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Editorial

More than COVID-19 it's after effects now needs concern not only the treating effects but also the side effects and the general health of the world population. Serious long-term complications appears less common but have been reported, especially in patients with severe COVID-19 who were hospitalized. These have been noted to affect different organs in the body systems which include: CARDIOVASCULAR: inflