On the other hand broad spectrum activity against Gram negative MDR bacteria was exhibited by only *C. copticum*, *C. spicatum, C. juncea, S. aromaticum* and *T. foenum graecumas* as evidenced from their activity against more than 3 test bacteria with fair size of zone of inhibition (Table- 4). Most potential plant extract was *S. aromaticum, C. copticum* and *C. juncea*. Other plant extracts have also demonstrated strong antibacterial activity against one or more bacteria. Our findings are correlated with reports of earlier workers on *S. aromaticum*, *T. foenum graecum, P. cubeba, C. longa* & *C. sinesnsis* (Ahmad et al., 1999; Lacobellis et al., 2005). While activity of *C. copticum, C. juncea, H. spicatum, A. barbadensis* against MDR bacteria are probably reported for the first time. Similarly, anticandidal activity of these 15 plant extracts demonstrated that 10 plants could exhibit varying level of activity (Table- 4). Highest activity in terms of radius of zone of inhibition was recorded in *C. copticum* followed by *C. juncea and S. aromaticum*. Five plants could not show any anticandidal activity. Similar findings have been reported by Mehamood et al., 1999 and few other workers (Aqil and Ahmad, 2007). Over all sensitivity of MDR bacteria against plant extracts showed that *P. aeruginosa* strain is more sensitive followed by *S. aureus, P. aeruginosa, B. subtilis, Shigella spp.* and *E. coli*.

On the basis of broad spectrum activity of plant extracts four less commonly studied plant extracts were evaluated for their potency in terms of minimum inhibitory concentration against a variety of MDR bacteria as shown in Table- 5. MIC values of *C. copticum* varied greatly from 0.51 mg/ml to 4.62 mg/ml against test bacteria. Similarly, MIC ranged from 2.26 mg/ml to 9.49 mg/ml (*C. juncea*), 1.70 to 6.25 mg/ml (*H. spictaum*) and 1.23 to 6.60 mg/ml (*Z. officinale*). Variation in MIC values might be due to difference in cell well composition and intrinsic tolerance of the test isolates, nature and composition of phytoconstituents. Phytochemcial analysis of active plant extracts of six plants was made for the presence of major phytocompounds like alkaloids, flavonoides, glycoside, phenols & tannins as depicted in Table- 6. The presence of one or more major phytoconstituents was detected in the crude extracts by colour test and or by TLC (Fig.1).The differences in their phytocompounds might be responsible for varied activity & MIC values. These observations were supported by many workers (Nakamura et al., 2002; Shanab et al., 2004). Thus our antimicrobial screening results also justify the traditional uses of these plants in ailments and localized skin infections caused by *S.aureus, E.coli, Shigella spp., P.aeruginosa,* and *Candida albicans*.

Synergism in plant extracts

In the traditional systems of medicine (Ayurveda and Unani-Tibbiya) formulation of herbal drugs are prepared as a mixture of many crude extracts in different preparations. It is commonly believed that various active phytoconstituents of plant extracts posses additive or synergistic activity. Therefore, 14 plant extracts were selected on the basis of their antimicrobial activity against *S. aureus* and were tested in different combinations by agar well diffusion method (Table- 7 and 8). Significant activity was detected in different combination such as (1) *C. sinesis and H.spicatum* (2) *C. sinensis and P. guajava* (3) *C. sinensis and C. longa* (4) *C. copticum and S. aromaticum* (5) *C. copticum and M. arvensis* (6) C. *copticum and Z. officinale* (7) *H. spicatum and P. guajava* (8) *C. copticum and C. sinensis* (9) *P .zeylanica and H. indicus* (10) *C. copticum and C. juncea*. The synergism in some of the above interaction is shown in plate- 1 and 2.

This preliminary investigation suggested that it would be wise to evaluate the possible additive, synergistic or antagonistic interaction of crude plant extracts in different combinations to obtain enhanced activity of herbal preparations, although, it will also require an additional data on *in vivo* studies.

Multiple antibiotic therapy is now considered an effective way to control infectious diseases caused by drug resistant bacteria. Phytocompounds which may have strong activity against antibiotic resistant bacteria is expected to give strong synergistic and additive effect with antibiotics. Considering this known fact, we have tried to see the possible synergistic effect between plant extracts *C. copticum* and antibiotics. *C. copticum* extract showed synergistic interaction with tetracycline, chloramphenicol, ampicillin and gentamycin against mutlidrug resistant *S. aureus* (MRSA) strain. The above findings show that synergistic interactions are specific and the possible reason may be found in the interaction of different phytoconstituents with antibiotics. This result agrees with the observation of synergistic interactions of medicinal plants with chloramphenicol as reported by Lee (1998) and Aqil et al., (2005).

Conclusion

This preliminary investigation indicated that potential plant extracts showing broad spectrum antimicrobial activity and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of various plants may also be exploited in preparation of herbal formulation of improved efficacy and quality.

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Table-1 Antibiotics resistant pattern and β-lactamase production by test strains

Name of bacteria	Strains code	β-lactamase hydrolyzing β-lactam antibiotics		Resistant pattern of used strains against antibiotics
		Ampicillin	Benzyl penicillin	
Staphylococcus aureus	SA-03	+	+	Cx, M, A. Pn, Cf, Do, Sm, Na
Staphylococcus aureus	SA-08	–	–	Cx, M, A, Pn, Cf, Sm,
Staphylococcus aureus	SA-11	+	+	Pn, Am, M, S, T, Do, Na, Cu,
Staphylococcus aureus	SA-21	+	+	Cx, M, A, Pn, Cf, Do, Sm,
Staphylococcus aureus	SA-22	+	+	Sensitive to all drugs
Staphylococcus aureus	SA-28	+	+	Pn, Am, Cx, Cf, M, Pc, Kt, T, S,
Staphylococcus aureus	SA-29	+	+	Cx, M, A, P,
E.coli	UP-2556	–	–	Pn, A, Cx, Do,
E.coli	EC-14	+	+	Pn, A, Cx, M, Ce, Cfx, Cep, Cu,
E.coli	EC-20	+	+	Pn, A, Cx, M, Ce, Cfx, Cu, Va, T, E,
Citrobacter sp	SM-06	+	+	Pn, A, Cx, M, Co, T, C, Do, Nx, Nf, Na, Cu
Shigellasp.	SM-07	+	+	Pn, Cx, M, Co, Cf, T Do C, Na
Shigellasp.	SM-08	+	+	Pn, A, Cx, Co, Ce, Cf, Cfxx, Na
Citrobacter sp	EN-06	+	+	Pn, Cx, M, T, C, Do, Nx, Na, E,
P. aeruginosa	*P.aeruginosa*	NT	NT	A, C, T, Na, Co, Cx, Am, M
B. subtilis	*BS*	–	–	Sensitive

Pn, Penicillin, A, Ampicillin; Cx, Cloxacillin, Ce, Cephotaxime; Cu, Cefuroxime; Cfx, Cefixime, Cefpodoxime; M, Methicillin; Va, Vancomycin; Nf, Nitrofurantoin, Nx, Norfloxacin, Nv, Novobiocin Co, Co-trimoxazole; Na, Nalidixic acid; T, Tetracyline; C, Chloramphenicol; Do, Doxycycline; E, Erthromycin.

Table 2 Ethanobotanical data and traditional uses of medicinal plants.

S. No.	Scientific Name (Family) V-Sp.-No.	Vernacular name	Part Used	Site of Collection	Known Phytocompounds	Traditional Uses
1	*Aloe barbadensis* Mill (Liliaceae) IOA-66/05	Ghee kavar	Leaves	Cultivated in R.A.K. Institute AMU Aligarh	Aloin , Iso-barbaloin, Emodin, Resin, Anthra quinone, Oxidase, Catalase, Lignan, Salicylic acid, Saponins, Sterols and Triterpenoids (Wendell and Combert; 2004)	Used in wound healing, treatment of burnt skin, Protection of skin from radiation and laxative (Wendell and Combert, 2004).
2	*Bombax cieba* L (Bombaceae) IOA-64/05	Semal	Flowers	Locally purchased	lup-20 (29) en-3b-ol, 2-hexyl-7, 8-dimethyl-1, 4-naphthaquinone (Ansari, 2004)	Used as Aphrodisiac, Laxative, anti- hemorrhagic and diarrhea (Oudhia, 2003).
3	*Carum copticum* Benth. & Hook. (Umbelliferae) IOA-62/05	Ajwain	Seeds	-do –	Essential oil, thymol (Chopra *et al.*, 1992).	Used in stomach, curmin, antiseptic, tonic, diarrhea, flatulence and cholera. (Chopra *et al.*, 1992)
4	*Camellia sinesis L.* IOA-62/05	Tea	leaves	-do-	Caffeine, tannins, catechin, theflavin (khare, 2007)	Stimulant, diuretic, astringent (khare, 2007)
5	*Crotalaria juncea L.* / Fabiaceae / IOA-63/05	Sana	Leaves	-do-	Juneceine, senecionins, seneciphylline (Morris, 1999)	Used in purification of blood, in impetigo, Psoriasis, Poisnous to livestock (Chopra *et al*, 1992). Also used as Antitumor hypotensive (Morris, 1999).
6	*Curcuma longa* L. (Zingiberaceae) IOA- 68/05	Haldi	Rhizomes	-do-	Curcumin, Alkaloids, Zingiberine, Ketone, Alcohol (Chopra *et al.*, 1992).	Used in Indian cuisine, mustard and curry powder providing colour and flavour. It is used as antitumor, anti-inflammatory, antioxidant, and anti-infectious activities (Chopra *et al*, 1992).
7	*Hedychium spicatum* Ham. ex smith. (Zingiberaceae) IOA-50/05	Kachri	Fruits	-do-	Essential oils, methyl-paracumarin, acetate, cinnamic ethyl-acetate (Harborne and Baxter, 1995).	Good in liver complaints, vomiting, diarrhea, inflammation and pain in snake bites, as tonic stomachic (Harborne and Baxter, 1995).
8	*Mentha arvensis* L (Labiatae) IOA-67/05	Peppermint	Leaves	CIMAP, Lucknow.	E.oil-d-carvone, carene,de-Sylvestrene & citronellol (Chopra *et al.*, 1992).	Used in fragrance, in get rid from bed breath, treatment of tonsillitis, Coryza and cough Oudhia, (2001).
9	*Piper cubeba* L. (Pipperaceae) IOA-65/05	Kabab chini	Seeds	Locally purchased	E.oils, cubebin (Chopra *et al.*, 1992).	Used in genito-urinary diseases like cystitis, gonorrhea (Chopra *et al.*, 1992).
10	*Piper nigrum* L (Piperaceae) HDCO-85/05	Gol mirch	Seeds	-do-	Alkaloids, Piperin, piperidine, E. oils (Chopra *et al*, 1992)	Used in weakness following fever, vertigo, coma, as stomach, in flatulence and in arthritic diseases (Chopra *et al.*, 1992).
11	*Psidium guajava* L (Rosaceae) IOA-23/05	Guava	Leaves	AMU campus Aligarh.	Astrin, Euginol (Chopra *et al.*, 1992) Flavonoids and Tannis (Nakamura *et al.*, 2002).	Used in ulcers, wounds cholera & diarrhea (Chopra *et al.*, 1992). Leukorrhia, and skin diseases (Nakamura *et al.*, 2002).
12	*Raphanus sativus L.* / Brassicaceae/ IOA-24/05	Muli	Leaves	-do-	Glucaldehyde, Enzymes and methylmercaptane (Chopra *et al*, 1992).	Used for urinary complaints, piles and gastrodynic pains. (Chopra *et al*, 1992).
13	*Syzygium aromaticum* L. (Myrtaceae) IOA-26/05	Clove oil	Buds	CIMAP, Lucknow.	E.oil-Euginol (Analyst; 1909). Vanilline (Harborne and Baxter, 1995).	Used in baked goods, sauces & as antidiabitic agents. (Chopra *et al.*, 1992).

14	*Trigonella foenum graecum* L. (Leguminosae) IOA-61/05	Methi	Seeds	Locally purchased	Alkaloids Trigonellin and cholline, E.oils, Saponins, Nicotinic acid (Chopra *et al*, 1992). Galactomannan and saponin (Ammar *et al.*, 1999).	Used in small pox, dysentry and for cooling (Chopra *et al.*, 1992). Ant diabetic and hypocholestrolemic (Ammar *et al.*, 1999).
15	*Zingiber officinale* Rosc. (Zingiberaceae) HDCO-01/05	Ginger	Rhizomes	-do-	Potassium-oxalate, camphene, β phellandrene, Zingiberene, cineol, citral, Borneol, E.oil (Chopra *et al.*, 1992).	Traditionally used in Asian and Indian dishes. Exhibited good antioxidant activity (Chopra *et al.*, 1992), cough, cold, fever, muscles aches and nausea. Also used as contraceptive and fertility agent (Ficker *et al.*, 2003).

Table-3 Antibacterial activity of plant extracts against Gram positive bacteria

S.No.	Scientific Name (Family)	Percent Yield	Antimicrobial activity (Radius in mm) ± SD							
			SA-03	SA-08	SA-11	SA-21	SA-22	SA-28	SA-29	MTCC 121*
1.	*Aloe barbadensis*	1.20	8.50± 0.50	6.33 ± 0.28	11.06± 0.1	6.33± 0.28	–	11.23±0.25	6.33±0.28	–
2.	*Bombex cieba*	5.40	–	–	–	–	–	6.30±0.26	–	–
3.	*Carum copticum*	6.25	–	12.2 ± 0.25	9.33± 0.41	8.5±0.24	18.2± 0.2	10.16±0.28	11.33±0.28	17.70±0.65
4.	*Crotalaria juncea*	4.00	7.40 ± 0.55	9.40 ± 0.17	8.13± 0.25	–	6.30±0.26	8.46± 0.41	8.23±0.25	9.20±0.15
5.	*Curcuma longa*	4.20	–	–	7.06± 0.11	–	–	–	6.23±0.25	8.16±0.50
6.	*Camellia sinesis*	10.20	9.20±0.12	8.23 ± 0.25	10.23±0.25	–	8.33±0.28	6.33 ± 0.28	8.33±0.28	–
7.	*Hedychium spicatum*	3.60	9.20± 0.12	6.23 ± 0.25	11.33±0.28	–	–	6.23 ± 0.25	7.4±0.58	6.26±0.25
8.	*Mentha arvensis*		7.06± 0.11	–	–	5.23±0.25	–	7.13±0.11	8.23±0.25	–
9.	*Piper cubeba*	7.17	–	–	7.16± 0.28	–	–	7.26± 0.25	–	8.23±025
10.	*P. guajava*	5.25	8.66± 0.50	8.33± 0.28	6.66± 0.28	–	–	–	6.33±0.28	–
11.	*Piper nigrum*	5.89	–	–	–	–	7.26±0.25	–	–	–
12.	*Racinus sativus*	5.25	–	8.33 ± 0.28	–	–	–	–	–	–
13.	*Sysygium aromaticum*		8.64± 0.55	7.33± 0.41	8.23± 0.25	7.26±0.25	–	11.23±0.25	7.26± 0.25	–
14.	*Trigonella foenum graecum*	2.94	–	8.23± 0.41	–	–	–	–	7.33±0.41	–
15.	*Zingiber officianle*	4.32	–	8.23 ± 0.28	9.16±0.28	–	9.13±0.11	6.26±0.25	–	–

* MTCC 121, *Bacillus subtilis,* SD – Standard deviation

Table-4 Antibacterial activity of plant extracts against Gram negative bacteria

S. No.	Scientific Name (Family)	Antimicrobial activity (Zone in mm) ± SD*							
		SM-06	SM-07	SM-08	UP 2566	EC-14	EC-20	P	CA
1	*Aloe barbadensis*	–	10.1±0.2	11.2±0.2	–	6.3±0.2	–	–	–
2	*Bombex cieba*	–	–	–	–	–	–	–	–
3	*Carum copticum*	12.1± 0.6	15.2±0.4	9.2± 0.1	13.0 ±0.4	8.2± 0.2	8.2± 0.2	6.4±0.2	18.3± 0.1
4	*Crotalaria juncea*	–	9.2± 0.1	10.1±0.2	–	13.0± 0.4	–	6.4±0.2	10.1±0.2
5	*Curcuma longa*	–	–	–	–	8.2± 0.2	–	6.3± 0.2	7.1±0.1
6	*Camellia sinesis*	–	10.2±0.2	9.3±0.3	–	–	–	–	6.2±0.2
7	*Hedychium spicatum*	6.2±0.2	8.5±0.3	7.0±0.2	7.5±0.2	8.8±0.4	8.5±0.5	6.4±0.2	8.2±0.2
8	*Mentha arvensis*	5.2±0.2	–	6.3±0.2	5.2±0.2	–	10.1±0.2	–	5.2±0.2
9	*Piper cubeba*	–	6.3±0.4	–	–	8.4±0.2	7.5±0.5	6.4± 0.2	8.2±0.2
10	*P. guajava*	5.2±0.3	–	–	6.4±0.5	7.2±0.2	7.5±0.5	–	–
11	*Piper nigrum*	–	–	–	–	–	–	–	7.2±0.2
12	*Racinus sativus*	–	–	–	–	–	–	6.4±0.2	–
13	*Sysygium aromaticum*	8.2± 0.2	19.1±0.4	8.2±0.2	7.3±0.4	8.2±0.2	8.2± 0.2	5.2± 0.2	8.6±0.5
14	*Trigonella foenum graecum*	–	13.2±0.1	12.4±0.4	13.8±0.4	11.6±0.4	12.3±0.8	–	–
15	*Zingiber officianle*	–	–	–	6.2±0.3	8.2±0.2	7.5±0.2	–	8.2±0.4

*SD – Standard deviation

- = No activity.

Table-No. 5 Activity profile of crude plant extracts in terms of Minimum inhibitory concentration (MIC)

S. No.	Plant Extract	Yield in mg/100 gm of dry powder	Minimum inhibitory concentration against test microorganisms (mg/ml)															
			SA						B	C		S		EC				
			SA-03	SA-08	SA-11	SA-21	SA-28	SA-29	MTCC	EN-06	SM-06	SM-07	SM-08	EC-M	EC-14	EC-20	P	CA
1	*C. copticum*	6.25	4.5	4.62	4.62	0.51	1.38	4.16	4.16	1.38	0.51	0.51	0.51	4.16	2.08	2.08	NT	1.38
2	*C. juncea*	4.0	8.54	2.84	2.84	NT	2.84	2.84	9.49	NT	NT	4.26	2.84	NT	4.26	NT	2.84	3.70
3	*H. spicatun*	3.6	4.21	4.21	3.33	NT	3.70	6.25	6.25	3.33	3.33	NT	1.70	1.70	NT	3.33	6.25	4.26
4	*Z. officinale*	4.32	3.33	3.33	3.33	3.33	3.0	6.66	6.66	NT	NT	NT	1.70	NT	1.70	NT	NT	1.23

NT - Not tested.

Organisms key : SA – *Staphylococcus aureus*, B – *Bacillus subtilis*, C - *Citrobacter* spp., S – *Shigella* spp., EC - *E.coli*, P – *Pseudomonas aeruginosa*, CA- *Candida albicans* .

Table-6 Phytochemical analysis of active plant extracts for major bioactive compounds

S. no.	Plant name	Part used	Phytocompounds detected					
			Alkaloids	Flavonoids	Glycosides	Phenols	Tannins Epi/ gallo	Condensed salts
1.	*Aloe barbadensis*	Leaves	+	–	–	+	+	–
2.	*Carum copticum*	Seeds	+	+	+	+	+	+
3.	*Crotalaria juncea*	Leaves	–	–	–	–	–	–
4.	*Hedychicum spicatum*	Fruit	+	+	+	–	–	–
5.	*Psidium guajava*	Leaves	+	+	–	+	+	–
6	*Zingiber officinale*	Rhizomes	+	–	+	–	–	–

Table 7 Synergistic interaction among plant extract

Strains Used	Plants Extract (A)	r_A (in mm)	Plant extract (B)	r_B (in mm)	Combined radius (r_A+r_A) in mm	Enlargement of Zone-size (in mm)	Synergism
S. aureus (SA-03)	*C. sinensis* *Z. officinale* *C. longa* *C. sinensis* *C. copticum* *C. copticum*	9.2 ± 1.1 7.9 ± 1.5 6.8 ± 0.4 17.1± 0.4 10.3 ± 1.5 10.3 ± 1.5	*Z. officinale* *C. sanctum* *P. guajava* *C. longa* *S. aromaticum* *M. arvensis*	7.9 ± 1.5 6.8 ± 0.4 8.3 ± 0.2 14.0 ± 0.7 8.2 ± 1.2 7.01 ± 0.60	17.1 ± 0.8 14.7 ± 1.8 15.2 ± 0.5 35.2 ± 0.4 24.2 ± 0.4 21.4 ± 0.1	– – – 4 6 4	– –. – + + +
SA-08	*C. copticum* *C. sinensis* *H. spicatum* *H. spicatum* *C. copticum* *C. copticum* *C. copticum*	16.0 ± 0.7 8.2 ± 1.2 9.0 ± 0.4 9.0 ± 0.4 12.1 ± 0.6 10.3 ± 1.6 10.3 ± 1.6	*C. sinensis* *Z. officianle* *R. sativus* *P.guajava* *Z. officinale* *S. aromaticum* *M. arvensis*	8.2 ± 1.2 7 ± 0.5 8.4 ± 0.3 9.3 ± 0.1 7.1 ± 0.8 7.1 ± 0.9 –	27.1± 0.8 15.2 ± 0.6 17.7 ± 0.6 22.5 ± 0.6 24.3 ± 0.9 23.6 ± 1.1 17.1 ± 0.8	3 – – 4 5 6 –	+ – – + + + –
SA-11	*P. guajava* *P. guajava* *H. spicatum* *C. spicatum* *C. copticum*	10.3 ± 0.4 10.3 ± 0.4 9.0 ± 0.8 9.3 ± 0.4 9.3 ± 0.4	*C. sinensis* *H. spicatum* *C. sinensis* *S. aromaticum* *M. arvensis*	7.3 ± 1.7 9 ± 0.8 7.2 ± 1.7 8.6 ± 1.0 –	17.1 ± 0.8 23.0 ± 0.8 19.5 ± 0.4 25.1 ± 0.3 –	– 4 3 8 –	– + + + –
SA-21	*Plumbago zeylanica* *Punica granatum*	15.2 ± 0.5 8.2 ± 0.9	*H. indicus* *H. antidysenterica*	10.3 ± 1.8 10.2 ± 1.8	32.1 ± 0.8 18.3 ± 1.4	7 –	+ –
SA-22	*Plumbago zeylanica* *Punica granatum* *Plumbago zeylanica* *Hemidesmus indicus*	15.2± 0.4 12.1± 0.6 14.6 ± 1.0 14.7± 1.7	*H. indicus* *H. antidysenterica* *Acorus calamus* *H antidysenterica*	14.6± 1.9 8.2 ± 1.0 15.2± 0.2 8.3 ± 1.0	33.2 ± 0.3 20.4 ± 0.8 32.9 ± 0.4 29.0± 0.6	4 - 3 7	+ - + +
SA-28	*C. sinensis* *P. guajava* *Z. officinale* *H. spicatum* *H. spicatum* *C. copticum* *C. copticum*	6.2 ± 0.2 6.6 ± 0.2 6.73 – 5.4 ± 0.5 10.4 ± 0.3 10.4 ± 0.5	*P. guajava* *Z. officianale* *C. longa* *Z. officinale* *P. cubeba* *S. aromaticum* *M. arvensis*	6.6 ± 0.2 6.7 ± 0.3 – 7.1 ± .02 7.1 ± 0.2 11.3 ± 0.3 7.4 ± 0.3	12.6 ± 0.7 13.0 ± 0.3 – 12.1 ± 0.230 12.1 ± 0.230 – –	– – – – – – –	– – – – – – –
SA-29	*C. copticum* *C. sinensis* *C. sinensis* *Z. officinale* *C. juncea* *C. copticum* *C. copticum*	11.1 ± 0.5 14.6 ± 1.5 – 6.7± 0.5 8.3± 0.1 7.4 ± 0.4 7.4 ± 0.4	*C.juncea* *P. guajava* *Z. officinale* *C. juncea* *H. longa* *S. aromaticum* *M. arvensis*	7.4 ± 0.4 9.0 ± 0.8 6.1 ± 0.5 8.0± 0.1 7.4 ± 0.2 7.4 ± 0.1 8.0 ± 0.6	21.2 ± 0.3 28.3 ± 0.1 – 14.7 ± 0.4 15.7 ± 0.1 14.8 ± 0.5 15.4 ± 1.0	3 5 – – – – –	+ + – – – – –
Shigella spp. (SM-07)	*C. copticum* *C. sinensis* *C. copticum* *C. copticum*	13.2 ± 0.2 8.0 ± 0.4 8.3 ± 0.2 9.0 ± 0.8	*C. sinensis* *C. juncea* *S. aromaticum* *M. arvensis*	8 ± 0.4 – 19.5 ± 0.2 –	8.0 ± 0.4 – 19.5 ± 0.2 –	– – – –	– – – –
SM-08	*C. copticum* *C. copticum* *C. copticum* *C. copticum* *C. copticum*	9.0 ± 0.8 9.0 ± 0.8 9.0 ± 0.8 6.3 ± 0.3 6.5 ± 0.2	*C. sinensis* *C. juncea* *C. longa* *S. aromaticum* *M. arvensis*	6.3 ± 0.2 7.4 ± 0.4 – 8.0 ± 0.7 6.5 ± 0.2	15.6 ± 1.2 16.4 ± 0.9 – 14.3 ± 0.9 12.5 ± 0.1	– – – – –	– – – – –

Table 8 Synergistic interaction between Plant extract and antibiotics

Strains Used	Plant extract (P)	r_P (in mm)	Antibiotic (A)	r_A (in mm)	Combined radius ($r_P + r_A$) in mm	Enlargement of Zone-size (in mm)	Synergism
SA-03	*C. copticum*	6.0 ± 0.4	C	16.8 ± 0.7	25.1 ± 0.4	3	+
	C. copticum	6.0 ± 0.4	T	17.1 ± 0.8	23.4 ± 0.5	–	–
	C. copticum	6.0 ± 0.4	Gm	14.0 ± 0.7	24.5 ± 0.5	4	+
	C. copticum	6.0 ± 0.4	Am	14.0 ± 0.2	23.0 ± 0.6	3	+
	C. copticum	6.0 ± 0.4	NA	–	–	–	–
SA-08	*C. copticum*	7.0 ± 0.8	C	11.0 ± 5	23.1 ± 0.5	5	+
	C. copticum	7.0 ± 0.8	T	12.0 ± 0.5	19.1 ± 0.3	–	–
	C. copticum	7.0 ± 0.8	Gm	12.0 ± 0.5	22.1 ± 0.4	3	+
	C. copticum	7.0 ± 0.8	Am	10.1 ± 1.0	17.0 ± 0.5	–	–
	C. copticum	7.0 ± 0.8	Na	10.0 ± 0.3	17.8 ± 1.5	–	–
	C. copticum	7.0 ± 0.8	Cf	16.2 ± 0.6	23.8 ± 0.6	–	–
SM-07	*C. copticum*	5.5 ± 0.2	C	11.3± 0.17	16.3 ± 0.4	–	–
	C. copticum	5.5 ± 0.2	T	5.1± 0.1	10.0 ± 0.3	–	–
	C. copticum	5.5 ± 0.2	Am	8.4 ± 0.2	13.6 ± 0.3	–	–
	C. copticum	5.5 ± 0.2	Na	–	–	–	–
	C. copticum	5.5 ± 0.2	Cf	8.2 ± 0.5	13.4 ± 0.7	–	–
EC-20	*C. copticum*	8.3 ± 0.3	C	14.3± 0.2	22.6 ± 0.5	–	–
	C. copticum	8.3 ± 0.3	T	16.3 ± 0.1	24.4 ± 0.2	–	–
	C. copticum	8.3 ± 0.3	Gm	15.2 ± 0.3	27.3 ± 0.3	–	–
	C. copticum	8.3 ± 0.3	Am	12.1 ± 0.1	20.4 ± 0.2	–	–
	C. copticum	8.3 ± 0.3	Na	11.4 ± 0.1	19.6 ± 0.2	–	–
	C. copticum	8.3 ± 0.3	Cf	20.4 ± 0.1	28.4 ± 0.2		

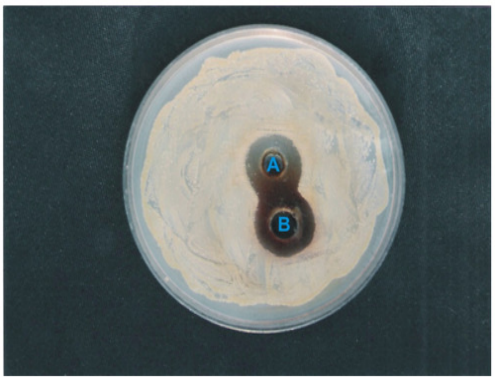


Plate 1 Synergistic interaction between plant extracts against *S. aureus* (SA-08)
(A) *P. guajava*
(B) *H. spicatum*

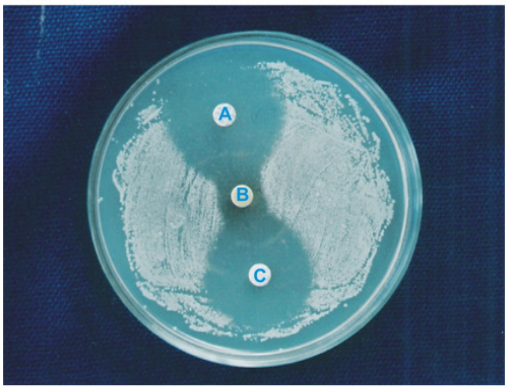


Plate 2 Synergistic interaction of plant extracts with antibiotics against *S. aureus* (SA-08)
(A) Gentamycin
(B) *C.copticum*
(C) Chloramphenicol

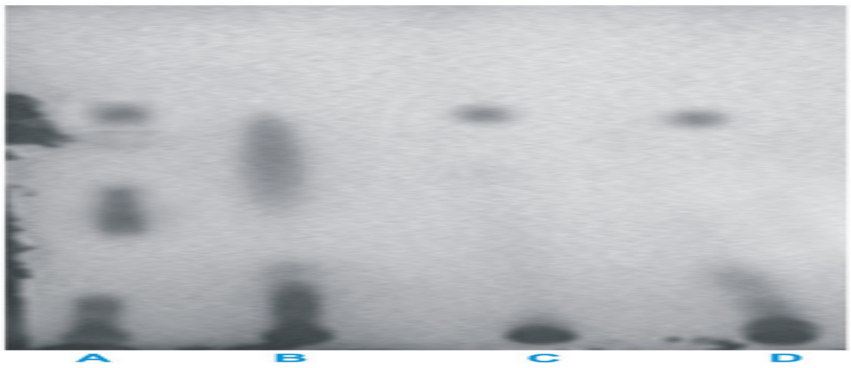


Figure 1 TLC profile for alkaloids of different plant extracts
Lane A: *A .barbadensis*; Lane B: *Z. officinale;* Lane C: *P. guajava and* Lane D: *H. spicatum*

Green Synthesis and Characterization of Zinc Oxide (ZnO) and Silver Nano particles (np) and Determination of Their Antibacterial Assay

S C Sati[1], Maneesha D Sati[1], I P Pandey[2] and Shikha Saxena

[1]Department of Chemistry, HNB Garhwal University (A Central University) Srinagar, Garhwal, Uttarakhand, India- 246174

[2]Department of Chemistry, DAV PG College, Dehradun, UK., India

Abstract- Development of reliable and eco-friendly processes for synthesis of metallic nano particles is an important step in the field of application of nanotechnology. The synthesis of nano particles has become the matter of great interest in recent times due to its advantageous properties and applications in different fields. Though physical and chemical methods are more popular for nano particle synthesis, the biogenic production is a better option due to eco-friendliness. The present abstract reports the investigation, synthesis and characterization of Zinc Oxide (ZnO) and Silver nano particles (NP), and their application on pathogenic bacteria. ZnO NP were synthesized by chemical reduction method using starch as capping agent and silver NP was prepared by green synthesis process from $AgNO_3$ solution through the extract of *Citrus sinensis* (Santra). The detail characterization of the nano particles was carried out using UV-Vis Spectroscopy,X-Ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), and Thermogravimatric (TGA) analysis. From SEM image analysis, the average particle size was found to be 90 nm and 50 nm, for ZnO and silver nano particles respectively. From the analysis of XRD pattern, UV-VIS spectroscopy and TGA, the formation of nano particles was confirmed. Antibacterial assay of synthesized ZnO and silver NP was carried out both in liquid and solid growth medium against different pathogens.

Introduction

Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. In the synthesis of metal nano particle by the reduction of the corresponding metal ion salt solutions. Nano particles are often referred to as clusters, nanospheres, nanorods and nanocups are just a few of the shapes at the small end of the size ranges from 1 to 100nm. Nano particles exhibit a number of special properties relative to bulk material and often have unique visible properties because they are small enough to confine their electrons and produce quantum effects [1]. Biosynthesis of nano particles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis [2]. A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nano particle using microorganisms and plants [3-4]. The rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and stable formation of metal nano particles has been reported. The shape and size of the nano particles synthesized using plants can be controlled and modulated by changing the pH [5]. The reduction of silver ions (Ag+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Medicinal herbs are the local heritage with global importance. Medicinal herbs have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolite in one or more parts of these plants.

Citrus sinensis belongs to the family *Rutaceae* is an evergreen tree of Garhwal Himalaya includes about 17 species distributed throughout the tropical and temperate regions .Citrus peels contain more bioactive compounds, such as phenolic acids, flavonoids, limonoids, and fibre[6]. In traditional Chinese medicine, the dried peel of the fruit is used to treat abdominal distension, to enhance digestion, and to reduce phlegm, and its various parts are used to cure cutaneous complaints, hemiplegia, snake bite, fever, loss of taste, chronic rheumatism, stomach ache, menorrhagia, spleenomegaly, edema and cardiac diseases, bronchitis and asthma [7].Experimental studies have demonstrated its analgesic, antibacterial, antimicrobial, antiviral, antiyeast antifungal, antidiarrheal, antiinflammatory, uricosuric activity, antimutagenic, antisplasmodic, antiatherogenic, antiperoxidative activity, anticarcinogenic activity, and radical scavenging activity[8].

In the present investigation, synthesis and characterization of Zinc Oxide (ZnO) and Silver nano particles (NP), and their application on pathogenic bacteria were investigated. ZnO NP were synthesized by chemical reduction method using starch as capping agent and silver NP was prepared by green synthesis process from AgNO3 solution through the extract of *Citrus sinensis* (sweet lime).

Material and Methods

Chemicals

Pure and analytical grade chemicals were used in all experiments including synthesis of ZnO and silver nanoparticles, media preparation for growth of bacterial cells. Zinc nitrate hexa -hydrate, sodium hydroxide (NaOH) starch and silver nitrate ($AgNO_3$), were purchased from Himedia laboratories Pvt. Ltd., Mumbai, India. The bacterial cultures of *E.coli, Bacillus subtilis, Psudomonas aeruginosa, Streptococcus pneumonia* were obtained from Department of Microbiology SBSPGCBS Balawala Dehradun and Antibiotics, (Ampicilin, Tetracycline, Ciprofloxacin, and Tobramycin) were purchased from Himedia, Mumbai, India. Beef extract, peptone, sodium chloride (NaCl) were purchased from Merck, India.

Glassware and apparatus

All glass wares (Conical flasks, Measuring cylinders, Beakers, Petri plates and Test tubes etc.) were purchased from borosil, India.

Synthesis of ZnO nano particles

The ZnO nano particles were prepared by wet chemical method using zinc nitrate and sodium hydroxides precursors and soluble starch as stabilizing agent. Soluble starch (0.5%) was dissolved in 500 ml of distilled water and treated in microwave oven for complete solubilization. Zinc nitrate, 14.874 g (0.1 mol), was added in the above solution. Then the solution was kept under constant stirring at room temperature using magnetic stirrer for one hour. After complete dissolution of zinc nitrate, 300ml (0.2 mol), of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 hrs after complete addition of sodium hydroxide. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged (Remi cooling centrifuge instrument, Model No-C30BL) at 10,000 × g for 10 min and the supernatant was discarded. Thus produced nano particles were washed three times using distilled water. Washing was carried out

Synthesis of silver nano particles from fruit peels extract of Citrus sinensis

to remove the byproducts and the excessive starch that were bound with the nano particles. After washing, the nano particles were dried at 80°C for overnight. During drying, complete conversion of Zn (OH)$_2$ into ZnO takes place.

Ag nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon resonance. On mixing the extract with aqueous solution of the Ag ion complex a change in the color from colorless to yellowish brown was observed. It was due to the reduction of Ag+ which indicates the formation of Ag nanoparticles shown in Figure.

Extraction

Weighing 25 g peels of *Citrus Sinensis* was thoroughly washed in distilled water, dried, cut into fine pieces and was smashed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper two or three times. The extract was stored in refrigerator for further experiments.

Synthesis

The aqueous solution of 1mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Citrus sinensis* extract was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 12 hrs at room temperature. Here the filtrate act as reducing and stabilizing agent for 1 mM of $AgNO_3$.

Characterization Techniques

UV-Vis Spectroscopy

The UV-Vis spectra of ZnO NP prepared with 0.5% concentration of soluble starch was shown in Figure (a). The absorption peak of the prepared nano ZnO was found at around 360 nm. The UV-Vis absorption spectra of the Ag NP were shown in Figure (b). Absorption spectra of Ag nano particles formed in the reaction media has absorbance maxima at 421 nm. A remarkable broadening of peak at around 350 nm to 480 nm indicates that the particles are polydispersed. It was observed that the peak was blue shifted in the absorption spectrum from 350nm to 480 nm with increasing reaction time.

The ZnO and Ag nano particles spectra obtained was followed the reported results (Vigneswaran et al., 2006 and Jain et al., 2009)

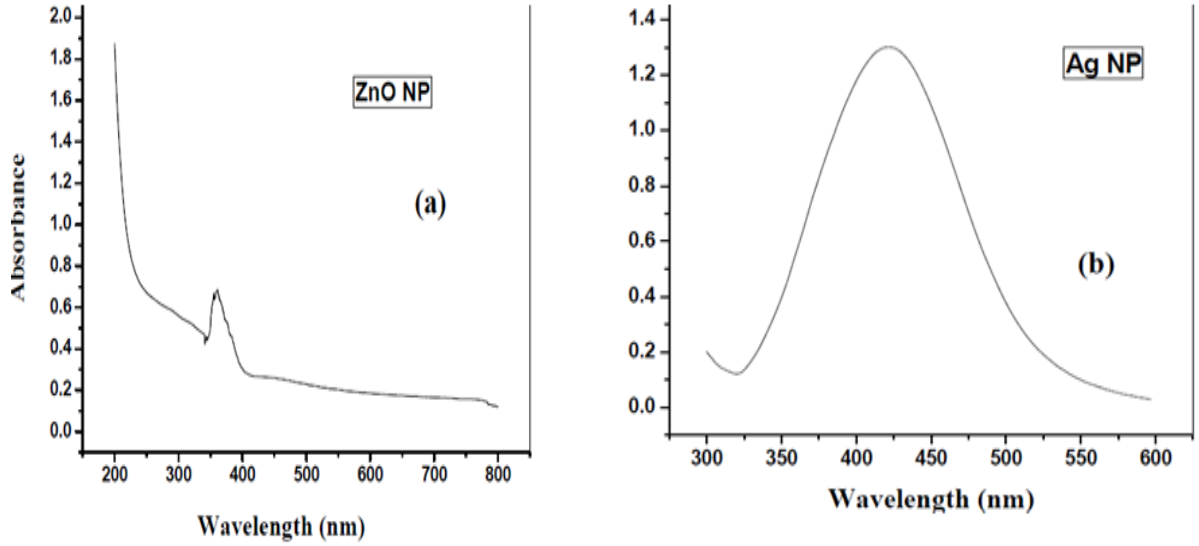
X-RAY diffraction method

The XRD pattern of bulk ZnO and nano ZnO were studied. All the peaks were hexagonal and approximately close to the reported information. Due to the crystal symmetry and related face velocities, the common crystal habit of ZnO is hexagonal in shape. Also the ZnO NP is the thermodynamically stable crystallographic phase. The width of the peaks in case of ZnO NP has increased due to the quantum size effect. The average particle size was estimated to be 42 nm using Scherer equation. The *Citrus sinensis* extract-mediated synthesized Ag nanostructure was confirmed by the characteristic peaks observed in the XRD image. All diffraction peaks correspond to the characteristic face centered cubic (FCC) silver lines. These diffraction lines observed at 2è angle 32.80, 38.20, 55.10° and

65.70 respectively, have been indexed as (111), (200), (220) and (311) respectively. XRD patterns were analyzed to determine peak intensity, position and width, full-width at half-maximum (FWHM) data was used with the Scherer formula. The typical XRD pattern revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver
nano particles. The average estimated particle size of this sample was 50 nm derived from the FWHM of peak corresponding to 111 plane with cubic and hexagonal shape.

Scanning electron microscope (SEM)

In this research work, Jeol JSM-6480 LV SEM machine were used to characterize mean particle size, morphology of nanoparticles. The ZnO powder sample and freeze dried sample of Ag NP solution was sonicated with distilled water, small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20 kV. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached



(a)UV- Vis spectra of the ZnO with 0.5% of soluble starch

Table–1. Zone of Inhibition of Antibacterial test of ZnO NP

Bioactive agent		Zone of inhibition (Diameter, cm)			
		E.coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Streptococcus pneumonia*
ZnO nanoparticle	5 mM	3.2	2.2	nil	0.2
	10 mM	4.7	4.1	nil	3.1
	15 mM	4.8	4.8	3.1	4.9
Erythromycin(10mcg/disc)		0.8	0.7	0.6	4.8
Vancomycin(10mcg/disc)		nil	nil	nil	0.6
Tobramycin(10mcg/disc)		nil	3.1	nil	3.2

Table–2. Zone of Inhibition of Antibacterial Test of Ag NP

Bioactive agent		Zone of inhibition (Diameter, cm)			
		E.coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Streptococcus pneumonia*
Ag nanoparticle	0.2 mM	2.5	3.2	3.1	3.2
	0.3 mM	3.4	4.2	3.3	3.4
	0.6 mM	4.2	4.3	4.2	3.8
Erythromycin(10mcg/disc)		nil	nil	0.6	4.1
Vancomycin(10mcg/disc)		0.8	nil	0.8	3.8

with the SEM. The EDX analysis of ZnO and Ag sample was done by the SEM (JEOLJSM 5800) machine. The EDX normally reveals the presence of phases.

The SEM image of ZnO and Silver nanoparticles synthesized by chemical reduction method and green synthesis process by using 10 % fruit extract and 1mM AgNO3 concentration was very clear. It gave a clear image of highly dense ZnO and silver nanoparticles. The SEM image showing silver nano particles synthesized using *Citrus sinensis* extract confirmed the development of silver nanostructures.

Thermo gravimetric analysis (TGA)

Thermal decomposition behavior of the gel has been studied using Netzsch (STA 449C) DSC/TG. The DSC/TG patterns were collected as a function of temperature up to 9000C under N2 atmosphere. The heating rate was 100C/min. in N2. Alpha alumina was used as reference material. TGA or thermo gravimetric analysis ZnO NP was carried out to observe characterstic weight loss with temperature. The TGA analysis of synthesized nano ZnO was studied. The synthesized nano ZnO is subjected to a heating from room temperature to highly thermal temperature of 1000°C.The initial weight loss took place at around a temperature of 180°C.The bulk ZnO did not show any weight loss when heated up to 1000°C but in case of soluble starch a weight loss at around 312°C was observed, which is matching the results reported in (Vigneswaran et al., 2006) equal to the degradation temperature of starch. These properties helped to characterize and confirm the formation of ZnO nano particles (range obtained from 90-100nm).

Antibacterial test

Antibacterial assays

ZnO and Ag nano particles bactericidal effect was studied against four different pathogenic bacteria *E.coli, Bacillus subtilis*, *Pseudomonas aeruginosa, Streptococcus pneumonia*. These nano particles were dispersed in autoclaved Millipore water by ultrasonication. Aqueous dispersion of ZnO and Ag nano particles of desired concentration was made. Disc diffusion test of ZnO and Ag nano particles was also done.

Results and Discussion

The effect of different concentration of ZnO NP like-5mM, 10mM, 15mM and 0.2mM, 0.3mM and 0.6mM of silver nanoparticles on bacteria was performed. As we increased the concentration of ZnO nanoparticles the antibacterial activity of ZnO nanoparticles increased. A clear inhibition zone treated with ZnO nanoparticles whereas the standard antibiotics like vencomycin, erythromycin, and tobramycin shows smaller zone of inhibition as compared to the nanoparticles treated discs. A clear inhibition zone treated with Ag nanoparticles whereas the standard antibiotics like vencomycin, erythromycin shows smaller zone of inhibition as compared to the nanoparticles treated discs (Table-1 and 2).

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Phytochemical Screening and Antioxidant Activity of *Andrographis paniculata* (Burm. f.) Nees by DPPH Scavenging Assay

Savita G. Aggarwal[1], Neetu Sharma[2],
[1] Department of Chemistry, Army Cadet College Wing, IMA, Dehradun, India
[2] Department of chemistry, Graphic Era University, Dehradun, India
email: goelsavishivi@gmail.com, Mobile: 9808305264

Abstract- The aim of this present study is to explore the antioxidant capacity and phytochemical analysis of the whole plant extracts of *Andrographis paniculata.* The screening of phytochemical tests revealed the presence of some active ingredients such as alkaloids, tannins, saponins, phenols, terpenoids and flavonoids. The different solvent extract were also evaluated for their total phenolic contents and antioxidant capacity by Folin Ciocalteu reagent method and Free radical scavenging capacity (DPPH assay). Butylated hydroxy toluene was used for antioxidant capacity and gallic acid for phenolic content as a standard. The EC_{50} value of Acetone extract was 25.80 µg/ ml compared to 17.75µg/ml, for BHT. The result shows that acetone extract of the whole plant of *Andrographis paniculata* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Key words- *Andrographis paniculata,* DPPH, Phytochemicals, Phenolic compounds and Antioxidant capacity.

Introduction

Plants have been used as an alternative source of medicine from ancient times before the advent of synthetic drugs. Medicinal plants have played an important role in treatment of the world health. According to an approximation of World Health Organization, nearly 80% of the population of developing countries relies on traditional medicine. Therefore, such plants should be investigated for better understanding of their therapeutic properties, safety and efficacy[1,2]. The traditional folk medicinal system uses the plant-products for the treatment of various infectious diseases. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[3,4]. Several Indian medicinal plants are known for their beneficial therapeutic effects which also might have antioxidant properties[5]. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA[6].

Andrographis paniculata(Burm. f.) Nees, one of the important medicinal plant, belongs to the family of Acanthaceae. It is plentifully found in south eastern Asia i.e., India, Sri Lanka, Pakistan and Indonesia. It is an annual herbaceous plant widely cultivated in India, China and some parts of Europe. It is found in wild throughout plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand. Andrographis paniculata, "Kalmegh" of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in north-eastern India as 'Maha-tita', literally 'king of bitters'. Mostly leaves and roots have been traditionally used over centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments or as a herbal supplement for health promotion.

The aim of the present study is the screening of the phytochemical constituents and antioxidant capacity of the extracts of Andrographis paniculata. Additionally, the inter-relationship between phenolic content and antioxidant capacity has been also carried out.

Material and Methods

Collection and authentification of plant

The plant sample *Andrographis paniculata* (Burm. f.) Nees was collected from Forest Research Institute, Dehradun. The authentification of plant sample has been certified by taxonomist. The whole plant was

dried in the shade and made into coarse powder using an electrical grinder. The powdered sample was preserved in airtight bags till further use.

Solvent extraction of plant material

The powdered material (100gms) was extracted for 24 hrs successively in Petroleum Ether, Chloroform, Acetone and Methanol by using Soxhlet apparatus. The extracts were concentrated in vacuum evaporator and kept in vacuum desiccators for complete removal of solvent and the weight of each extract was measured.

Preliminary phytochemical screening

The various solvent extracts of the plant samples were then subjected to qualitative chemical tests for the identification of various phytochemical constituents like Alkaloids, Carbohydrates, Glycosides, Proteins, Tannins, Sterols, Saponins, Amino acids etc ,which are responsible for pharmacological as well as biological activity of plant.

Detection of alkaloids

Small portion of the solvent free extract was stirred with a few drops of diluted HCl and was filtered. The filtrate may be tested for following color tests Mayer's reagent (cream colored precipitate); Hager's reagent (yellow colored precipitate and Wagner's reagent (reddish brown precipitate) to detect the presence of an alkaloid.

a) Mayer's test: Test solution with Mayer's reagent (1.36 g of mercuric chloride in 60 ml distilled water + 5.0 g of potassium iodide in 20 ml distilled water + 20 ml of distilled water) gave cream precipitate.

b) Hager's test: Test solution with Hager's reagent (saturated aqueous solution of picric acid i.e. 1.0% (v/v) solution of picric acid in hot water) gave yellow precipitate.

c) Wagner's test: Test solution with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 5 ml of water and 100 ml distilled water) gave reddish brown precipitate.

Detection of carbohydrates and glycosides

Small quantity of extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate may be subjected to Molish's Test; Selivanoff test; Barfoed's test; Fehling's test and Benedict's test to detect the presence of carbohydrate qualitatively.

a) Molish test: Test solution with few drops of Molish's reagent (10 gm napthol) in 100 ml of 95% of alcohol and 2 ml of conc. H2S04 added slowly from the side of test tubes showed a purple ring at the junction of the two liquids.

b) Selivanoff's test: An appearance of pink colour on addition of resorcinol crystals and conc. HCI in test solution, detected the presence of carbohydrates.

c) Fehling's test: Test solution neutralized by addition of sodium hydroxide solution and treated with Fehling A and B (added in equal volume) gave precipitate.

d) Barfoed's test: Test solution treated with Barfeod's reagent (12 gm of copper acetate in 200 ml distilled water and addition of 12.5 ml of 8.5 % of lactic acid solution) when boiled on water bath gave brick red precipitate.

e) Benedict's test: Test solution treated with Benedict's reagent and allowed to boil on a water bath, showed reddish brown precipitate.

Detection of sterols

Sterols were detected by performing following tests.

a) Salkowaski's test: When a few drops of conc. H_2So_4 were added to the test solution, shaken and allowed to stand, lower layer turned red, indicating the presence of sterols.

b) Liebermann – Burchard's test: The test solution was treated with few drops of acetic anhydride. When concentrated sulphuric acid is added from the sides of the test tube, it showed a brown ring at the junction of the two layers and upper layer turned green.

c) Hensen's test: Portion of dried extract were taken in 10ml of chloroform. To this few drops of conc. H_2SO_4 was added. Both the acid and chloroform layers turning red which shows the presence of sterols.

Detection of saponins

Dilute 1 ml of the test solution with distilled water to 20 ml and shake in a graduated cylinder for 15 mins. A 1 cm layer of foam indicated the presence of saponins.

Detection of phenolic and flavonoid Compounds

Small quantity of tests dissolved in water and subjected for following tests to detect the presence of phenolic compounds and tannins.

a) $FeCl_3$ solution (5%) test: Test solution treated with a few drops of ferric chloride solution, the presence of intense green colour indicates presence of phenolic compounds.

b) Vanillin HCl acid test: Test solution was treated with

vanillin reagent (19ml Vanillin in 10 ml alcohol and 10 ml concentrate HCl), presence of red color indicate presence of phenolic compounds.

c) Zinc hydrochloric acid reduction test: Test solution with zinc dust and few drops hydrochloric acid, shows magenta red color.

Detection of proteins and free amino acids

Dissolved small quantities of test solution in a few ml of water and subjected the solution to following test:

a) Millon's Test: Test solution when treated with Millions reagents (1 gm mercury in 9 ml fuming HNo_3, volume maked upto 100ml with distilled water) and heated on a water presence of red precipitate, indicates presence of amino acids.

b) Ninhydrin test: Test solution treated with Ninhydrin reagent (0.1 % *w/v* of ninhydrinin an n-butanol) presence of blue violet colour indicates presence of amino acids.

c) Biuret test: Test solution treated with 40% NaOH and dil copper sulphate solution, presence of blue colour indicates presence of amino acids.

Test for tannins

Portion of extracts were taken separately in a few ml of alcohol. To this few drops of freshly prepared FeCl3 (1.62gm in 1L of .001M HCl) was added. Development of greenish violet color shows the presence of tannins.

Free radical scavenging capacity (DPPH assay)

The hydrogen donating ability of each extract in the presence of DPPH (2, 2-diphenyl-1-picrylhydrazyl) stable radical was examined according to method developed by Blois[7] and Cao *et al*[8]. The stock solution of DPPH was prepared and was kept at 20°C. 2ml of a volume of 25 to 200 µl of the sample was then poured into an optical glass cuvette and the stock solution was added to a final volume of 4ml. After 30 min incubation at 28 to 30°C in dark, the absorbance at 517 nm using spectrophotometer (Thermofisher Scintific UV-2700) was measured against a blank of pure methanol and DPPH (2ml each). Synthetic antioxidant Butylated Hydroxy Toluene (BHT) was used as a standard. The experiment was repeated thrice. The antioxidant capacity was expressed as Ec50 value, which represents the sample concentration necessary to decrease the initial DPPH concentration by 50%.

The Ec50 value was calculated as follows-

$$\%\ DPPH\ radical - scavenging = \left[\frac{Absorance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} X\ 100\right]$$

Ec50 of the extract was determined by using SPSS software.

Determination of the total phenolic content

Total phenolic contents were determined by Folin Ciocalteu Reagent Method[9] using gallic acid as a standard. Appropriate dilutions of the samples (0.25ml) were oxidized with Folin-Ciocalteau reagent for five minutes at room temperature followed by addition of 7.5% (w/v) Sodium carbonate solution. The absorbance of the resulting blue color was measured at 740nm on UV-VIS spectrophotometer after heating at 50°C for 5.0 minutes in a water bath. Quantification was done on the basis of the standard curves of gallic acid prepared from 1ml of each of the 200,150,100 and 50 ppm gallic acid solutions assaying in the manner similar to that used for the extracts. The experiment was repeated thrice and the results were expressed in term of % gallic acid equivalents (GAE).

Results and Discussion

The result of the screening of phytochemical constituents of *Andrographis paniculata* is summarized in Table – 1.0

From the Table-1 it is clear that *Andrographis paniculata* possesses variety of phyto- chemicals. Among all the solvent extracts, the acetone and methanol extracts are rich in phyto chemical constituents. So these two solvent extract were further examined for antioxidant activity. The antioxidant capacity was determined using DPPH free radical scavenging assay. DPPH is a stable free radical which accepts electrons and becomes a stable diamagnetic molecule. The purple color or violet color of the DPPH solution changes to yellow and the absorbance at 517nm wavelength maximum decreases. The decrease in absorbance of DPPH caused by antioxidant activity is due to the reaction between the antioxidant molecule and its radical which results in the scavenging of the radical by electron donation. Acetone extract shows good antioxidant activity; so the results of acetone extract was only shown. Table-1.2 reveals that the acetone extract had DPPH radical scavenging activity which ranges from 51% to 90% at a concentration of 17ìg/ml. In acetone extract possess scavenging more than 90% compared to the BHT control. The BHT control was assayed at 100 ìg/ ml corresponding to the maximum allowable concentration for BHT additions to food stuffs[10]. The EC50 value for

Table-1.0 Phytochemical constituents of *Andrographis paniculata*

S. No.	Phytochemical Test	Petroleum ether extract	Chloroform extract	Acetone extract	Methanol extract
1.	**Test for carbohydrates**				
i	Fehling's test	(-)	(-)	(-)	(+)
ii	Molish's test	(-)	(-)	(-)	(-)
iii	Barfoed's test	(-)	(-)	(-)	(-)
iv	Benedict's test	(-)	(-)	(-)	(+)
v	Selivinoff's test	(-)	(-)	(+)	(+)
2.	**Test for proteins and amino acid**				
i	Million's test	(-)	(-)	(+)	(-)
ii	Biuret test	(-)	(-)	(+)	(-)
iii	Ninhydrin test	(-)	(-)	(+)	(-)
3.	**Test for steroids**				
i	Salkowski test	(+)	(+)	(+)	(+)
ii	Gilberman-Buchard's test	(+)	(+)	(-)	(-)
iii	Hensen's test	(-)	(-)	(+)	(+)
4.	**Test for alkaloids**				
i	Wagner's test	(-)	(-)	(+)	(+)
ii	Hager's test	(-)	(+)	(+)	(+)
iii	Mayer's test	(-)	(-)	(-)	(-)
5.	**Test for phenolic and flavonoid compounds**				
i	Vanillin-HCl test	(-)	(-)	(+)	(+)
ii	Ferric chloride test	(+)	(+)	(-)	(+)
iii	Zinc hydrochloric acid reduction test	(-)	(-)	(-)	(-)
6.	**Test for tannins**	(-)	(-)	(-)	(+)
7.	**Test for saponins**	(-)	(-)	(+)	(+)

(+)present, (-) absent

Table-1.1 Free Radical Scavenging Capacity of Acetone Extract

S. No.	Concentration of Acetone extracts (µg/ml)	Antioxidant activity (%)
1	25	54.50
2	50	61.84
3	75	66.81
4	100	76.65
5	125	83.16
6	150	75.16
7	175	80.41
8	200	85.41
9	Ec_{50}	25.80

Table-1.2 Results of Total Phenolic Content in Acetone Extract

Solvent Used	Total Phenol content (%)	Ec_{50} (%)
Acetone	0.213	25.80

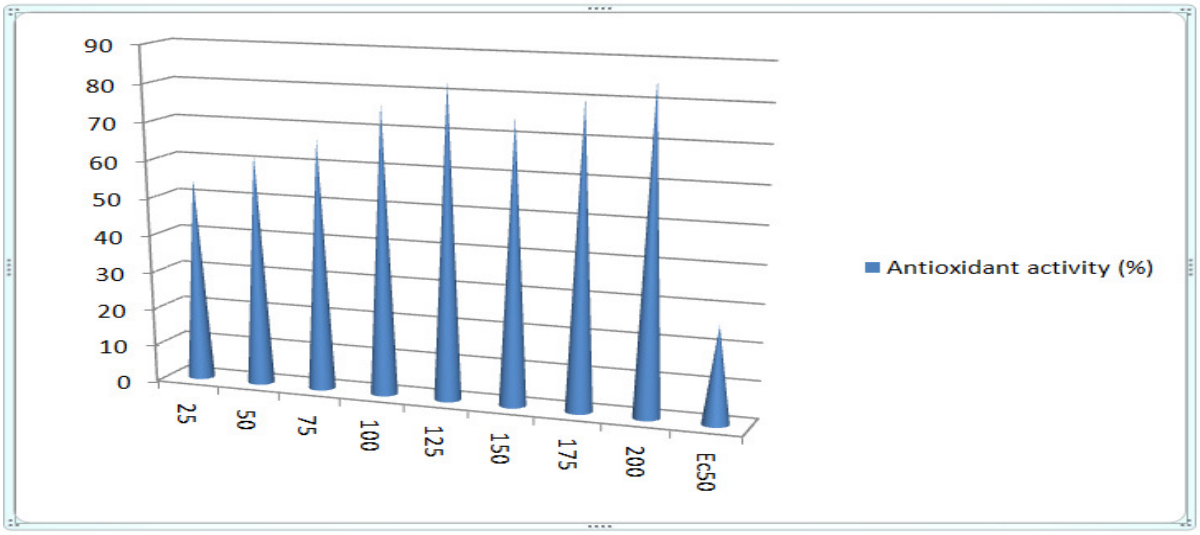

Fig. Antioxidant Activity (%) of Acetone extract at different concentration

acetone was found to be 25.80 ìg/ ml, which is higher than that of the standard value. DPPH free radical scavenging capacities of the plant extractive are attributed to their phenolic constituents. A large number of studies have been reported[11, 12] wherein the free radical scavenging capacity of the plant positively correlated with their phenolic constituents. Thus the acetone extracts was also examined for its phenolic constituents. Total phenolic constituents is determined by Folin Ciocalteu reagent method For the acetone extract the value comes around 0.24% Gallic Acid Equivalents (GAE). It was observed that the phenolic contents of acetone extracts are associated with an increased radical scavenging capacity. This effect may be due to the electron donating ability of the phenolic compounds present in the acetone extract.

Conclusion

The above data confirms that the plant *Andrographis paniculata* is rich in phyto-chemical constituents. Further study explores the in-vitro antioxidant capacity of acetone extracts with results comparable to those of the standard compounds such as Butylated Hydroxy Toluene (BHT), gallic acid. *Andrographis paniculata* can therefore be proposed as a new potential sources of natural additives for the pharmaceutical industries. The data clearly indicated that the acetone extracts of *Andrographis paniculata* possessed good antioxidant activity among all three solvent extract. However, further work needs to be conducted to isolate the components responsible for the antioxidant activities of the extracts and to identify bioactive compounds.

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Comparative Phytochemical Screening and *in-vitro* Free Radical Scavenging Activity and Anti-microbial Activity of Leaves and Rhizomes of *Acorus calamus* Linn.

Ajay S Bisht*, Monika Bisht and Divya Juyal
Himalayan Institute of Pharmacy and Research, Atak Farm, Rajawala, Dehradun, India

Abstract- The present communication attempts to evaluate the comparative *in-vitro* 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 *Acorus calamus* were 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. The free radical scavenging activity of methanolic extract of leaves and rhizomes of the plant was investigated *in-vitro*, using spectroscopic method against 1, 1-diphenyl-2- picrylhydrazyl (DPPH). The leaf extract was found to possess strong activity against DPPH as compared to rhizome. Antimicrobial activity was also performed using ethanolic extract through cold percolation method against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherischia coli and Salmonella typhi.* The extract was found to have positive results against all.

Keywords: *Acorus Calamus* Linn., ethanopharmacological, free radical scavenging, DPPH, Antimicrobial.

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 Kashmir and Sirmoor in Manipur and Naga Hills.[2] The roots and rhizomes are used medicinally since ancient times. They possess antispasmodic, carminative and anthelmintic properties and are also used for the treatment of epilepsy, mental ailments, chronic diarrhoea, 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 up and stored with dry foods[8], antihyperlipidemic activity, antidiabetic 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. Pro-oxidant conditions dominate either due to the increased generation of the free redicals or due to the excessive oxidation stress of the current life or the poor scavenging / quenching in the body due to the depletion of dietary antioxidants. Keeping in view of the above observations, in the present study, methanolic extract of leaf and rhizome of the plant were subjected to in-vitro antioxidant activity against the DPPH generated free radicals.

Material and Methods

The leaves were wildly collected from catchment of Bhimtal Lake in Uttarakhand located in North India, proclaimed as to have ethano-pharmacological importance. These were preserved in 70% ethyl alcohol for various studies.

Screening for free radical scavenging activity

Chemicals- 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Rutin, methanol, Standard DPPH solution: 0.135 mM solution, Sample stock solution: 0.1 mg/ml solution for all sample methanolic extract (1 mg/10 ml methanol).

Methodology- The effect of extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1 ml of extract in methanol containing 0.02-0.1

mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of mixture was measured spectrophotometrically at 517 nm; ascorbic acid, rutin were used as references. The ability to scavange DPPH radicals was calculated by this equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(Abs_{control} - Abs_{sample})}{(Abs_{control})} \times 100$$

where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract / reference.

Screening for antibacterial activities

Chemicals- 10% aqueous dimethylsulfoxide (DMSO), Gentamicin (4µg/ml),

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 inoculum consisting of 10^6CFU/ml was used. Antibiotics such as Gentamicin (4µg/ml) and solvent 10% DMSO without the test compound were used as positive and negative controls respectively. The tests were conducted in triplicate.

Dtermination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the methol extract was evaluated by Tube dilution method . The methanol extract MIC was determined by dilution of the extract to various concenterations (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 calamus* Linn. 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
1.	Carbohydrates	Benedict's test	+	+	+	+	+
		Molisch's test	+	+	+	+	+
		Caramelisation	+	+	+	+	+
2.	Glycosides	Fehling's test	-	+	+	+	+
3.	Steroids	Libermann burchard test	+	+	+	-	-
		Salkowski reaction	+	+	-	-	-
4.	Proteins & Amino acids	Biuret test	-	-	-	+	+
		Ninhydrin test	-	-	-	-	+
5.	Saponins	Foam test	-	-	+	+	+
6.	Tannins	$FeCl_3$ test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanillin hydrochloride test	-	-	-	+	+
7.	Triterpenoids	Libermann burchard test	+	+	+	-	-
8.	Alkaloids	Dragendroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
9.	Resin	Resin	-	-	-	-	-
10.	Flavonoids	Alkaline reagent test	-	+	+	+	+
		Shinoda's test	-	-	+	+	+

Table-2 Phytochemical screening of Rhizome of *Acorus calamus* Linn.

S. No.	Constituents	Tests	Hexane	Chloroform	Ethylacetate	Methanol	Water
1.	**Carbohydrates**	Benedict's test	-	-	+	+	+
		Molisch's test	-	+	+	+	+
		Caramelisation	-	+	+	+	+
2.	**Glycosides**	Fehling's test	-	+	+	+	+
3.	**Steroids**	Libermann burchard test	+	+	+	-	-
		Salkowski reaction	-	+	-	-	-
4.	**Proteins & Amino acids**	Biuret test	-	-	-	+	+
		Ninhydrin test	-	-	-	-	+
5.	**Saponins**	Foam test	-	-	+	+	+
6.	**Tannins**	$FeCl_3$ test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanillin hydrochloride test	-	-	-	+	+
7.	**Triterpenoids**	Libermann burchard test	+	+	+	-	-
8.	**Alkaloids**	Dragendroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
9.	**Resin**	Resin	-	-	+	-	-
10.	**Flavonoids**	Alkaline reagent test	-	-	-	-	-
		Shinoda's test	-	-	-	-	-

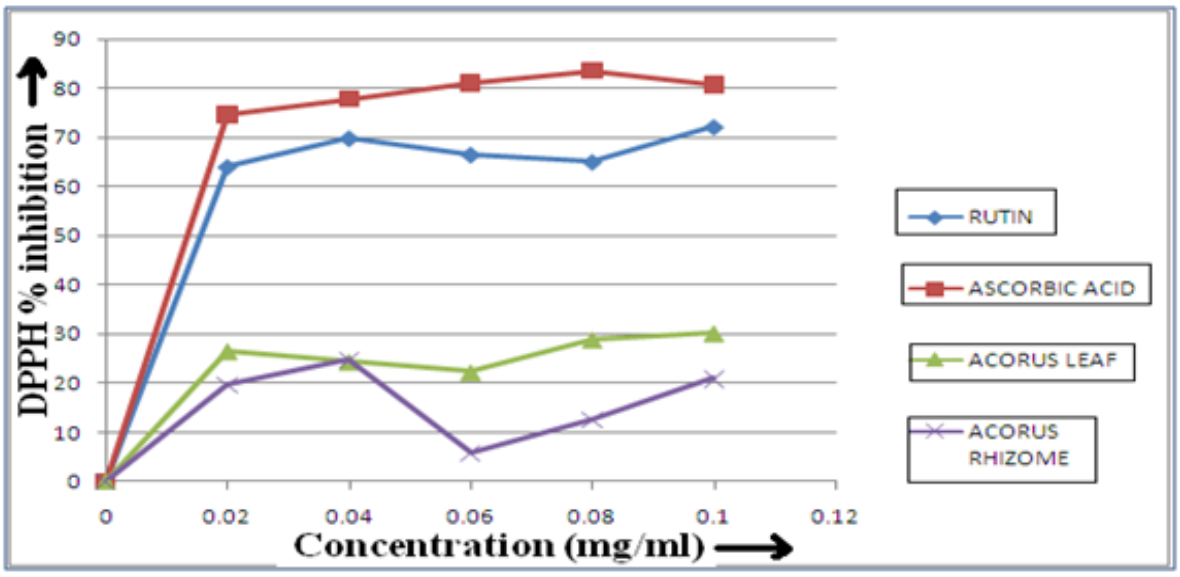
Table- 3 Absorbance at various concentrations

Concentrations →	0.02	0.04	0.06	0.08	0.1
Ascorbic acid	0.153	0.133	0.114	0.099	0.116
Rutin	0.217	0.182	0.202	0.211	0.168
Leaf	0.443	0.450	0.469	0.429	0.421
Rhizome	0.485	0.454	0.569	0.528	0.477

Table- 4 Percentage of Standards and Sample

Concentration	0.02	0.04	0.06	0.08	0.1
Ascorbic acid	74.62%	77.94%	81.09%	83.58%	80.76%
Rutin	64.01%	69.81%	66.5%	68.01%	72.13%
Leaf	26.53%	24.37%	22.22%	28.85%	30.18%
Rhizome	19.56%	24.70%	5.63%	12.43%	20.89%

Fig.1 DPPH % Free radical inhibition Vs Concentration mg/ml.



Free radical scavenging activity- In-vitro DPPH free radical scavenging activity of the methanolic extract of the leaf and rhizome of *Acorus calamus* Linn. Compared with Ascorbic acid and Rutin (standard used) it was observed that extract of the leaves shows higher activity than that of the rhizome. At a concentration of 0.1 mg/ml the scavenging activity of the leaves reached 30.18% while at the same concentration rhizome have 20.89% activity. Absorbance of control was 0.603. Results are shown in table- 3, 4 and Fig. 1

Antimicrobial activity (cup plate method)

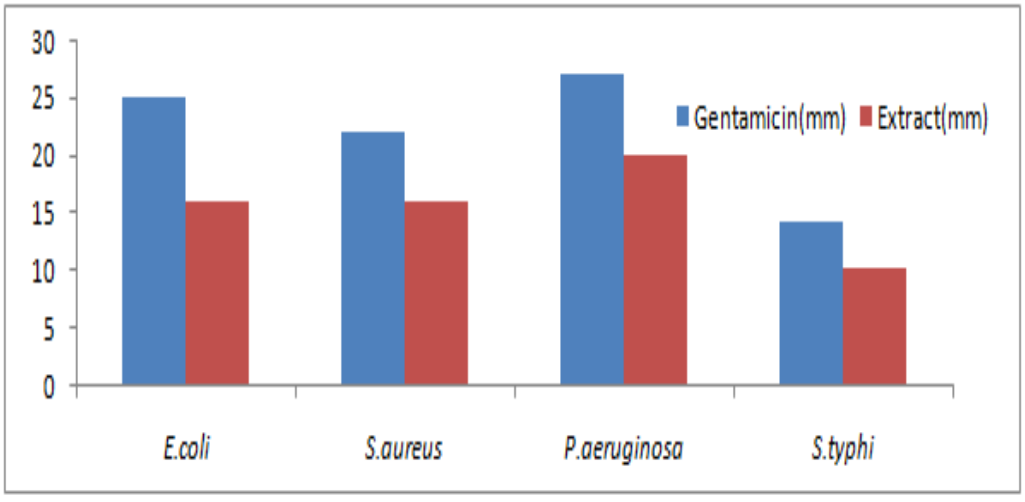
Table- 5 Zone of inhibition (mm)

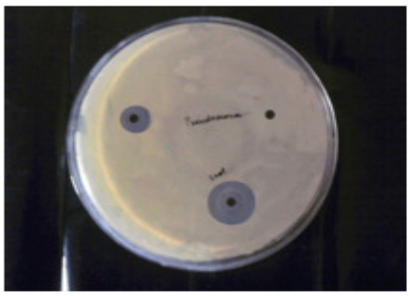
	E.coli	*S.aureus*	*P.aeruginosa*	*S.typhi*
Gentamicin	25	22	27	14
Extract	16	16	20	10

Table- 6 Minimum Inhibitory Concentration (mg/ml) of Extract

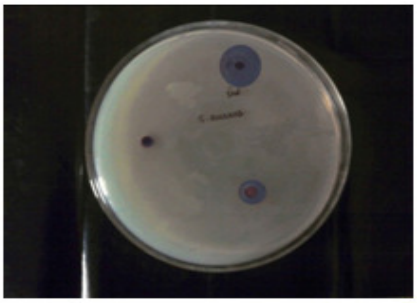
E.coli	*S.aurues*	*P.aeruginosa*	*S.typhi*
125	125	62.5	250

Fig.2 Comparative graph between Gentamicin and EtOH extract showing zone of inhibition (mm)

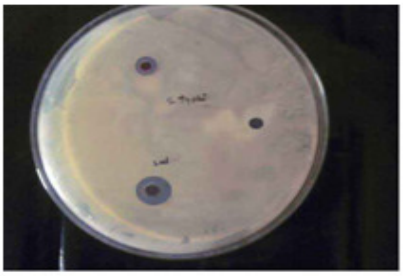




P.aeruginosa (std= gentamicin, B=blank, E=Extract)



S.aureus (std=gentamicin, B=blank, E=Extract)



S.typhi (std= gentamicin, B=blank, E=Extract)

E.coli (std= gentamicin, B=blank, E=Extract)

Fig.3 Plates showing zone of inhibition against various micro-organisms.

Acknowledgment

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Conclusion

Results of the proposed study suggest that there is possible use of leaf and rhizomes of *Acorus calamus* Linn. as a natural antioxidant. As it is already known that free radicals have implicated in causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, CNS disorders etc. Study of Acorus *calamus* Linn leaf and rhizome shows considerable antioxidant activity. Thus, it may be concluded that the plant can be effectively used further in the treatment of above mention ailments. It may also be concluded that the present property of the plant is due to the presence of high level of polyphenolic compounds including flavonoids, flavonols, proanthocyanidines etc. Plant also shows positive and considerable anti-microbial activity against all micro-organisms used.

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Synthesis of Zno Nano Particles Using Plant Extract, Their Characterization and Antibacterial Activity

Sulaxna Sharma[1]*, Sarika[2], Awanish Kumar Sharma[3]

[1]Department of Chemistry, THDC Institute of Hydropower Engineering and Technology, B. Puram Tehri.

[2]Department of Chemistry,Graphic Era University,Dehradun-247667.

[3]Department of Physics,Graphic Era University,Dehradun-247667.

*Corresponding author, email: sulaxnasharma@gmail.com

Abstract - Zinc is a trace element and essential nutrient in humans. It plays a vital role in all physiological processes. The aim of the present study is to synthesize ZnO nano particles by sol-gel process using zinc acetate dihydrate as the starting material and ethanolic extract of sunflower seeds as surfactant. The prepared sample was then calcined at two different temperatures 300° and 600 °C. The synthesized samples were characterized using XRD, FE-SEM and EDAX to determine their morphology, composition, particle size and structure. The results obtained show agglomeration up to certain extent and the particle size is close to ~30 nm for calcination at 600 °C.

Antibacterial activity was performed using the MIC test for *E.Coli* (gram-negative) bacteria. Optical density of the samples was measured after the 14 hour incubation period and compared to the positive controller to determine the efficiency. Both the samples gave almost similar results probably due to high degree of agglomeration. The calcination temperature when increased, particles tend to obtain a specific shape such as acicular or rod like. In case of antibacterial testing, a refined shape gives better results if the particle size is similar. If not, then the efficiency of the process will go down as the particle size increases, shape remaining the same.

Keywords: ZnO, sunflower seeds extracts, *E.Coli* bacteria, Antibacterial activity

Introduction

Zinc is an essential nutrient for human health. It is a natural element found in all plants and animals and plays a crucial role for the growth of skin, teeth, bones, hair, nails, muscles, nerves and brain function. In other words, Zinc is a trace element and plays a vital role in all physiological processes in humans. Its function in cells and tissues depends on those metallo-proteins and enzymes with which it is associated[1]. It has been used as a drug in the prevention and treatments of diseases since 14th century A.D.[2], as a number of zinc-containing formulations including Rasaka or kharpara (zinc ore or zinc carbonate), Yasada (zinc metal), pushpanjana (zinc oxide) and pittala (brass) are used as therapeutic agents in Ayuveda[3]. Therapeutic uses of zinc oxide in modern system of medicine have also been used. Zinc oxide is widely used in cosmetics as it shows antibacterial action along with UV protection effect[4, 5].

It has been well established that nano materials have different properties as compared to their bulk counterpart, therefore, use of ZnO nano particles as an antibacterial agent is widely investigated these days[6, 7]. ZnO nano particles are also considered to be non-toxic, bio-safe and biocompatible. The antibacterial activity of ZnO has been studied largely with different pathogenic and non-pathogenic bacteria such as *S. aureus and E. coli*.[6, 7]. Several reports have addressed the harmful impact of nano materials on living cells, but relatively low concentrations of ZnO are nontoxic to eukaryotic cells 8-11. ZnO nano particles significantly inhibit growth of a wide range of pathogenic bacteria under normal visible lighting conditions[7]. Several studies suggest that different morphologies (particle size and shape) of ZnO have different degrees of antibacterial activities[7, 12]. The most accepted mechanism of the antibacterial activity of ZnO is that it develops Reactive Oxygen Species (ROS) including hydrogen peroxide, hydroxyl ions and singlet oxygen, which migrate into the bacterial cell and eventually disrupts the membrane[7]. Other reasons cited for the same are electrostatic forces in ZnO nano particles that directly kill bacteria[13] and the surface abrasiveness of ZnO nano particles which was reported to produce disorganization of both cell wall and cell membrane of *E. Coli*[12].

In the present paper, synthesis of ZnO nano-particles has been carried out by sol-gel method using alcoholic (ethanol) extract of sunflower seeds (oleic acid as one of the constituent[14]) as surfactant and potassium hydroxide as precipitating agent and were then calcined. The synthesized and calcined samples were characterized by different characterizing techniques XRD, FE-SEM, EDAX. Antibacterial testing of the prepared samples using *E. coli* bacteria were evaluated.

Experiment

Synthesis of natural surfactant

Dried powder of sunflower seeds were taken in a round bottom flask to this ethanol was added in 1: 5 w/v ratio. The mixture was refluxed at 60 °C for 24 h. The solution thus obtained was cooled to room temp. and then filtered to remove the residue. The volume of etanolic solution was reduced up to half the volume by rotary evaporator and was used as surfactant.

Synthesis of ZnO nano-particles

In the present study sol-gel method is used to prepare ZnO nano-particles[15]. Aqueous solution of zinc acetate dihydrate (50 ml, 0.2M) and DMSO (50 ml) were taken in a beaker and stirred magnetically. To the resulting solution 5 ml of ethanolic extract of sunflower seeds[14] was added as surfactants and the stirring was continued for half an hour. To this, 1.2M potassium hydroxide solution was added drop-wise to initiate the precipitation reaction and stirring was continued for another two hours. Centrifugation of the sample was done to obtain the precipitate. The precipitate thus obtained was washed with water repeatedly to remove the impurities of K^+. It was then washed with ethanol to remove the impurity of organic solvents. The precipitate obtained after washing was dried at 60-70 °C.

Characterization

Thermal analysis of the milled ZnO powder was carried out using TG/DTA (Perkin-Elmer Diamond) in air at a heating rate of 10 °C/ min to determine the calcination temperature. The structure of synthesized ZnO powder was studied by the Powder X-Ray Diffractometer (XRD, D8 Bruker AXS Diffractometer) with CuKá radiation (ã= 0.1541 nm) and a monochrometer 50 kV and 300 mA with the scanning rate and step being 2 °/min and 0.02°, respectively. The surface morphology of the powder and coatings was examined by a Field Emission Scanning Electron Microscopy (FESEM, Quanta FEI-200) and a Scanning Electron Microscope (SEM, ZEISS EVO-18). Qualitative elemental analysis of the coatings was carried out using an Energy Dispersive Analysis of X-rays (EDAX, PENTA FET Precision) an attachment with the FESEM equipment.

Antibacterial testing of the prepared samples

ZnO nano particles as prepared were subjected to a quantitative antibacterial testing procedure. MIC test was employed and Optical Density (OD) values were measured at 600 nm. To determine the antibacterial activity an inoculum of *E.Coli* bacteria (gram negative) was prepared using LB broth and nutrient agar as a medium. All the test tubes were then put in autoclave for killing of any residual impurities. The samples were measured for 5 different weights including 0.5, 0.75, 1, 1.5 and 2 mg. Samples were then put in the bacteria medium and kept for incubation for 14 hours. Optical density of the tubes was then measured at 600 nm for various samples. A positive control was kept in which no ZnO particles were added. Optical Density value of the samples was then compared with the positive control and efficiency was calculated.

Results and Discussion

TGA results

Thermal Gravimetric Analysis (TGA) was carried out for the sample as prepared. In TGA, weight loss of a substance is measured under controlled heating rate as a function of temperature. This was done to determine suitable temperatures for calcination. Weight loss is observed when the temperature is suitable for the reaction. Hence in a particular temperature range (25-1000 °C) with heating rate (10 °/min) and in air atmosphere (with gas flow 200 ml/min), a gradual decrease in weight is observed from the thermogram shown in Fig. 1.

The weight loss observed in the temperature range 22-141 °C is not considered for calcinations as this temperature range is associated with weight loss due to moisture and melting of some foreign substances. From the TG curve, it has been observed that a signifi-

cant weight loss is observed in the temperature range 400 °C to 555 °C. This indicates that some structural or phase change takes place within this temperature range. Hence, calcination temperatures were chosen as 300 °C and 600 °C so that the effective phase change may take place for comparison.

XRD analysis

XRD of the calcined samples was carried out to confirm the presence of ZnO compound. It also indicated the impurities if any, present in the samples. XRD spectra are shown for the samples calcined at 300 °C (Fig. 2) and at 600 °C (Fig. 3).

In the XRD spectra of the powder obtained after calcination at 300° and 600 °C, it has been observed that the compounds of only ZnO (JCPDS card No. 00-036-1451) and ZnO_2 are present with minimum noise distortion or impurities. However, for the sample calcined at 600 °C sharp peaks of ZnO indicate the presence of particles in highly crystallized format (JCPDS card No. 01-080-0074). In the spectra mostly the peaks obtained are of ZnO. Whereas, $K_2Zn_6O_7$ peaks are also observed indicating that complete washing of potassium ions from the samples did not take place.

FESEM analysis

FE-SEM of the calcined samples was done to determine their morphology, particle size, extent of agglomeration, etc. The HV was kept at 20kV and images up to a magnification of 100,000X were obtained as shown in Fig. 4.

The average particle size obtained for the sample calcined at 300 °C is around 20-30 nm. A lot of agglomeration is evident from the figure, which indicates that the amount of surfactant used for the present study is not sufficient to prevent the agglomeration effectively. However, it has been observed from the figure that the shape of the particles is mostly spherical and globular. For the sample calcined at 600 °C, no apparent shape or morphological change has taken place in comparison to the sample calcined at 300 °C. Particle size around 20-30 nm, is almost similar to the sample calcined at 300 °C.

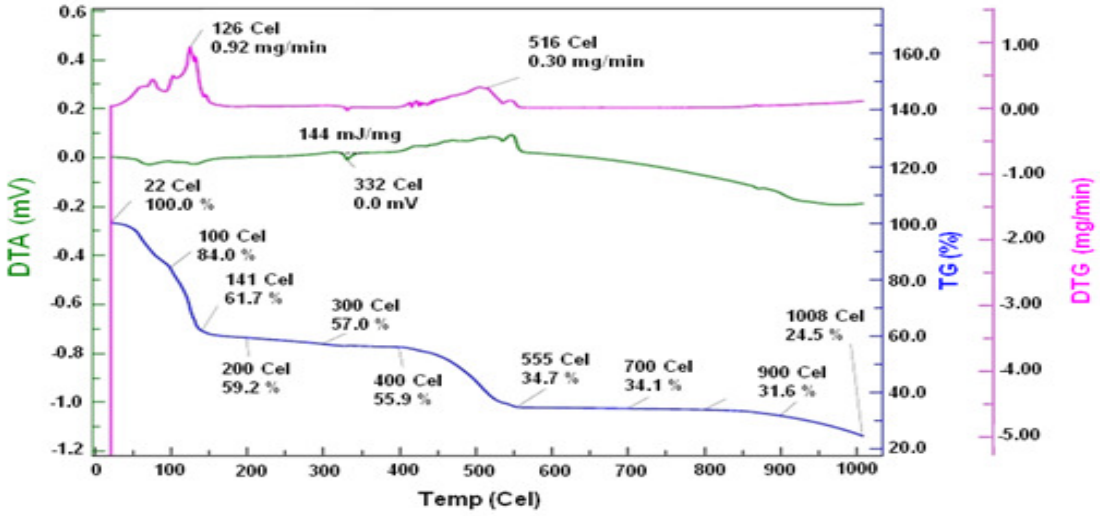


Fig. 1 Thermal Gravimetric Analysis curve of as-synthesized sample.

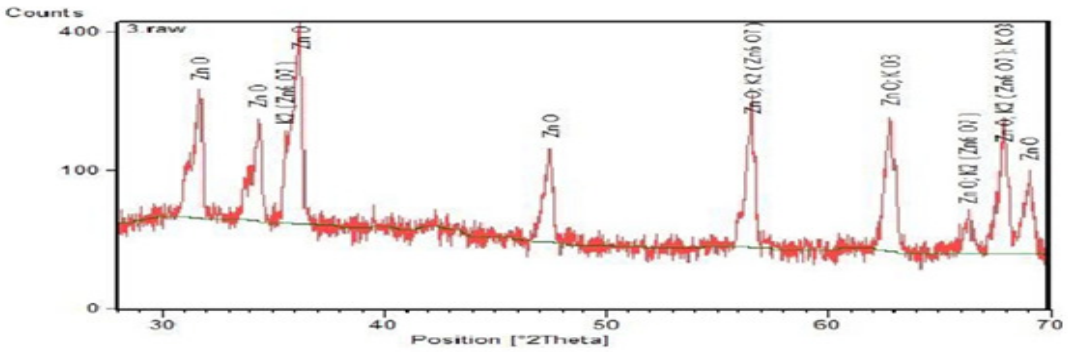


Fig. 2: XRD spectrum of synthesized sample using alcoholic extract of sunflower seed as surfactant and calcined at 300 °C.

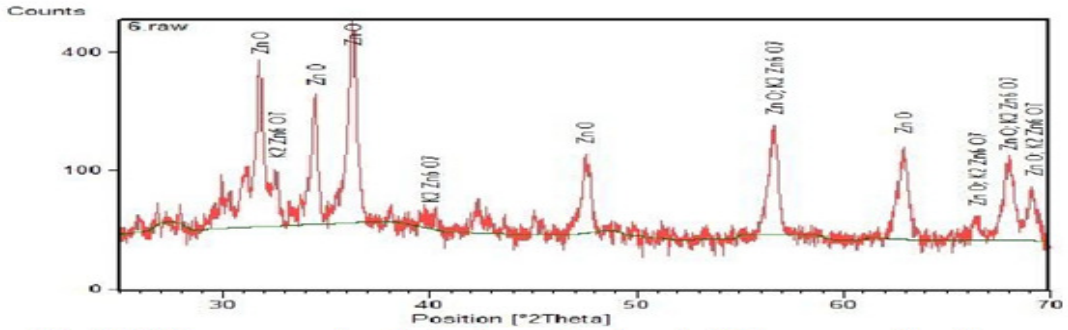
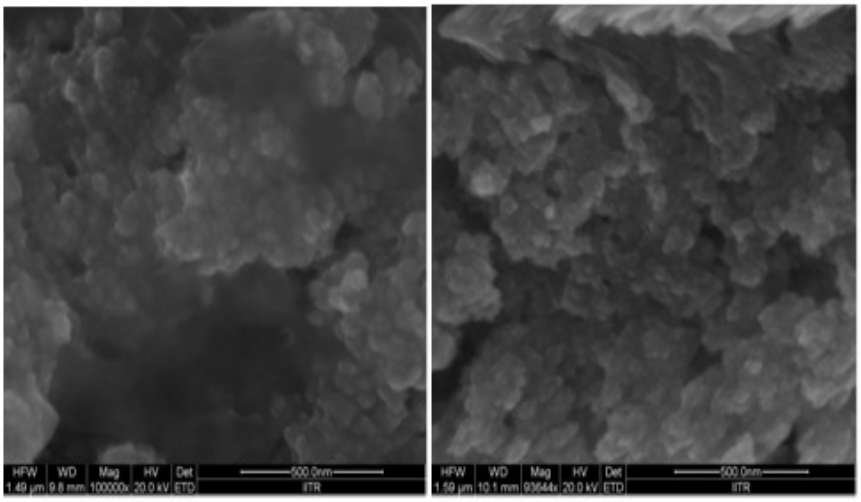


Fig. 3: XRD spectrum of synthesized sample using alcoholic extract of sunflower seed as surfactant and calcined at 600 °C.



(a) (b)

Fig. 4 FESEM images of synthesized nano particles obtained on calcination at (a) 300 and (b) 600 °C.

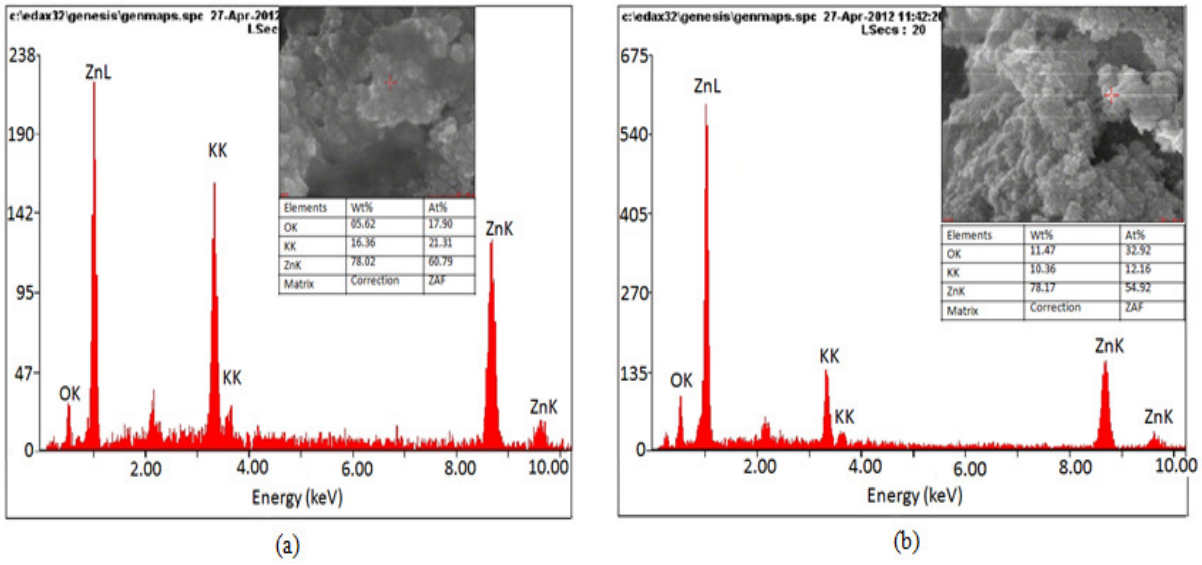


Fig. 5: EDAX micrograph of the sample calcined at (a) 300 °C and (b) 600 °C.

EDAX analysis

Energy dispersive X-ray spectroscopy (EDS) is an analytical technique used for the elemental analysis or chemical characterization of a sample. The prepared samples were characterized using EDAX technique (Fig. 5) to determine the elemental composition. Mostly peaks from the Zn K and O K shells were obtained. Zn L peaks were obtained in the graph; however, it was not incorporated in the table.

During synthesis, KOH was used as precipitating agent. After synthesis, repeated washing was done to remove K^+ ions, but from the XRD analysis, it has been observed that K^+ ions were not completely removed even with repeated washing. This is also clearly evident from the elemental composition table (see Fig 5), though, the major element is still Zn with sharp and well-defined peaks of K and L shell. Since point imaging was done to obtain the EDAX analysis results *i.e.*, on varying the location report of elemental analysis may vary. This might be the reason for a deviation from the elemental composition obtained for sample calcined at 300 °C in comparison to sample calcined at 600 °C. Amount of K^+ ions vary in this case that means K^+ is present in free form and particles formed are mostly of ZnO. The atomic percent of Zn as obtained from its K- shell area is 60.79.

Antibacterial activity test

The samples prepared were tested for their antibacterial properties against *E.Coli.* (gram negative) bacteria. Optical Density (OD) values were measured after an incubation period of 14 hours and compared to the positive controller to determine the efficiency of the process. Each sample was tested for 5 different concentrations such as 1.5, 1.875, 2.5, 3.75 and 5 mM. Following equation was used to determine the efficiency of the sample and the data obtained is given in the Table- 1.

$$\frac{\text{ODE value positive controller - ODE sample}}{\text{ODE value positive controller}} \times 100 = \text{Efficiency}$$

Sample (calcinations temp.)	Weight (mg) for 5ml	Concentration (mM)	Optical Density value	Efficiency %
Positive control	0	-	1.723	-
300	0.5	1.25	1.137	34.02
300	0.75	1.875	0.964	44.06
300	1.0	2.5	0.896	48.01
300	1.5	3.75	0.671	61.06
300	2.0	5	0.548	68.20
600	0.5	1.25	1.062	38.38
600	0.75	1.875	0.907	47.37
600	1.0	2.5	0.876	49.17
600	1.5	3.75	0.713	39.18
600	2.0	5	0.593	65.59

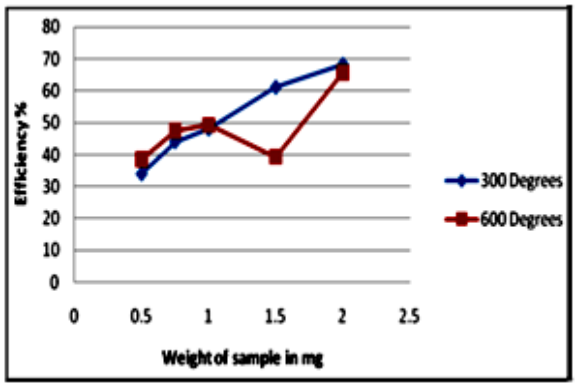


Fig. 6: Efficiency curve for 300 and 600 °C.

Efficiency of samples calcined at 300 °C and 600 °C were compared and given in Fig. 6. It can be observed that efficiency for both the samples is almost similar with results slightly better for 600 C sample. This might be due to the highly crystalline structure of the sample as suggested by XRD. Slight dip in the efficiency for the sample calcined at 600 °C might be due to inhomogeneity of the suspension or sample contamination. The efficiency of both the samples is in the range of 30-70%. This % efficiency may be explained in terms of degree of agglomeration. Particles were not separated properly which accounts to lower their effectiveness. Sample at 600 °C was slightly less agglomerated, hence its efficiency is better.

Conclusions

The following conclusion has been drawn from this study that Sol-Gel route is a cost effective route for synthesis of ZnO nano particles. However, the amount of surfactant/ capping agent is not sufficient to prevent the agglomeration of the sample but it effectively arrests the growth of nano particles (*i.e.*, 20-30 nm in size) as determined by FESEM. Calcination temperature has a pronounced effect on the morphology of particles but in the present case, only partial development of the morphologies took place which suggests that a higher temperature or greater calcinations time is needed to complete the transformation. For antibacterial applications, in the present case two forces are acting together (i) Particle size – the lesser the particle size, the greater is the efficiency against bacteria (ii) Extent of agglomeration has an inverse relation with bacterial efficiency. As when the particles are separated they will be better available for attack. The use of natural surfactant shows slightly better antibacterial activity to that of commercially available chemicals (ethylene diamine and citric acid monohydrate) used as surfactants reported in our previous paper[16] where antibacterial tests were carried out by zone of inhibition method.

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Phytochemical, Antioxidant and *In Vitro* Antibacterial Activity of Aqueous and Acetone Fruit Extracts of *Kigelia Africana*

P. K. Chauhan[1], V. Singh[2], Somesh Sharma[1] and I. P. Pandey[3]

[1]School of Bioengineering and Food Technology, Shoolini University, Solan, HP, India

[23]Department of Microbiology, Himachal Institute of Life Sciences, Paonta Sahib, HP, India

[3]Department of Chemistry, DAV (PG) College, Dehradun, UK., India

Abstract- Phytochemicals present in plants probably explain the various uses of plants for traditional medicine. In this study *Kigelia Africana* fruit was selected for assessing the level of various Phytochemicals, enzymatic and non- enzymatic antioxidants and antimicrobial activity. Results showed the presence of Alkaloids, Glycosides, Terpenoids & Flavonoids and Tannins in both the extracts whereas reducing sugars and saponins were absent in acetone fruit extract. The levels of different enzymatic and non- enzymatic antioxidants were also assessed in different extracts of *Kigelia africana* for catalase, peroxidase, ascorbate oxidase and vitamin C activities, which were found to be maximum in aqueous fruit extract. Antimicrobial activity of these extracts was then studied using agar well plate method against the bacteria strains isolated from the Urine samples of stone patients. Antimicrobial activity of aqueous extract was also greater than that of the acetone extract.

Keywords: *Kigelia africana*; phytochemicals; antioxidants; antimicrobial activity.

Introduction

Human use of plants as medicines agent pre-dates recorded history. Ethnomedicinal plant-use data in many forms has been utilized in the development of formularies and pharmacopoeias, providing major focus in global healthcare, as well as contributing substantially to the drug development process[1]. Secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds. The presence of these secondary metabolites in plants probably explains the various medicinal and antioxidant activities of these plants[2]. Antioxidants help to prevent the free radical damage that is associated with cancer and heart disease. *Kigelia africana* is a plant used for this study. It is found mostly in riverine areas where distribution is restricted to the wetter areas. Antibiotics are sometimes associated with adverse effects on hosts which include hypersensitivity, depletion of beneficial gut, mucosal microorganisms, immune suppression and allergic reactions. Bacteria have the genetic ability to transmit and acquire resistance to drugs[3]. Essential oils and extracts of certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods[4]. The investigation of certain indigenous plants for their antimicrobial properties is very useful[5] and there is increasing interest in plants as source of agent to fight microbial diseases and treatment of several infections[6]. This study mainly concerned with the phytochemical antioxidant and antibacterial activity of the aqueous and acetone fruit extracts of *Kigelia africana* using clinical isolates of bacteria from urine samples of stone patients.

Material and Methods

Collection of plant materials

The plant material used was the dried fruit of *Kigelia Africana* collected from FRI, Dehradun and were identified by Botanical Survey of India, Dehradun.

Extraction of plant material

The plant material taken for the study was stored under refrigerated condition till use. The extracts were prepared by using Sonicator and soxlet apparatus and evaporated on water bath then crude extracts were obtained and stored at the temperature of 40°C till use for investigation.

Phytochemical investigations

Tests for alkaloids and flavonoids

Tests for Alkaloids and Flavonoids were performed by the method of Harborne J B *et al.*[7].

Test for saponins

Foam test: 1ml of aqueous extract was diluted sepa-

rately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Test for tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic, tannins and green black for catecholic tannins.

Test for glycoside

Glycoside test was performed by the method of Siddiqui A A *et al.*[8].

Test for terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoids and green bluish color for steroids.

Test for reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitates.

Antioxidant activity

Catalase activity was assayed by the method of Sinha *et al.*,[9]. Peroxidase activity was carried out by the method of Addy and Goodman *et al.*[10]. Ascorbate Oxidase activity was carried out by the method of Vines and Oberbacher *et al.*,[11]. The ascorbic acid activity was carried out by the method of Sadasivam, S *et al.*,[12].

Determination of antibacterial activity

The antimicrobial activity of *Kigelia africana* fruit extract was carried out by using *in vitro* agar well diffusion method. Muller Hinton agar was sterilized by using autoclave, then it was poured in petri plates and allowed to get solidify. The wells of desired diameter (8 mm) were made with the help of borer. Bacterial suspension of each strain is applied and grown overnight. The fruit extract was poured in each particular labeled well with the help of sterile micropipettes by maintaining the asceptic environment. These petri plates were then kept for incubation at 37°C for 24hrs. After the completion of incubation period, the zones of inhibition were measured and recorded. The antimicrobial activity of *Kigelia africana* fruit extract was performed against bacterial strains i.e. *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris.*

Results and Discussion

Phytochemical investigation of *Kigelia Africana*

Qualitative analysis showed the presence or absence of phytochemical constituents and the results are summarized in **Table - 1.** Results showed the presence of Alkaloids, Glycosides, Terpenoids & Flavonoids and Tannins in both the extracts whereas reducing sugars and saponins were absent in acetone fruit extract. The phytochemical screening revealed

Table - 1 Phytochemical analysis of both the extracts of *Kigelia Africana*

Plant extracts	Alkaloids	Glycosides	Terpenoids	Flavonoids	Tannins	Reducing Sugars	Saponins
Aqueous extract of *Kigelia africana*	+	+	+	+	+	+	+
Acetone extract of *Kigelia africana*	+	+	+	+	+	-	-

Antioxidant activity of aqueous and acetone extracts of Kigelia Africana

that *Kigelia africana* fruit extract contain alkaloids, flavonoids, tannins, cardiac glycosides, cyanogenic glycosides, anthraquinone glycoside, antho-cyanosides (anthocyanin pigment) and reducing compounds[13]. Terpenoids reduces sugar level in the blood hence *Kigelia Africana* shows anti diabetic activity[14].

Antioxidant activity of aqueous and acetone extracts of *Kigelia Africana*

Enzymatic antioxidants

The levels of Enzymatic Antioxidant assessed in different extracts of *Kigelia africana* were collectively represented in **Table 2.** Among the two extracts of *Kigelia africana*, the highest activity of catalase was observed in aqueous extract (3.267 mg protein) and lowest in acetone extract (1.802 units/mg protein). Peroxidase activity was found to be very high in acetone extract (2.942x106 units/mg protein/min). In plants, antioxidant enzyames namely catalase[15] and peroxidase have been shown to increase when subjected to stress conditions. The ascorbate oxidase activity was highest in aqueous extract (0.0315 units/mg protein) and lowest in acetone extract 0.0123 units/mg protein.

Non-enzymatic antioxidants

The concentration of non enzymatic antioxidant in *Kigelia africana* extracts was also assessed and the results are represented in **Table - 3**. Vitamin C content was low in acetone extract (0.144 mg/ g tissue), whereas high in aqueous extract (0.427 mg/g tissue). Ascorbate has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells and shown to function as a reductant for many free radicals[16].

Antibacterial activity of aqueous and acetone extracts of *Kigelia africana*

The *in-vitro* antibacterial activity of aqueous and acetone extracts of *Kigelia africana* against *Proteus vulgaris, E.coli* and *Staphylococcus aureus* bacteria strains isolated from the Urine sample of stone patients were examined by Agar well diffusion method and the results are represented in **Table- 4**. It has been found that in case of Gram +ve bacteria aqueous extract of *Kigelia africana* showed maximum antibacterial activity i.e. against *Staphylococcus aureus* (20 mm) while acetone extract of *Kigelia Africana* showed minimum antibacterial activity against *Staphylococcus aureus* (16 mm). In case of Gram -ve bacteria acetone extract of *Kigelia africana* showed minimum antibacterial activity against *E coli* (7 mm) and against *Proteus mirabilis* (6 mm) while aqueous extract of *Kigelia africana* also showed maximum antibacterial activity against *E. coli* (17 mm) and against *Proteus vulgaris* (11mm).

Table - 2 Enzymatic Antioxidant analysis in both the extracts of *Kigelia Africana*

Samples	Catalase μ/moles of H2O2 decomposed/min/g protein	Peroxidase IU/L	Ascorbate oxidase μ mole/ml
Aqueous extract of *Kigelia Africana*	3.267	2.904x106	0.0315
Acetone extract of *Kigelia Africana*	1.802	2.942x106	0.0123
	1 unit = μ/moles of H2O2 decomposed/min/g protein	1 unit = μ moles pyrogallol oxidized/min	1 unit = 0.01 O.D change/min

Table-3 Non-Enzymatic Antioxidant Activity (Ascorbic acid)

Samples	Vitamin C (mg/g)
Aqueous extract	**0.427**
Acetone extract	**0.144**

Antibacterial activity of aqueous and acetone extracts of *Kigelia africana*

Table- 4 Zone of inhibition of aqueous and acetone extracts of *Kigelia Africana*

Bacterial species	Aqueous extract (mg/ml)	Acetone extract (mg/ml)
Staphylococcus aureus	20 mm	16mm
E.coli	17 mm	7 mm
Proteus vulgaris	11 mm	6 mm

Plants extracts are usually more active against gram positive bacteria than gram negative bacteria[17]. According to Abu-Shanab *et al.,* 18 gram negative bacteria are more resistant to plants extract as compared to gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism and this is in support of the present finding which showed that both the extracts of *Kigelia africana* were effective on the growth of *S.aureus* which is gram positive bacterial species. So from the present study it has been concluded that aqueous extract of *Kigelia Africana* possess significant amount of phytochemicals, antioxidant enzymes and antimicrobial agents than the acetone extract. Further studies are needed to isolate the exact active component which are responsible for antimicrobial activities. It is hoped that this report will serve as a basis of information for future project to be embark on in order to evaluate the potentials of *K. pinnata* (Lam) Benth as a strong medicinal plant in improving human health status.

Conflict of interest statement

We declare that we have no conflict of interest.

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Phytochemical and Antimicrobial Studies on the Leaves of *Sapium Sebiferum Roxb*

S. Rawat[1]*, O.P. Sati[2] and A. Tomar[1]
[1]Department of Chemistry,Dolphin (PG)Institute Of Biomedical and Natural Sciences,Dehradun , UK., India
[2]Department of Chemistry,H.N.B.Garhwal Central univercity ,Uttarakhand UK., India
E. mail: sumanman.2008@rediffmail.com

Abstract- The present study was aimed at detecting the phytochemicals and evaluating antimicrobial activities of ***Sapium Sebiferum Roxb.*** known for its medicinal properties in folk medicine. Phytochemical screening was carried out on the leaves of *Sapium Sebiferum Roxb*.The results show the presence of alkaloids, proteins, carbohydrates, phenolic compounds, tannins, amino acid, fats and fixed oil and saponins The assessment of antifungal activity was performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar PDA) against Aspergillus niger, M.gypseum and T. flavurusclem. The antibacterial effect was studied by the agar direct contact method using B.cereus, B.pumilus, M.luteus and Escherichia coli strains.. These phytochemicals were isolated from the plant with yields of 16.18 % of Petroleum ether extract, 3.51% of Chloroform extract, 23.16 % of Acetone extract and 11.11% of methanol extract. The results revealed that the acetonic extract exhibited significant antimicrobial activity of concentration of 100-500 ì/ml respectively against tested organisms, particularly more effective against B.cerus, B.pumilus and T. flavurusclem than the other extract when compared to the standard drug (chloramphenicol).

Keywords: *Sapium Sebiferum Roxb*, Antimicrobial activity, Phytochemical Studies

Introduction

Sapium Sebiferum Roxb is a plant belonging to family *Euphorbiaceae*[1]**.** It is monoeciouces, deciduous small tree upto 13 m tall, stem often gnarled bark whitish grey with vertical cracks, containing white latex. Leaves are alternate, broad rhombic to ovate in shape 3.8-8.5 cm long and have smooth edges, heart shaped .Fruits are three- lobed, three-valved capsules.[2] Its wood has been used to make various implements, toys, furnitures, inferior quality pencils, cricket bats and Chinese printing blocks.[3] In Chinese medicine, its oil is used as purgative and emetic. Resin from root bark is considered as purgative. The seed is antidote, emetic and purgative.[4]

Material and Methods

Collection and identification of leaves of *Sapium Sebiferum Roxb*

Leaves of *Sapium Sebiferum Roxb* were collected from FRI, Dehradun (India). Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), Botanical Survey of India, Northern regional centre, Dehradun (BSI).

Extraction of leaves of *Sapium Sebiferum Roxb* in different solvents (Non-polar to Polar)

The collected plant material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (500 gm) of ***Sapium Sebiferum Roxb*** were crushed. The crushed leaves were extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone and methanol by hot percolation method using Soxlet apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure.

Phytochemical analysis of different extracts

The different extracts of leaves of ***Sapium Sebiferum Roxb*** were tested for various components:

Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dilute HCl and filtered. The filtrate was then tested for following colour test.

(i) Mayer's test (a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. **(b)** 5 gms of potassium iodide was dissolved in 20 ml of distilled water. **(a)** and **(b)** was mixed and the volume was adjusted

to 100ml with distilled water .Appearance of cream colour precipitate with Mayer's reagents showed the presence of alkaloids.

(ii) Wagner's Test- 1.27 gm of iodine and 2 gm of potassium iodide was dissolved in 5 ml of water and made up the volume to 100ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloids.

(iii) Hager's test- Take 20 ml of saturated solution of picric acid and add few drops of it to 2-3 ml of extract. A yellow color was observed.

Detection for carbohydrates and glycosides

(i) Molisch's test- 10 gm of alpha naphthol was dissolved in 100 ml of 95% alcohol. Extract was treated with this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides of the test tube, purple or violet color appeared at the junction.

(ii) Benedicts test- The test solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed if reducing sugars were present.

(iii) Fehling's Test- 6.932 gm of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 gm of potassium sodium tartarate and 10 gm of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solution was mixed in equal volume prior to use and few drop of sample was added and boiled, a brick red precipitate of cuprous oxide was formed, if reducing sugars were present.

Test for sterols and triterpenoids

Salkowski test: Extract was treated with few drops of conc. Sulfuric acid, shake well and allowed to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

Test for proteins and amino acids

(i) Ninhydrin test- 1gm of ninhydrin (indane1, 2, 3 trione hydrate) was dissolved in n-butanol and make the volume to 100ml. Extract treated with this solution gave violet colour on boiling.

(ii) Biuret test- To 3ml test solution 4% w/v NaOH and few drops of 1% w/v copper sulphate solution were added. A blue color was observed.

Test for sponins

Foam test- 1ml of extract was diluted with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicated the presence of sponins.

Anti-microbial activity of different extracts

The anti-microbial activity of the leaves of ***Sapium Sebiferum Roxb*** was carried out. The leaves extract were screened for anti bacterial and anti fungal activities.

Anti bacterial activity of leaves extract

In this study, the anti bacterial activity was studied against the micro organism and the bacterial cultures used were- (i) B. cerus (ii) B. pumilus (iii) M. luteus (iv) Escherichia coli

These bacterial cultures were maintained on nutrient agar slants at first, being incubated at 37° c for about 18-24 hours and then stored at 4° 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°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 HI-Media Laboratories, Mumbai. The media used for anti-bacterial activity was Muller-Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121°C at 15 psi for 15-30 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 solidification, 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 asceptic 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 100µl of each leaf extract individually was loaded. This method depends upon the diffusion of leaf extract from hole through the solidified agar layer of petridish to such an extent

that the growth of added micro organism is prevented entirely in a circular area or Zone around the hole containing leaf extract.

Incubation- Petri plates were incubated overnight at 37°C ± 0.5°C 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 Percentage yield of different extracts *Sapium Sebiferum Roxb leaves*

Sl. No.	Solvent system	%age yield
1.	Petroleum Ether	16.18 %
2.	Chloroform	3.51 %
3.	Acetone	23.16 %
4.	Methanol	11.11 %

Table-2 Qualitative Phytochemical Chemical Analysis of Extract of *Sapium Sebiferum Roxb* leaves

Test performed	Pet. Ether Extract	Chloroform Extract	Methanol Extract
Test for Alkaloids			
Mayer's test	_	_	_
Hager's test	_	_	+
Wagner's test	_	_	+
Test for Carbohydrates			
Fehling test	_	-	+
Molish test	_	_	-
Benedict test	_	-	+
Test for phenolic compounds and Tannins			
Vanillin HCL acid test	_	-	+
Dil. $FeCl_3$.test	_	-	+
Lead Acetate Test	_	+	+
Test for Sterols / Triterpenoids			
Salkowaski test	+	+	
Test for Saponins			
Saponins Test	_	_	+
Test for Proteins and acids			
Ninhydrin test	_	_	-
Biuret test	_	_	_

Key (-) Absence, (+) Presence

Table-3 Antibacterial activity of different extracts of *Sapium Sebiferum Roxb* and standard drug chloramphenicol

S. No.	Test organism	Inhibition zone in mm						
		Pet. Ether	Chloroform	Acetone	Methanol	Standard drug		
						Ampicillin	Streptomycin	Chloram-phenicol
1.	E. coli	10	20 mm	20	-	20mm	17mm	25mm
2.	Bacillus cereus	18	16 mm	25	-	15mm	16mm	36mm
3.	B. pumilus	-	20	25	22	_	16mm	20mm
4.	M. luteus	-	10	14	16	30mm	26 mm	30 mm

Table- 4 Antifungal activity of different extract *Sapium Sebiferum Roxb* and standard drug clotrimazole

S. No.	Test Organism	Inhibition zone in mm					
		Pet. Ether	Chloroform	Acetone	Methanol	Standard drug	
						Amphotericin-B	Clotrimazole
1.	*Aspergillus niger*	_	_	-	-	_	11mm
2.	*M. gypseum*	18	14 mm	20 mm	-	_	_
3.	T. flavurusclem	12 mm	12 mm	22 mm	-	_	12mm

Discussion

Phytochemical studies reveal that acetonic extract was the richest extract for phytoconstituents. It contains all tested phytoconstituents as shown in table-2. Acetonic extract showed good antibacterial activity against B. cereus and B. pumilus and good antifungal activity against T. flavurusclem in comparison to the standard drug.

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Quantitative Evaluation of Carbohydrate Levels in Different Vegetables by UV-Visible Spectrophotometer

Sandeep Negi, R. Dhoundiyal, Harish Chandra, H.V. Pant and Deepali Singhal
Department of Chemistry, SGRR (PG) College, Patharibagh, Dehradun, UK., India
*E-mail: negisandeep1969@gmail.com

Abstract- A study was carried out to determine the soluble carbohydrate content of some selected vegetables which include Carrot, Moringa, Spinach, Sorrel leaves, Curry leaves, Fenugreek leaves, Drumstick leaves and Lettuce. Soluble carbohydrate was determined by Anthrone method Spectrophotometry at wavelength of 750 nm. It was found that curry leaves had the highest percentage of carbohydrate followed by drumstick, while Lettuce and spinach had least percentage.

Key words- Carbohydrates, Vegetables, UV-Visible Spectrophotometer, Wavelength, Anthrone, Absorbance.

Introduction

Carbohydrates are compounds made up of carbon hydrogen and oxygen, thus they are regarded as hydrates of carbon represented as C (H_2O). They act as the primary source of energy which is converted into glucose to generate energy essential for metabolism in every cell of the body (Flitsch SL., et. al, 2003, M. Sarvan Prasad et.al., 2012) and are of special importance as they constitute more than 50% of the dry weight of most plants (Lehninger, 1993). These compounds perform a number of functions ranging from stores of potential energy in animals to source of energy supporting tissues in plants (Dyke, 1960).Polysaccharides serve for the storage of energy (e.g., starch and glycogen), and as structural components (e.g., cellulose in plants and chitin in arthropods) (M.Sarvan Prasad et.al.,2012). The 5- carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD, and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA(2). Carbohydrates are the most abundant bio-molecules on earth; each year photosynthesis converts more than 100 billion metric tonnes of carbondioxide (CO_2) and water (H_2O) into cellulose and other plant products (Herman, 1968). For most people, between 40% and 60% of total calories should come from carbohydrates, preferably from complex carbohydrates (starches) and naturally occurring sugars (Carl A. Burtis et.al.,2000).

This study was aimed at evaluating the soluble carbohydrate content of some selected vegetables consumed locally viz, sorrel (inflorescence), carrot and *Moringa* leaves with a view to determine whether they meet the dietary requirement of consumers. It is envisaged that the findings of the investigation would provide additional information on the nutritional status of the vegetables.

Material and Methods

Instrumentation- Ultraviolet visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Ultraviolet-visible spectroscopy was recorded on ELICO SL-160, India.

Sample preparation

The vegetables used for this investigation were obtained from field to ensure a supply of fresh samples throughout the period of the study. Moringa leaves, carrot, spinach, sorrel leaves, curry leaves, fenugreek leaves, drumstick leaves, mint, coriander, lettuce were taken as samples. A sample of each vegetable was washed and ground to a fine pulp using pestle and mortar.

Chemicals and Reagents

Anthrone, Sulphuric acid were purchased from Merck Specalities pvt.Ltd.

Reagent Preparation

0.2 grams of anthrone was weighed accurately and dissolved in 100 ml sulphuric acid , made by adding

500ml of sulphuric acid to 200 ml of water. The reagent was allowed to stand for 30-40 minutes with occasional shaking until it was perfectly clear.(Trevelyan & Harrison, 1952).

Procedure

One gramme(1g) of macerated sample was placed in 25ml of volumetric flask, 10ml of distilled water was then added and shaken vigorously followed by addition of 15 cm^3 of 52% perchloric acid. This was stirred continuously for 30minutes and the mixture was later filtered using Whatman no1 filter paper. Pipetted out 1ml of each extracted sample into a 25ml of volumetric flask and add 2ml of freshly prepared anthrone reagent in each volumetric flask and finally make up the volume up to the mark with distilled water. Reference was prepared by taking 2ml of anthrone reagent in a 25ml of volumetric flask and made up the volume up to the mark with distilled water. The wave length of the above prepared samples was checked in uv-visible spectrophotometry and wavelength was set at 750nm. At that wavelength the developed colour absorbances were noted for the samples. The total soluble carbohydrate was then estimated using the standard curve of Glucose (Pearson *et al*., 1976).

Results and Discussion

Soluble carbohydrates content of Morigna leaves, carot, Spinach, Sorrel leaves, Curry leaves, Fenugreek leaves, Drumstick leaves and Lettuce is given in the table. It was found that curry leaves contain maximum carbohydrate percentage and Lettuce contain least carbohydrate percentage. The recommendation for the general population is that carbohydrate should supply 50% to 55% of total calories and 130 gm per day (520 calories per day) for male and female adults and for athletes in between 55% to 65 % of total calories.

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Table

S.No	Name of Vegetable	Carbohydrate contents (%)
1.	*Moringa leaves*	0.240 %
2.	*carrot*	0.185 %
3.	Spinach	0.073 %
4.	Sorrel leaves	0.155 %
5.	Curry leaves	0.995 %
6.	Fenugreek leaves	0.430 %
7.	Drumstick leaves	0.79%
8.	Lettuce	0.0675%

Aroma Profile and Comparison of Chemical Composition of Thymus Serpyllum From Lower Hill of the Garhwal Himalaya

Alok Maithani*[1], Harish Ch Andolah[2] and Versha Parcha[1]

1. Department of Applied Chemistry, Sardar Bhagwan Singh PG Institute, Balawala, Dehradun, UK., India
2. School of Environmental Science and Natural Recourse (SENR) Doon University, Dehradun, UK., India

*Corresponding Author: Dr Alok Maithani, Asst Professor, Sardar Bhagwan Singh PG Institute, Balawala, Dehradun, UK., India;
email: alok_maithanii@rediffmail.com

Abstract- The Essential oil content in inflorescence of *Thymus serpyllum* cultivated in the hill of Uttarakhand was found to be 0.70 % on dry weight basis. The oil analyzed by capillary GC and GC-MS. Eighteen constituents, representing 98.71% of the oil were identified. The major components of the oil were thymol (36.8%), terpinen-4-ol (21.4%) and limonene (12.60%). The quality of lavender oil produce in Garhwal Himalyan region of India was found to compete with standard quality of oil from other parts of globe.

Keywords- Tymol, *Thymus serpyllum*, essential oil, GC, GC-MS

Introduction

Thymus serpyllum L. is a perennial herb indigenous in central and southern Europe, Africa and Asia. It is widely used in folk medicine in the treatments of variety of diseases such as gastro enteric and bronchopulmonary disorders, as well as due to its anthelmintic, carminative, sedative and diaphoretic properties[1].

Thyme oil is among the world's top 10 essential oils also used as a preservative for food[2]. The aromatic and medicinal properties of the Thymus species have made it one of the most popular herbs. The genus Thymus has numerous species and varieties and their essential oils have been studied earlier[3]. Thyme was used medicinally by the Egyptians, Greeks, and Romans. Most of the present day research has centred on thyme's ability as an antibacterial and anti-infectious agent, even when diffused in the air. There are several species of thyme oil in use, and although the strongest is red thyme and the gentlest is Linaloöl, their uses are the same. The difference is in their relative strength.

Although the essential oil compositions of thyme grown in different parts of the globe have been investigated[4]. But the present study aims to examine the chemical composition of *Thyme* from Garhwal region of Uttarakhand Himalaya and to compare it with previous reports.

Material and Methods

Collection of plant material

The samples of wild growing *Thymus serpyllum* plants were collected during the flowering stage in May 2013 from Kalsi (Dehradun), which is located on the top of foot of lower Shiwalik mountain (Himalaya) at altitude about 700 m (a.s.l). The plants were identified by Dr Sandeep Dhyani, Faculty of Biotechnology, GRDPG IMT, Rajpur Dehradun, Uttarakhand. The samples were dried in shadow at room temperature for 10 days. Voucher specimens where deposited in Herbarium of Botanical Department of BSI, Dehradun.

Isolation of the essential oils

Air-dried aerial parts of *Thymus serpyllum* deprived from wooden parts (100 g) were subjected to hydrodistillation, using Clevenger-type apparatus for 3 h according to the standard procedure. Essential oils thus obtained were dried over Na_2SO_4 and stored in a sealed dark vials, then kept at 4°C prior to further analysis. The essential oil content was determined as percentage on fresh weight basis as average of three independent extractions of each site to minimize error (Percentage yield of essential oil was determined on fresh weight basis which was found by taking average of three extractions). Extracted oils were combined for further analysis.

GC Analysis

Analyses of the oil samples was carried out by using HP7890 GC manufactured by Agilent equipped with a flame ionization detector (FID) and a HP-5 fused silica column (30 m x0.32 mm x0.2ìm film thickness). The

sample was injected directly into the column. The injector and detector temperatures were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed from 60°C to 220°C with an increase in rate of 3°C/min injected 0.2ìl oil.

Gas chromatography-mass spectroscopy (Gc-Ms)

Analysis of the oil was performed out on a Agilent mass spectrometer (Model 5975C) coupled to a Agilent GC with a 60 m x0.32 mm x 0.2ìm film thickness column (DB5). The sample was injected directly split less mode. Helium was used as the carrier gas (flow rate 1ml/min). The oven temperature was programmed from 60°C to 220°C at 3°C/min. Other conditions were other conditions were the same as described under GC. The identity of the constituents of the oils was established on the basis of GC retention indices, by comparing their 70 eV mass spectra with those reported in literature.

Identification of components

The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, C_9-C_{32} under experimental conditions, co-injection with standards (Aldrich and Fluka), MS library search (NIST and WILEY), and by comparing with the MS literature data (Adam 2007). The relative amounts of the individual components were calculated on the basis of GC peak area (FID response) without correction factors.

Result and Discussion

The essential oil content in the dry at room temperature (25- 30°C), inflorescence of *T serpyllum* cultivated in the lower Himalayan tract of Garhwal Himalaya situated in western Himalaya region was found to be 0.55 %.

Our results on chemical profiling of *T. serpyllum* essential oil are in agreement with several other studies (Raal et al., 2004; Verma et al., 2009; Verma et al., 2011;). Eighteen compounds were identified in T. serpyllum oil, which accounted for 98.71% of the total oil; the major components of the oil were thymol (36.8%), terpinen-4-ol (21.4%) and limonene (12.60%). á-terpinyl acetate (5.7%), Delta-3 carene (3.7%), Borneol (3.6%), á-terpinene(2.1%), and á-pinene(1.8%) are among the major terpenoids present in the oil. In a similar study carried out with the *T. serpyllum* oil from Serbia showed highest concentration of Thymol (38.5%), p-cymene (8.9), Borneol(6.0%), and carvacrol (4.7%). However most of the components were mearly in similar concentrations[5].

It means that oil produces in Kalsi Dehradun region compete with international market. The variation (Qualitative and quantitative) could be due to difference in location, elevation, genetic makeup of

Table-1 Essential oil composition of *Thymus serpyllum* from Dehradun

S. N.	RI	Component name	Area %	
			(Kalsi Thyme)	(Serbian Thyme)
1	932	α-pinene	1.8	2.0
2	988	β-myrcene	0.98	1.3
3	1014	α-terpinene	2.1	1.1
4	1408	β-caryophyllene	1.1	1.3
5	1179	*p*-cymen-8-ol	0.56	-
6	1298	carvacrol	0.48	4.7
7	1020	*p*-cymene	0.23	8.9
8	1002	Phellandrene	0.21	-
9	946	Camphene	0.27	2.0
10	1008	Delta-3 carene	3.7	
11	1141	Camphor	0.58	0.7
12	1232	Thymol methyl ether	5.40	-
13	**1289**	**Thymol**	**36.8**	**38.5**
14	**1024**	**Limonene**	**12.6**	**0.6**
15	**1174**	**terpinen-4-ol**	**21.4**	**0.7**
16	1506	α -bisabolene	1.2	-
17	1346	α-terpinyl acetate	5.7	-
18	1165	Borneol	3.6	6.0
19	1093	Linalool	-	2.4
20	1226	Thymol Methyl Ether	-	3.8
21	1050	λ – terpenene	-	7.2
22	883	β –myrcene	-	0.2
23	1190	Cis-dihydrocarvone	-	0.1
24	1426	Aromandendree	-	0.1
25	1566	Spatulol	-	0.3
Total			**98.71**	**99.51**

the plants or due to adaptive process to particular ecological conditions. According to observation of Lawrence difference in quantity of lavender oil components may depend upon plants genotype, cultivation area, altitude and microclimatic features of the habitat[6-7]. A lot still needs to be done in the area of investigating the pharmacological activities of lavender oil. There is a need to identify active compounds in the extracted oil responsible for the various associated pharmacological activities. This will help in specific therapeutic application of the oil. There is also the need for exhaustive characterization of all the compounds in a given oil extract as this will help in a more accurate chemo typing. Chemo typing could be more meaningful and accurate if the sample size and geographic coverage are enlarged while also noting the ecosystem (including soil type, climate/weather condition, season, etc.) and time of collection of cultivation or wild growth. There is the need to also match the various chemo types with the level and type of pharmacologic activities displayed.

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In *vitro* Assessment of Antioxidant Activity of Selected Essential Oils

Mohammad Shavez Khan [1], Garima Sharma[1], Zafar Mahmood[2] and Iqbal Ahmad [1*]

[1] Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India

[2] The Himalaya Drug Company, Dehradun, UK., India

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

Abstract- Essential oils are the liquid mixture of volatile compounds obtained by steam distillation from various plants. Many essential oils are known for biological properties. Considering the growing demand of natural antioxidants, three essential oils were tested for their antioxidant activity using DPPH radical scavenging assay and Ferric reducing antioxidant power (FRAP). These oils were also assayed for their total phenolic content. Free radical scavenging activity was found maximum in Thyme oil followed by Eucalyptus oil and Peppermint oil. However in FRAP method, the order of antioxidant power was maximum for Thyme oil followed by Peppermint oil > Eucalyptus oil. A concentration dependent increase in antioxidant activity was also recorded in both assays. Further HPTLC and GC-MS based detection of active compound is needed to understand the mechanism and synergy among various compounds.

Keywords- Essential oil, Antioxidant activity, Polyphenolics, Free radical scavenging.

Introduction

Medicinal plants and their derived products are important therapeutic utility for number of ailments; it has been recorded since ancient times that different parts of medicinal plants are used to cure specific ailments. Owing to wide acceptance for the safety and reliability of these natural entities as compared to expensive and possible adversarial effects comprising synthetic drugs, there is widespread interest in drug derived from plants (Gordon et al., 2001). The custom of indigenous plants in traditional health practices has a long past. Medicinal plants are used to preserve and promote healthy life, prevent disease and cure ailments. It has been assessed that even today, 80% of the world population rely on herbal traditional medicines for their primary health care (Cassady et al., 1990; Cragg et al., 2005; Ahmad et al., 2006).

Aromatic and medicinal plants are the source of natural antioxidants due to their secondary metabolites such as polyphenols. Phenolics can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (Lapornik et al., 2005). Essential oils also called volatile or ethereal oils are aromatic oily liquids obtained from different plant parts and widely used as food flavours (Burt, 2004). The use of essential oils has been well established in food preservation, pharmaceuticals, alternative medicines and natural therapies (Bruckdorfer, 1996; Lis-Balchin and Deans, 1997). Essential oils are complex mixture of terpenes and their compounds. Because of their relatively safe status, essential oils and their constituents are gaining increasing interest, and their exploitation for potential multi-purpose functional use (Ormancey et al., 2001; Sawamura, 2000).

In the recent past, essential oils are reported for their various biological properties such as antiseptic, antifungal, antibiofilm, anti-Quorum Sensing, anti-inflammatory as well antioxidant activities (Ahmad et al., 1998; Ahmad et al., 1999; Beg and Ahmad 2002; Khan and Ahmad 2012; Hussein et al., 2013; Amorati et al., 2014). Further, there is an increase demand for safe antioxidant agents to be used in cosmetics, pharmaceuticals, food preservations and healthcare (Amorati et al., 2014). Though the chemical antioxidants (BHA, BHT etc.) have shown to be toxic in nature (Sasaki et al., 2002), yet the role of essential oil in traditional system of medicine, especially in aromatherapy is well established (Buttner et al., 1996). The search for natural antioxidants has generated interest among scientific community to reinvestigate essential oils as safe and promising antioxidant agent (Wannes et al., 2010; Tongpoothorn et al., 2012; Kapoor et al., 2014). Some reports on essential oil property are contradictory also. Therefore, using more than one method for assessing antioxidant property of essential oil will provide comparative analysis which may be useful in se-

lecting essential oils for different purposes. In this study, we have selected commonly used three essential oils to evaluate their antioxidant activity using two different assays. The polyphenolic content and concentration dependent effect was also determined. Such information is valuable, as essential oils containing high antioxidant activities can prove valuable for maintenance of health and support scientific validation of traditional claims of well-known culinary essential oils.

Table- 1 Physiochemical characteristics of selected essential oil

Name/Common name/Family	Physiochemical Parameters				
	Part used	Colour	Refractive Index	Specific gravity	Solubility in 90% alcohol
Thymus vulgaris (Linn.)/ Thyme oil/Lamiaceae	Seeds	Golden yellow	1.500	0.900	Soluble
Eucalyptus sp (Labill.)/ Eucalyptus oil/Myrtaceae	Leaves	Colourless	1.465	0.940	-do-
Mentha piperata (Linn.)/ Peppermint oil/Lamiaceae	Aerial Part	Colourless	1.463	0.908	-do-

Material and Methods

Collection of essential oil samples

The essential oils were purchased from Hi-media Laboratory Mumbai (Peppermint oil and Eucalyptus oil) and Thyme oil was kindly provided by Fragrance and Flavour Development Center, Kannauj, UP.

Evaluation of physiochemical characteristics of essential oils

Physical and chemical properties of essential oils such as colour, refractive index, specific gravity and solubility in alcohol were determined at Flavour and Fragrance Development Center, Kannauj, India (Table 1).

Total phenolic content

The total phenolic content of the essential oil was determined by the method of Spanos and Wrolstad (1990), as modified by Lister and Wilson (2001). To 0.5 ml of each sample (containing different concentrations (ìg/ml) of oil), 2.5 ml of 1/10 dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45 °C for 15 min. Each experiment was performed in triplicates. The absorbance of all samples was measured at 765 nm using a UV/Vis Spectrophotometer (Spectronic 20 D+, Thermo Scientific, USA). The standard curve of gallic acid is plotted and results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

DPPH radical scavenging assay

Free radical scavenging activity of essential oils against stable 1, 1,diphenyl-2-picrylhydrazyl (DPPH) was determined spectrophotometrically by slightly modified method of Gyamfi et al. (1999) as described below. When DPPH reacts with an antioxidant, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm on a UV/visible light spectrophotometer (Spectronic 20 D+, Thermo Scientific, USA). Fifty µl of essential oil in DMSO, yielding different concentrations was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 ìl of 50 mM Tris-HCl buffer (pH 7.4). DMSO (50 ìl) was used as a vehicle control in the experiment. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured spectrophotometrically. Ascorbic acid and butylated hydroxytoluene were used as positive controls. Inhibition percent was calculated from the following equation-

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

FRAP assay (Ferric reducing antioxidant power assay) Reducing power was measured by the direct reduction of $Fe^{3+}(CN^{-})^{6}$ to $Fe^{2+}(CN^{-})^{6}$, and was determined by measuring absorbance resulted from the formation of the Perl's Prussian blue complex following the addition of excess ferric ions (Fe^{3+}). Hence, the ferric reducing antioxidant power (FRAP) method of Oyaizu (1986) with little modification was adopted to measure the reducing capacity. Different concentrations (µg/ml) of essential oils in 0.75 ml of distilled water were mixed with 1.25 ml of 0.2 M (pH 6.6) sodium phosphate buffer and 1.25 ml of 1% potassium ferricyanide [$K_3Fe(CN_6)$]. After 20 min of incubation at 50 °C for 20 min, the reaction mixture was acidified with 1.25 ml of trichloroacetic acid (10%). Finally, 0.5 ml of $FeCl_3$ (0.1%) was added to this solution, and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicates greater reduction capability.

Results

Total phenolic content

The total phenolics of all the three oils were determined as micrograms of gallic acid equivalents per gram of essential oil (Nakatani et al.,2000; Elizabeth et al.,2007) by computing with standard calibration curve constructed for different concentrations of gallic acid (50ìg to 800ìg). The graph was linear between these concentrations. Thyme oil showed highest amount of total phenolics (388 ± 4.2 mg/g of gallic acid equivalents) while lemon grass oil showed the least (288.89 ± 1.64 mg/g of gallic acid equivalents) as depicted in Table- 2.

Antioxidant activity

A total of three essential oils under study were subjected to antioxidant scrutiny by two standard methods namely DPPH free radical scavenging activity and reducing power activity by FRAP. The oils were ten times diluted in dimethyl sulpho-oxide and were tested at concentration ranging from 100-500 µg/ml. Of these three essential oils, thyme oil demonstrated strong DPPH scavenging activity (>80% decolorization) at 500 µg/ml concentration. The order of activity among these essential oils was maximum for Thyme oil, followed by Eucalyptus oil >peppermint oil as shown in Table- 3. The values were comparable to commercial standards ascorbic acid (94.9%) and BHT (92.8%)

Similarly by ferric reducing antioxidant power (FRAP) method, Thyme oil demonstrated powerful ferric ions (Fe^{3+}) reducing ability; (absorbance e" 1.0). In this assay, the higher absorbance values correspond with higher reducing ability meanwhile moderate ferric ion reducing ability was shown by the two essential oils (Eucalyptus oil and Peppermint oil) when compared to standards (ascorbic acid and BHT). The reducing power of all essential oils increased with increasing concentration of the oil as shown in Table- 4. The comparative analysis of essential oils determined by the two methods suggested, that Thyme oil, possessed promising antioxidant activity whereas Peppermint and Eucalyptus oils possessed relatively low antioxidant activity.

Discussion

The antioxidant potential of essential oils highlights their therapeutic potential both in traditional and modern phytomedicines. Since essential oils consist of terpenes and other different kind of phenolic compound, it would seem reasonable that their mode of action might be related to those of other phenolic compounds (Tassou et al., 2000) and contribute to their antioxidant activity. The hydroxyl groups of phenols donate hydrogen to free radicals and thus inhibit the free radical chain reaction and hence the phenolic contents may contribute directly to the antioxidative action (Chatterjee et al., 2007). Consequently, the antioxidant activities of essential oils are often explained by their total phenolics. In this study, Thyme oil has shown maximum free radical scavenging ability by DPPH method and maximum absorbance by FRAP assay indicating strongest antioxidant property. However, Peppermint oil and Eucalyptus oil have shown moderate antioxidant activity. Our findings are in agreements with reports of various other workers who have investigated for similar antioxidant potential of essential oils and their active compounds from India as well as other parts of the world (Dorman et al., 2000; Manuel et al., 2010; Zahin et al.,2010; Vaibhavi et al., 2010). However, the activities slightly differ due to difference of minor phytoconstituents and the variability in the quantity of major active constituents

which may arise due to different agroclimatic condition, plant varieties and extraction processes.

Conclusion

On the basis of preliminary investigation of the present study, it can be concluded that the above tested oils and their major active compounds have the potential for application in real food system and healthcare.

Acknowledgement

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Table- 2 Total phenolic concentration as gallic acid equivalents of essential oils.

Essential oil	*Total phenolics (mg/gm of oil)*
Thyme oil	388±4.2
Eucalyptus oil	300±1.94
Peppermint oil	288.89±1.64

Table- 3 Free radical scavenging activity of essential oils by1,1,-Diphenyl picrylhydrazyl method.

Name of the Essential oil	**Concentration of oil (µg/ml)**				
	Percent decolorization				
	100	**200**	**300**	**400**	**500**
Thyme oil	48.02±2.67	55.24±1.78	63.28±1.98	76.60±2.61	80.26±1.67
Eucalyptus oil	16.46±3.23	37.84±3.22	40.79±1.99	47.52±2.99	50.56±2.67
Peppermint oil	13.73±3.01	21.46±1.78	31.64±3.46	37.74±4.23	44.35±1.86
Control (BHT)	87.41±1.21	88.80±1.23	88.90±3.04	89.20±1.25	90.20±2.15

The above data are the mean ±SD of three replicates.
BHT: Butylated hydroxyl Toluene

Table- 4 The ferric reducing antioxidant power of essential oils by FRAP method.

Name of the compound	**Concentration of oil (µg/ml)**				
	Reducing power at Absorbance (700nm)				
	100	**200**	**300**	**400**	**500**
Thyme oil	0.757±0.09	0.793±0.04	0.971±0.01	1.91±0.11	2.05±0.12
Eucalyptus oil	0.31±0.01	0.402±0.03	0.481±0.01	0.719±0.02	0.991±0.07
Peppermint oil	0.185±0.04	0.318±0.03	0.654±0.03	0.981±0.10	1.710±0.06
BHT (Control)	1.321±0.12	1.432±0.06	1.987±0.12	2.122±0.05	2.542±0.32

The above data are the mean ± SE of three replicates.
BHT: Butylated hydroxyl Toluene

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Enzymatic and Non-Enzymatic Antioxidant Nature of Essential Oils Extracted from Curry Patta , *Pine Apple* and *Chakotra*

Ajay Singh
Department of Chemistry, Uttaranchal University, Dehradun, UK., India
Corresponding author Email: ajay21singh@yahoo.com

Abstract- In the present study enzymatic and non-enzymatic antioxidant activities of essential oil of Murraya koengii (curry patta), Citrus maxima (chakotra) and Pineapple (Ananas comosus) were studied. Estimation of Antioxidant activity were carried out by b-carotene bleaching method, Thiocyanate assay and Deoxyribose assay. In Thiocyanate assay it was observed that all plant essential oils showed antioxidant activity at low and moderate concentration. The maximum antioxidant nature was shown by Chakotra followed by pine apple and curry patta. In Deoxyribose assay all plant essential oils showed antioxidant activity at lower concentration. Curry patta showed the maximum activity followed by chakotra and pine apple. In b-carotene bleaching method, Chakotra essential oils showed maximum antioxidant activity.

Keywords: antioxidant, enzymatic, non-enzymatic activity, essential oils, Murraya *koengii, Citrus maxima* & Ananas comosus.

Introduction

Essential oils are secondary metabolites which are volatile in nature, insoluble in water, highly soluble in organic solvent, vegetable and minerals oils. These oils consist of terpenes, alcohols, esters, aldehydes, ketones and phenols etc. Citrus fruits are notable for their fragrance or essence, partly due to flavonoids and limonoids contained in the rind, and most of them are juice-laden (German, 1999). The juice contains a high quantity of citric acid giving them their characteristic sharp flavor. They are also good sources of vitamin C and flavonoids (Knight, 1998). Essential oils contain many biochemical properties like antioxidant, anti-microbial, antihistaminic, etc. They are used as flavoring agent or disinfectant or as perfume in food industry, perfume industry or pharma industry.

Citrus fruits are important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez- Lopez *et al.,* 2005; Jayaprakasha and Patil, 2007; Ebrahimzadeh *et al.,* 2004). Flavanones, flavones and flavonols are three types of flavonoids which occur in Citrus fruits (Calabro *et al.,* 2004). The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin (Mouly *et al.,* 1994; Schieber *et al.,* 2001).

Antioxidants are substances that reduce, neutralize, and prevent the damage done to the body by free radicals which are the species containing simply electrons that are no longer attached to atoms. Instead of circling the nucleus of an atom (much like the earth circles the sun), free radicals are both free and radical enough to go careening through our cells (Meister, 1994). A process called oxidation creates free radicals and this process happens in the context of normal metabolic processes and our everyday exposure to our environment. In other words, eating, breathing, and going out in the sun, all contribute to the process of oxidation, free radical formation, and the resulting damage that is caused to the cells of our bodies. It causes the deterioration of bone, joints and connective tissue; the wearing out of organs; the decline of the immune system; the irritating advance of the visible effects of aging; and even, possibly, to some extent, the aging process itself (Padayatty et al., 2003). Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in biochemistry of living organisms (Jeffrey, 1986).

Much like the immune system itself which operates at a cellular level, the hardworking vitamin C reaches every cell of the body. The concentration of vitamin C

in both blood serum and tissues is quite high. In fact, this nutrient plays a major role in the manufacture and defence of our connective tissue, the elaborate matrix that holds the body together. It serves as a primary ingredient of collagen, a glue-like substance that binds cells together to form tissues (Padayatty *et al*., 2003). As a water-soluble antioxidant, vitamin C is in a unique position to "scavenge" aqueous proxy radicals before these destructive substances have a chance to damage the lipids. It works along with vitamin E, a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop free radical chain reactions (Valco *et al*., 2007).

Material and Methods

For experiment Curry Patta, Pineapple and Chakotra were collected from local market of Dehradun. All the fruits were peeled off. After peeling, juice was extracted with the help of juicer and mixer in laboratory. Equal volume of the juices was taken and mixed with 50% ethanol. Centrifuged it at 5000 rpm for 10 minutes. From supernatant, discarded the pellet. Then Protein content was estimated by using Lowry's method and Bradford Method (Sawhney and Singh, 2007).

Enzymatic antioxidant activity: Superoxide Dismutase (SOD) activity assay

In this method four solutions A, B, C and D were prepared.

Solution A: 0.1mM EDTA containing 50mM Na_2CO_3.

Solution B: 90µM Nitroblue tetrazolium in solution A.

Solution C: 0.6% triton X in solution A.

Solution D: 20mM hydroxylamine HCl (pH-6)

To, 1.3 ml of solution A and 0.5ml solution B, 0.1 ml of solution C and 0.1 ml of solution D were added and mixed well. 5µl juice extract was added to that reagent mixture and immediately absorbance at 560nm at interval of 20 second was taken. SOD was calculated.

Estimation of ascorbic acid (Vitamin C) of samples was done by using method given in Introductory Practical Biochemistry book by Sawhney and Singh.

β-Carotene bleaching method

In this procedure, the plant extracts, Vitamin E and BHA (butylated hydroxy anisole) were applied on TLC plates and after developing with a suitable solvent system, plates were sprayed with a betacarotene solution and exposed to daylight until discolouring of the background (6h.) The active compounds were seen as orange colour on the plate. Methanolic extracts of Citrus plants, Vitamin E and BHA were used as positive controls. Extracts which showed strong antioxidant activity were subjected to further tests. The same experiment was performed for the isolated fractions and compounds.

Thiocyanate assay

The peroxy radical scavenging activity was determined by thiocyanate method using Vitamin C as standard. Increasing concentration of the fractions in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04M, pH 7) in a test tube and incubated in darkness at 37°C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentage scavenging inhibitions were calculated and were compared with the standard, Ascorbic acid. A control was also prepared replacing water with plant extract.

Observations

Experimental results of protein content in fruit samples are given in Table-1, Ascorbic acid and SOD activities are given in Table-2. Antioxidant activities by b-carotene bleaching method are given in table-3.

Results and Discussion

From Table- 1, it is observed that for 1ml juice, 3.97 gm of curry patta, 4.17 gm of Chakotra and 4.07 gm of Pine apple were needed. Curry patta was found to have maximum protein content i.e. 125µg/ml, followed by pine apple and chakotra.

Ascorbic acid & SOD in citrus fruits, Superoxide dismutase i.e. enzymatic antioxidant property was found to be maximum in Lemon (18.5unit/mg of protein) followed by chakotra (17.6 unit/mg). Ascorbic acid in the citrus fruits was observed maximum in lemon juice (0.65 mg/ml) followed by chakotra (0.62 mg/ml). Ascorbic acid on the basis of weight of fruit was observed maximum in Chakotra then in lemon

and close result by pine apple followed by mousambi and orange (Table 2).

Pine apple and other citrus fruits contain different chemical compounds like terpenes i.e. limonene is present in lemon which gives there characteristic taste and smell. Lemons contain significant amount of citric acid which provides low pH and sour taste to juice. They also contain vitamin C (ascorbic acid) which is essential to human health. Antioxidant property of citrus fruits is mainly due to vitamin C (ascorbic acid), terpenes like limonene and flavonoids compounds. The results reported by Gavy and Singh also support our findings (Padayatty *et al*., 2003).

β-Carotene bleaching method

Antioxidant activity by b-Carotene Bleaching method was determined by taking 10 and 20 mL of essential oils. Results are given in Table- 3. In this method, Chakotra has shown maximum activity followed by curry patta & pine apple. Similar trend was obtained when concentration of essential oil was increased up to 20 mL. But on very high oil concentration, antioxidant property might vary. Thiocyanate method also showed that Chakotra has maximum antioxidant property, followed by curry patta and pine apple.

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Table- 1 Protein content in samples

S.No.	Samples	Wt. of sample for 1ml of juice (gms)	Protein in Juice Extract µg/ml	Protein in sample µg/gm
1.	Curry Patta	50/12.6 = 3.97	118	118/3.97=29.5
2..	Chakotra	50/12=4.17	115	115/4.17=27.6
3..	Pine apple	50/12.2=4.09	120	120/4.09=29.3

Table- 2 Ascorbic Acid and SOD in citrus fruits

S.No.	Fruit Sample	SOD Activity(unit/mg)	Ascorbic acid mg/ml of juice	Ascorbic acid in per gram of sample
1.	Curry Patta	**18.5**	**0.65**	0.65/5=0.13
2.	Chakotra	17.6	0.62	0.62/4.17=0.14
3.	Pine apple	16.9	0.53	0.53/4.09=0.129

Table- 3 Antioxidant activity by β-carotene bleaching method

S.N	Fruit Sample	Antioxidant activity when Quantity of essential oil -10µL	Antioxidant activity when Quantity of essential oil - 20µL
3	Curry Patta	69.3	72.0
4	Chakotra	70.4	**72.9**
5	Pine apple	68.3	70.0

Screening of Medicinal Plants for Antityphoidal Activity Against Multidrug Resistance in *Salmonella Typhi*

Neha, Chauhan, S., Sharma, Y., Tagore, T., Alam, A., Khan, M.A. and Farooq, U. *

Molecular and immune-parasitology research laboratory, Department of microbiology, faculty of biotechnology, Shoolini University of biotechnology and management Sciences, Solan, 173212, Himachal Pradesh, India.

*Corresponding Author

Dr. Umar Farooq,

Email:ufarooq8@gmail.com

Abstract- Typhoid is an major public health problem in tropical and subtropical countries including India. Development of multidrug resistance in *Salmonella typhi* is a major problem. There is a great need to develop some new promising natural drugs with high efficacy and lesser side effects as compared to synthetic drugs available in the market for the treatment of typhoid fever. In the present study, four different medicinal plants species,*Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) were screened invitro for their anti-*Salmonella* activity. The aqueous and methanolic extracts of all the four medicinal plants were prepared and the efficacy of these extracts was screened against MDR strains of *S. typhi.* A total of 14 laboratory adopted *S. typhi* strains was used to check the efficacy of aqueous and methanolic extracts of *Ficus benghalensis*, *Ocimum sanctum*, *Ocimum basilicum*and *Mentha arvensis.* Antibiotic susceptibility of all strains were also check to confirm MDR strains of *S. typhi.*It was observed that these strains were found resistant to Trimethoprim, Co-Trimoxazole and Sulfonilamide, and sensitive to Penicillin G, Amikacin, Amoxicillin, Levofloxacin and ciprofloxacin. Furthermore, the aqueous extracts of *Ficus benghalensis, Mentha arvensis* showed maximum inhibitory activity whereas the aqueous extract of *Ocimum sanctum* and *Ocimum basilicum* showed moderate activity against *S. typhi* isolates. While methanolic extracts showed low efficacy against all isolates. The study suggested that the aqueous extracts of *F. behgalensis* and *M. arvensis* have potent antibacterial activity against MDR strains.

Keywords- Anti-salmonella activity, MDR- multidrug resistance, *Salmonella typhi,* aqueous and methanolic extract.

Introduction

Typhoid fever is a worldwide disease primarily found in countries where sanitary conditions are poor. It is considered to be one of the most under-reported diseases in the developing world. In some areas, it has been estimated that typhoid fever is responsible for 2 to 5% of all deaths. *S. typhi*, the etiological agent of typhoid fever, is a gram-negative, flagellated, non-capsulated, non-sporulating, facultative anaerobic bacillus. It has asomatic (O), antigen (oligosaccharide), flagellar (H), antigen (protein) and an envelope (K) antigen (polysaccharide); and has a Lipopolysaccharide macro-molecular complex called endotoxin that forms the outer portion of the cell wall (Zaidi *et al.,* 2003). The multiple drug resistance (MDR) among these bacterial strains is increasing day by day and MDR strains have become more popular. So there is the need to develop new drug based on natural product to overcome this situation. Herbs are the most reliable resources to be studied to discover the chemical entities for health care. Today, there is a widespread demand for drugs derived from plants, and people believe that medicines derived from plants are safe and dependable compared to synthetic drugs that have adverse effects (Ravikumar *et al.,* 2010). Therefore, there is a need to develop alternative antimicrobial medicines from other sources such as plants for treatment of infectious diseases. The present study was planned to screen medicinal plants for antityphoidal activity.

Material and Methods

Microbial strains

Standard of *Salmonella typhi* (MTCC-733)was obtained from IMTECH, Chandigarh and 14 clinical cultures were obtained from the Molecular and Immune-parasitology

research laboratory, Department of Microbiology, Faculty of Biotechnology, Shoolini University, Solan, H.P.

Characterization and Identification of *S. typhi*

Strains were done by using different specific media and compared to standard. All the clinical isolates were identified by biochemical tests.

Plant materials

Fresh parts of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) were used for the study by preparing their Aqueous and methanolic extracts.

Antityphoidal activityassay

All 14 isolates with standard were cultivated in specific media and screened for 8 antibiotics (i.e., Levofloxacin, Amikacin, Amoxicillin, Penicillin G, Trimethoprim, Sulfanilamide, Co-Trimoxazole and Ciprofloxacin) according to Kirby-Bauer method for to identified MDR strain. All MDR and sensitive strains were then screened with aqueous and methanolic extracts of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) by using Agar well diffusion method.

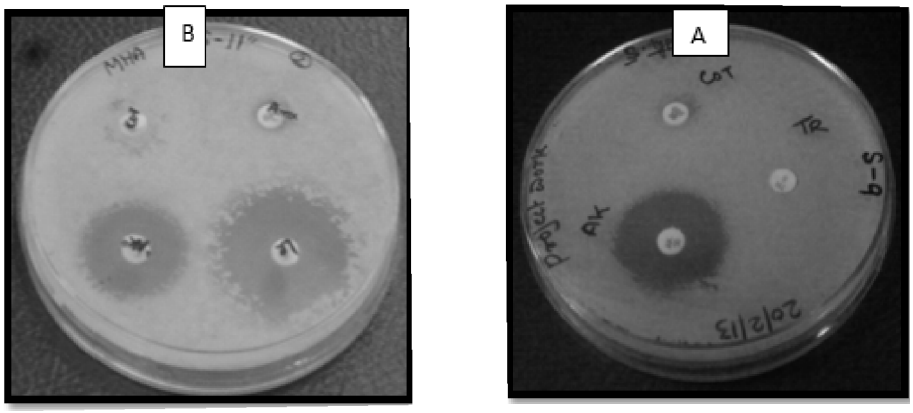

Figure-1 Showing the efficacy of Antibiotics- (A) resistant to Co-trimoxazole, Trimethoprimand sensitive to Amikacin. (B) Resistant to Amoxycillin, Co-trimoxazole and sensitive to Levofloxacin and Amikacin.

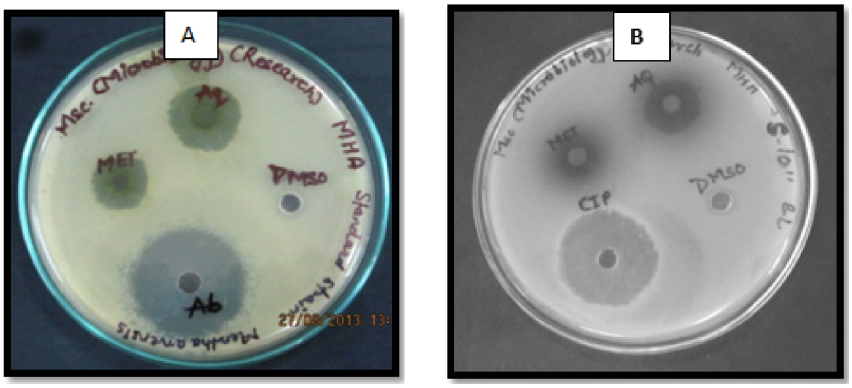

Figure-2 (A) Showing the zone of inhibition against aqueous and methanolic extract of *Mentha arvensis* against *S. typhi* and (B) Showing the zone of inhibition against aqueous and methanolic extract of *Ocimum sanctum*.

Results and Discussion

The biochemical analysis showed that all 14 strains and standard strain is *S. typhi*. The isolates were found highly resistant to Trimethoprim, Co-Trimoxazole and Sulfanilamide, 70% resistant to amoxicillin and 70-100% sensitive for Penicillin G, Amikacin, Amoxicillin, Levofloxacin and ciprofloxacin (Table-1, Fig.-1). All sensitive and MDR isolates were screened with the aqueous and methanolic extract of all four medicinal plants. It was observed that the aqueous extracts showed more efficacy against MDR and sensitive strains of *S. typhi* (Table-2, Fig-2) while methanolic extract of leaf of all plants were found less sensitive and showed very low efficacy against all strains of *S. typhi.*

Typhoid fever is a major public health problem in tropical and subtropical countries including India. The development of MDR strains in causative agent *s. typhi* is an alarming situation in endemic region. To overcome this problem, development of a new drug is an urgent need. The resistance against various drugs has increased due to indiscriminate use of commercially available antimicrobial drugs commonly used for the treatment of infectious diseases. This has led to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. Nearly 80% of the world population depends on the traditional medicine for primary health care, mainly including the use of natural products (Shetty*et al*., 2006).

In the present study, four plants were screened for their antimicrobial activity. The aqueous and methanolic extracts of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) were screened against MDR and sensitive strains of *S. typhi.*We observed that the aqueous extract of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) have potent anti *Salmonllla* activity and large zone of inhibhition was observed on agar plates. The methanolic extract of all four plants screened showed no or small zone of inhibitions. This suggested that the methanolic extracts of leaf of all plants do not harbor any molecule which has antibacterial activity while the aqueous extract of leaf harboring the natural molecules has antibacterial activity. Similar to our study, Coutinho et al (2008) reported that the *Mentha arvensis* have potent antibacterial activity and capable of controling MDR strains. The author also observed that the *Mentha arvensis* could serve as a source of plant-derived natural products with antibiotic resistance-modifying activity to be used against multidrug resistant and other pathogens (Coutinho *et al*., 2008).The present study has determined that the aqueous extract of *Mentha arvensis* and *Ocimum sanctum* have high efficacy against the MDR strains of *S. typhi* and able to control the growth of *S. typhi* invitro, which may be used to derive natural molecule. Another fact may be implied that the antimicrobial action of aqueous extract may be due to synergistic action of different phytochemical constituents probably present in the extracts (Bibitha *et al*., 2002).

Table -1 Antibiotic susceptibility analyses for *S. typhi* isolates

Isolates	Zone of Inhibition (in mm)							
	Levofloxacin	Amikacin	Ciprofloxacin	Amoxicillin	Penicillin-G	Co-Trimoxazole	Trimethoprim	Sulfanilamide
S*	17mm	17mm	27mm	18mm	19mm	NZ	NZ	NZ
S-3	30mm	26mm	26mm	15mm	15mm	NZ	NZ	NZ
S-4	36mm	31mm	27mm	17mm	22mm	NZ	NZ	NZ
S-5	19mm	26mm	28mm	29mm	19mm	NZ	NZ	NZ
S-6	30mm	27mm	26mm	10mm	22mm	NZ	NZ	NZ
S-7	28mm	25mm	26mm	9mm	10mm	NZ	NZ	NZ
S-9	28mm	25mm	27mm	26mm	10mm	NZ	NZ	NZ
S-10	28mm	25mm	27mm	9mm	NZ	NZ	NZ	NZ
S-11	33mm	26mm	26mm	10mm	17mm	NZ	NZ	NZ
S-12	26mm	20mm	26mm	17mm	20mm	NZ	NZ	NZ
S-13	29mm	25mm	27mm	17mm	19mm	NZ	NZ	NZ
S-14	32mm	29mm	27mm	18mm	18mm	NZ	NZ	NZ
S-15	25mm	28mm	27mm	17mm	10mm	NZ	NZ	NZ
S-16	30mm	26mm	27mm	9mm	14mm	NZ	NZ	NZ
S-17	36mm	33mm	26mm	34mm	11mm	NZ	NZ	NZ

Where, S*- Standard strain of *S. typhi* (MTCC 733); highly sensitive >25; Sensitive >17; Intermediate 11-16; Resistant <13; NZ- No zone formation; mm- millimeter

Table-2 Antimicrobial activity of methanolic and aqueous extract of different plants against MDR and sensitive isolates of *S.typhi*

Isolates	Zone of Inhibition (in mm)									
	Ficus benghalensis		*Ocimum sanctum*		*Ocimum basilicum*		*Mentha arvensis*		DMSO	Ciprofloxacin
	(AE)	(ME)	(AE)	(ME)	(AE)	(ME)	(AE)	(ME)	-ve control	+ve control
S*	16	8	20	16	14	6	23	20	NZ	27
S-3	15	7	19	16	14	5	22	19	NZ	26
S-4	17	8	20	16	14	5	23	20	NZ	27
S-5	16	7	18	15	13	5	23	20	NZ	28
S-6	16	6	20	14	14	7	22	20	NZ	26
S-7	15	7	20	16	12	5	21	20	NZ	26
S-9	16	8	20	14	14	5	23	19	NZ	27
S-10	16	7	20	16	12	6	23	19	NZ	27
S-11	17	7	19	15	14	5	23	20	NZ	26
S-12	17	6	20	16	14	5	22	19	NZ	26
S-13	16	9	20	16	14	7	22	19	NZ	27
S-14	16	8	20	16	14	6	22	19	NZ	27
S-15	16	7	20	10	12	5	22	19	NZ	27
S-16	16	7	19	15	14	5	23	20	NZ	27
S-17	16	7	19	15	14	5	23	19	NZ	26

Where, NZ- no zone formation; Resistance - < 7; Sensitive - >15; Intermediate 6-7; Highly sensitive- > 20; S*- Standard strain of *S. typhi* (MTCC 733); (AE) –Aqueous extract; (ME) – Methanolic extract

Conclusion

The present study suggested that the antibacterial activity of aqueous leaf extract of selected plant materials against MDR strains of *Salmonella typhi* was observed high determined that these plants are capable in controlling the MDR strains. Further research is required to explore the natural products from these plants to develop new drugs.

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Inhibitory Activity of *Ageratum conyzoides* Leaves Extract Against Pathogenic Bacteria

S.Farooq, A.K.Dixit[1] and Zafar Mehmood[2]
[1] Email: dixit.arunesh@gmail.com
[2]Email: zafarmehmood31@gmail.com

Abstract- Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. With the continuous use of antibiotics, microbes have become resistant causing clinical problems in the treatment of infectious diseases. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Plant materials remain an important recourse to combat serious diseases in the world. One approach is to screen local medicinal plants and to determine their antimicrobial active compounds. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the therapies involves the use of plant extracts or their active constituents. Keeping in view of the importance of herbs traditionally used for the treatment of infectious diseases, this study is designed to evaluate the antimicrobial activity of *Ageratum conyzoides* used in the Indian system of medicine for the treatment of manifestations caused by micro organisms.

Keywords- *Ageratum conyzoide* leaves extract, pathogenic bacteria.

Introduction

Natural plant products known as herbal medicines have long been used to control microorganisms causing plant and human diseases[1]. Medicinal plants are excellent antimicrobial agents because they possess a variety of chemical constituents that are antimicrobial in nature. Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular plant species[2] because of the need for alternative sources of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics[3].

There is thus continuous effort for synthesis of new chemicals having antimicrobial activity. But most of these chemicals are potentially toxic and are not free of side effects on the Host[4]. This has urged microbiologist for formulation of new antimicrobial agents[5, 6] and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents[7].

The aim, therefore of this work was to evaluate the antibacterial efficacy of *A. conyzoides* Leaves extracts on pathogenic bacterial cultures.

Ageratum conyzoides L. is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities. In Central Africa, it is used to treat pneumonia, but its most common use is to cure wounds and burns. Traditional communities in India use this species as a bactericide, antidysenteric, and antilithic whereas in Asia, South America, and Africa, aqueous extract of this plant is used as a bactericide. In Cameroon and Congo, traditional use is to treat fever, rheumatism, headache, and colic. The whole plant is used as an antidysenteric. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhoea, rheumatism, spasms, or as a tonic.

The leaves extract has been potentially active against bacterial infections, fungal derived skin disease and cancer of cervix, eczema, itchiness of eye and to kill lice. Efficacy of this plant has been determined against II and IV star larvae of *Anapheles stephensi*[8].

Material and Methods

The aerial parts of *Ageratum conyzoides* was collected from from the surroundings of Dehradun city located

in Uttarakhand (India) in April 2014. The plant was properly identified and authenticated.

Method of extraction

The aerial parts of *A. conyzoides* at flowering stage were collected, washed, air dried in shadow and grinded by mixer grinder. After grinding, 300 gm of plant material was extracted in 1.2 liters of different solvents (methanol, hexane, and water) separately three times at 40°C to 45°C for 6 hours. The organic solvent was filtered by whatman filter paper till clear solution was obtained. Solvent was evaporated in a rotatory evaporator under reduced pressure (vacuum) at 40°C and the semi solid crude extract was placed in a vacuum oven at 40°C for dryness. The crude extra it was stored in air tight container at dark place[9].

Screening for antibacterial and antifungal activity

The antibacterial and antifungal (anticandidal) activity was carried out by employing 24h cultures of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Activity of aqueous and methanolic and Hexane extracts of *Ageratum conyzoides* was tested separately using Agar well diffusion method[10, 11,12,13,14]. The medium was sterilized by autoclaving at 121°C (15 lb/in^2). About 30 ml of the Agar medium with the respective strains of bacteria and fungi was transferred aseptically into each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in 6mm diameter well. Antibacterial assay plates were incubated at 37 ± 2°C for 24 hrs, antifungal (anticandidal) assay plates were incubated at 28 ±2°C for 48 hrs. The Ciprofloxacin solution was used as a positive control for antibacterial activity, whereas Clotrimazole was used as positive control for antifungal (anticandidal) activity, and diameter of the zone of inhibition was measured.

Results and Discussion

Table- 1 and 2 shows the antibacterial and antifungal (anticandidal) activity of the crude aqueous, Hexane, Acetone and methanolic extracts of *Ageratum conyzoides* on *Staph. aureus, E. coli and Candida albicans*. The methanolic and acetone extract of *A. conyzoides* shows the highest antibacterial activity with the diameter of zone of inhibition ranged 15-20 mm against *Staph. aureus and E. coli.* Hexane and aqueous extract shows the least range with 10-15 mm as the zone of inhibition while no zone of inhibition observed in aqueous extract against *E. coli*. Against *Candida albicans*, methanolic and acetone show the highest zone in the range of 15-20 mm followed by aqueous extract in the range of 10-15 mm while no activity was detected in Hexane extract as depicted in table-2.

The results obtained in this study revealed antimicrobial efficacy of extracts of *A. conyzoides* L. leaves. The active components of these plants may be due to their high non polar compounds. This is similar to the findings of Ijeh et al.[15] (2006), but in contrast to the findings of Obi and Onuohau[16], (2000) who documented ethanol as the best solvent for the extraction of plant active substances of medicinal importance. Methanol extracts were the most potent of all the extracts, suggesting that the active component must be a highly non polar compound.

The antimicrobial activities of these extracts, (methanol and acetone) appeared to be broad spectrum since both the Gram-positive and Gram negative bacteria were sensitive to their inhibitory effects. The choice of these microorganisms used in the work was made due to the fact that some of them are causative agents of intestinal wound and skin infection in human.

Conclusion

It was clearly evident from the study that *A. conyzoides* L. possess antibacterial and antifungal properties. When the antibacterial activity of the extracts of the plant were compared in water, acetone and methanol extracts, significant difference was noticed in their activity. The antibacterial activity of the extracts could be enhanced, if the components are purified. These plants therefore, are potential sources of new drugs for treating infections caused by these clinical pathogens.

Further investigation using bioassay guided fractionation to isolate and characterize the active constituents is under progress.

Antibacterial activity of different extract of *Ageratum conyzoides*

Test organism	Diameter of zone of inhibition (mm)				
	Hexane extract	Methanol extract	Aqueous extract	Acetone extract	Ciprofloxacin
E.coli	1+	2+	NAD	2+	3+
Staph. aureus	1+	2+	1+	2+	3+

*1+; 10-15 mm diameter of zone of inhibition 2+; 15-20 mm 3+; 20-25 mm 4+; Above 25 mm

NAD; No Activity Detected

Antifungal activity of different extracts of *Ageratum conyzoides*

Test organism	Diameter of zone of inhibition(mm)				
	Hexane extract	Methanol extract	Aqueous extract	Acetone extract	Clotrimazole
Candida albicans	ND	2+	1+	2+	3+

*1+; 10-15 mm **diameter of zone of inhibition 2+; 15-20 mm 3+; 20-25 mm 4+; above 25 mm**

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Phytochemical Analysis and Antioxidant Activity of *Glycyrrhiza glabara Linn.*

Suman Lata Chhimwal, Shalini Pathak and Sadhana Pandey Tiwari
Oil Extraction, Environmental and Disaster Management Laboratory, Department of Chemistry, DAV (PG) College, Dehradun, UK., India

Abstract- The Licorice plant (*Glycyrrhiza glabara*, family leguminosae), also known as "sweet roots" (contains a compound 50 times sweeter than sugar) and is therefore used in confectionary industry and also finds its application in pharmaceutical industry too; this is attributed to a credible biologically active root component Glycyrrhizin. A study was conducted to evaluate preliminary phytochemical studies and explore the free radical scavenging activity of licorice root extract. Extracts show the presence of various phytochemicals. The methanolic extract shows significant antioxidant activity.

Keywords-*Glycyrrhiza glabara,* Phytochemical analysis, Antioxidant activity.

Introduction

Since the origin of Human life, plants continue to play a therapeutic role to improve Human health. They have been used throughout the world as drugs and remedies for various diseases. Licorice (or liqourice) is a plant of ancient origin and steeped in history. It grows in subtropical climates in Europe, the Middle East, and Western Asia. Licorice extracts and its principal component Glycyrrhizin has extensive uses in food, tobacco products and snuff and in traditional herbal medicines. It is a perennial herb which possesses sweet taste and is, therefore used as a flavouring agent in ayurvedic medicines to disguise the unpleasant flavour of other medications (Biondi et al., 2005). Usually in traditional system of medicine, roots and rhizomes of *Glycyrrhiza glabara* are used. These have extensive pharmacological properties. It contains triterpene, saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances. Hispaglabridins A and B have significant antioxidant activity and both the glabridin and glabrene possess estrogen like activity.

Licorice has been shown to have great antioxidant free radical scavenging (*Haraguchi et al., 1998; Di Mambro & Fonseca, 2005*) and anticonvulsant activities (*Nassiri-Asl et al., 2007*).

It has been shown to decrease circulating levels of testosterone in men *(M.M Rafi et al 2002, D Armanini et al 2002)*. The effect of Glycyrrhizin(GR) on HIV replication in cultures of peripheral blood mononuclear cells (PBMS) from HIV infected patients has also been investigated. The antioxidant and antimicrobial constituents of licorice were studied by some researchers. The antimicrobial Flavanones from leaves of licorice were studied by *Fukui, H..K, et al, and Li.WY*.

Material and Methods

Plant material- *Glycyrrhiza glabara* was collected from the garden of Himalaya Drug Company, Dehradun where medicinal herbs are cultivated.

Extraction

The air dried material (150gm) was finely pulverized and extracted by percolation with water, MeOH (methanol) and hexane for one week at room temperature. The combined extracts were filtered and concentrated using Soxhlet apparatus to obtain crude extract of about 10 gm each of water, methanol and hexane.

Phytochemical screening

The extracts were analyzed for the presence of various phytochemicals such as Tannins, Flavonoids, Saponins, Steroids, Terpenoids, Alkaloids, Glycosides, Proteins. The following tests were performed. (Siddiqui and Ali, 1997).

Tannins

Ferric chloride test- Extract was treated with ferric chloride; appearance of blue colour indicated hydrolysable tannins and green colour condensed tannins.

Flavonoids

Alkaline reagent test- To the test solution few drops of sodium hydroxide solution(1ml 10% NaOH to 3ml extract) was added, intense yellow colour was formed which turned to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Saponins

Froth formation test- 2ml solution of extract was placed in water in a test tube; on shaking stable foam was formed.

Steroids

Salkowski test- Treated the extract with few drops of concentrated sulphuric acid (about 5 drops to 1 ml), red colour at lower layer indicated presence of triterpenoids.

Triterpenoids

Salkowski test- Extract was treated with few drops of concentrated sulphuric acid; formation of yellow coloured, lower layer indicated the presence of triterpenoids.

Alkaloids

1 ml of 1% HCl was added to 3ml extract in a test tube. The mixture was heated for 20 mins, cooled and filtered. About 2 drops of Mayer's reagent to 1ml of extract was added. A creamy precipitate formation indicated presence of alkaloids.

Antioxidant activity

The antioxidant activity (free radical scavenging activity of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Brain-Williams et al. (1995).

The ability of plant extract to scavenge DPPH radical was calculated by the equation-

$$\%age = \frac{(\text{Abs. of blank})-(\text{Abs. of sample})}{(\text{Abs. of blank})} \times 100$$

Table- 2 % Inhibition of roots of *Glycyrrhiza glabra at different concentration*

Conc.of Samples	Abs.-1	Abs.-2	Mean (Absorbance-sample)	DPPH radical scavenging activity (%)
Blank	0.064	0.064	0.064	0.00
0.05mg/ml	0.037	0.039	0.038	40.625
0.10mg/ml	0.034	0.032	0.033	48.438
0.15mg/ml	0.029	0.029	0.029	54.687
0.20mg/ml	0.023	0.022	0.0225	64.843
0.25mg/ml	0.020	0.020	0.020	68.75
0.30mg/ml	0.018	0.016	0.017	73.437

Conclusion

The DPPH antioxidant assay provides information on reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. The degree of reduction in absorbance measurement is indicative of the radical scavenging potential of the extract. The roots of *Glycyrrhiza glabara* showed the presence of various phytochemicals as tannins, flavonoids, glyceroids, terpenoids, etc. to which the medicinal properties of the plant are attributed. From the present study, it has been concluded that the *Glycyrrhiza glabra is* the good source of phytochemicals and anti-oxidants. Further screening is needed to identify the bioactive compounds responsible for antioxidant activities and its use in treatment of various diseases.

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Karela (*Momordica charantia L)* plant with Leaves, Flowers, Fruits and Seeds shown on the cover page.

Botanical Name: *Momordica charantia L*
Hindi Name: Karela
Sanskrit Name: Sushavi
English Name: Bitter Mellon or Bitter Gourd
Tamil Name: Pavakkachedi
Assamese Name: Karal

Plant description

M. Charantia (bitter melon or bitter gourd) is a flowering vine in the family Cucurbitaceae. It is a tropical plant that is widely cultivated in Asia, India, East Africa, and South America for its intensely bitter fruits that are commonly used in cooking and as a natural remedy for treating diabetes. It is a climbing perennial that usually grows up to 5 m, and bears elongated fruits with a knobby surface. It is a useful medicinal and vegetable plant for human health and one of the most promising plants for diabetes. The plant, which is green when it is young and yellowish-orange when it is ripe, fruits around September or October.

Medicinal properties

Bitter melon is a powerful nutrient-dense plant composed of a complex array of beneficial compounds. These include bioactive chemicals, vitamins, minerals and antioxidants which all contribute to its remarkable versatility in treating a wide range of illnesses. The fruits contain high amounts of vitamin C, vitamin A, vitamin E, vitamins B1, B2 and B3, as well as vitamin B9 (folate). The caloric values for leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively.The fruit is also rich in minerals including potassium, calcium, zinc, magnesium, phosphorus and iron, and is a good source of dietary fiber (bitter melon "monograph", 2008). Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates, all of which confer a bitter taste. The antidiabetic effects are triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds

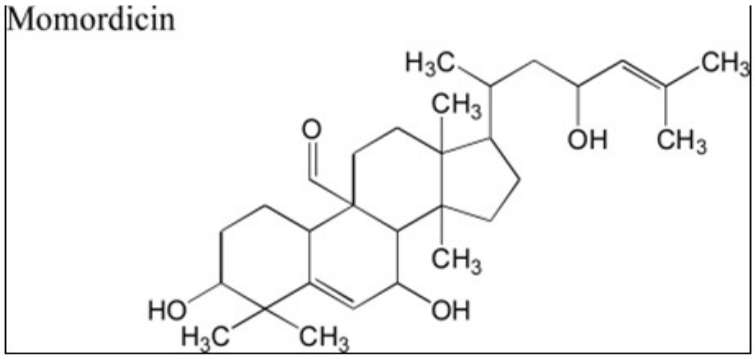
Phytochemistry

Bitter melon is used both as a medicine and as a vegetable Fruit has medicinal properties such as antidiabetic, anticancer, anti-inflammation, antivirus, and cholesterol lowering effects. It contains many phenolic compounds that may have the potential as antioxidant and antimutagen. The fruit, stems, leaves and roots of bitter melon have all been used in traditional medicine to help treat ailments such as hyperlipidemia, digestive disorders, microbial infections and menstrual problems. Bitter melon has been shown to possess powerful antiviral properties that can stimulate the immune system and activate the body's natural killer cells to help fight off viruses such as white

spot syndrome virus and human immunodeficiency virus.

Studies have also shown that bitter melon has anti-carcinogenic properties and can be used as a cytotoxic agent against many types of cancer. Studies have also shown that bitter melon modulates signal transduction pathways for inhibition of breast cancer cell growth and can be used as a dietary supplement for prevention of breast cancer.

The main constituents of bitter melon which are responsible for Several glycosides have been isolated from the *M. charantia* stem and fruit and are grouped under the genera of cucurbitane-type triterpenoids. In particular, four triterpenoids have AMP-activated protein kinase activity which is a plausible hypoglycaemic mechanism of *M. charantia*.



Active constituent of Karela (*Momordicin*)

Forth Coming Events

- **Dates**: 13 Jan 2015 to 18 Jan 2015.
  Location: Vancouver, BC, Canada
  Abstract: The PI3K-Akt-mTOR signaling pathway is one of the primary mechanisms for controlling cell growth, survival, and motility in response to intracellular signaling and extracellular cues. This meeting brings together scientists and clinicians from academia and industry to discuss the opportunities and liabilities of targeting the PI3K and related pathways in disease.
  Topics: biochemistry, cancer, immunology, cell biology, drug discovery
  Weblink: http://www.keystonesymposia.org/15J2
  Contact: Attendee Services; Phone: [970-262-1230 or 800-253-0865]; Email: info@keystonesymposia.org
  Related subject(s): Biology; Oncology

- PHARMAceutical EXPO 2015
  23-25 January 2015, Hyderabad, Telangana, India.
  Venue: Hitex Exhibition Centre, Hyderabad, Telangana, India.
  The event will be attended by more than 8000 business delegates and eminent speakers. The delegates will include CEOs & top executives of the pharmaceutical industry, officers from regulatory departments, pharmacists from trade & profession, research & development personnel, pharmaceutical consultants, hospital administration, top officials from centre & state agencies, academicians and teachers from medical & pharmacy colleges. The expo would showcase latest technological developments in pharmaceuticals, drug & formulations; display of latest pharmaceutical machinery, plants, laboratory equipment, analytical instrument & cleanroom equipments; direct access to highly targeted senior pharma executives, buyers, procurement managers & contract manufacturers; and meeting wit business development managers who are looking for new supplies, building strategic partnerships or entering into new ventures.
  Parallel Event: 66th Indian Pharmaceutical Congress (IPC).
  Organizers: Kamal Bhardwaj, Deputy Director, Federation of Indian Chamber of Commerce and Industry (FICCI)- Trade Fairs, Federation House, Tansen Marg, New Delhi- 110001, India.
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  URL: www.pharmaexpo.in

- **OMICS Group Conferences** invites all the participants across the globe to attend the **"5th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems"** (**Pharmaceutica-2015**) slated on March 16-18, 2015 at Crown Plaza, Dubai, UAE.
  Pharmaceutica-2015 conveys recent developments in Nano-drug delivery systems, novel preformulation, formulation approaches and strategies have reoriented the focus of almost all the Pharmaceutical and Biopharmaceutical R&D's towards Novel Drug Delivery Systems (NDDS). To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. A complete knowledge of the relevant therapeutic and physicochemical properties of the drug enables determination of its proper formulation and delivery method.
  Email:pharmaceutica@pharmaceuticalconferences.com

- **3rd Pharma Pro & Pack Expo 2015**
  13-15 May 2015, Mumbai, India.
  ***Venue*:** Mumbai Exhibition Centre, Mumbai, India.
  A third international exhibition on total pharma manufacturing technologies. The exhibitor profile include: pharma machineries & equipments; processing, packaging & analytical lab; pharma materials; packaging materials& consumables; lab

glassware & consumables; environment control equipments & services; water management products & services; and turnkey contractors.
***Co-located Show*: iPHEX 2015** – An international exhibition for pharma and healthcare.
***Special Pavillion*: PharmaLab Expo 2015** – A complete range of analytical lab equipments.
***Organizers:* IPMMA and GPE Expo Pvt. Ltd.,** Global, 402-403, Abhijyot Square, B/h Divya Bhaskar, S.G. Highway, Ahmedabad- 380 051, India.

- **2nd Myanmar Medi-Pharm Expo 2015**
14-16 May 2015, Yangon, Myanmar.
Venue: Tatmadaw Exhibition Hall, Yangon, Myanmar.
The exhibitor profile of the 2nd international medical, hospital and pharmaceutical exhibition include: medicines & pharmaceutical materials; pharmaceutical production / packaging equipment / technology & cleanrooms; medical equipment & technology; laboratory & analytical / testing equipment & reagents; chemical, biotechnology & diagnostics; dental equipment & supplies; cosmetic & beauty products; disinfection & disposal systems; environmental & hospital waste treatment; and healthcare & spa.
***Cost of Participation:* Shell scheme:** USD 2650 per booth of 9 sqm, **Raw Space:** USD 270 per sqm (minimum 18 sqm).
Organizers: Ms Thai Tuyet Huong, Vice General Manager, **M/s Vietnam National Trade Fair and Advertising Company (VINEXAD)**, 9 Dinh le, Hanoi, Vietnam.
Phone: + (844) 382 555 46 (Ext. 433).
Fax: + (844) 393 630 85.
Mobile: +84 90415 4438.
Skype: thai.tuyet.huong
Email: thaihuyan@gmail.com, thaihuyan@hotmail.com, thaituyethuong@vinexad.com.vn, exhvinexad@hn.vnn.vn, huong.vinexad@yahoo.com
*URL:*www.vinexad.com.vn, www.mediapharmexpo.com

- 9th in-PHARMA Japan 2015
1-3 July 2015, Tokyo, Japan.
Venue: Tokyo Big Sight, Tokyo International Exhibition Center, Japan.
A ninth international pharmaceutical ingredients expo & conference which is Japan's most important exhibition for active pharmaceutical ingredients (API)s, intermediates, excipients and formulations. The exhibit profile include pharmaceutical ingredients, APIs, intermediates, additives, functional ingredients, natural extracts, fine chemicals, outsourcing, OEM, various analytical services and equipment. The visitor profile includes marketing & sales, purchasing, production, R&D, management, quality control and engineering.
***Co-located Show:* INTERPHEX Japan 2015** – The twenty-eight international pharmaceutical R&D and manufacturing expo / conference.
Organizers: Ms Megumi Yanai, in-Pharma Japan Show Management, **M/s Reed Exhibitions Japan Ltd.**
Phone: + 813 3349 8519
Email: yanai-pi@pijapan.jp
URL: www.pijapan.jp/en/

- **Dates:** 25 Aug 2015 - 27 Aug 2015
Location: Valencia, Spain
Abstract: Toxicogenomics-2015 is a remarkable event which brings together a unique and International mix of large and medium pharmaceutical, biotech and diagnostics companies, leading universities and clinical research institutions making the Symposium a perfect platform to share experience, foster collaborations across industry and academia, and evaluate emerging technologies across the globe.
Topics: Toxicology Approaches, Concept of Toxicogenomics, Omics Technologies in Toxicogenomics, Biomarkers in Toxicogenomics, Drug monitoring, Predictive Human Toxicity and ADME/Tox Studies, Genomic Approaches to Predictive Toxicology, PK and PD Tools for DNA-Damage Pathways, Adverse Outcome Pathways
Weblink:http:/ toxicogenomics.conferenceseries.com/
Contact: Phone: [+1-650-268-9744]; Email: toxicogenomics@conferenceseries.net
Related subject(s): Pharmacology and Drug Development; Genomics and Bioinformatics

Instructions to Contributors

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The Journal has a Helping Board of Editors which assists young researchers free of charge for modification, correction and preparation of proper format of Research paper for publication. In that event such paper/s may be addressed to 'The Director Helping Board of Editors', Universities' Journal of Phytochemistry and Ayurvedic Heights, 1- Inder Road, Dehradun (U.K.). The papers are accepted for publication on the advice of the refrees.

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3. Tables & Figures should be double spaced on separate pages, numbers consecutively in Roman numerals and placed at the end of manuscript.
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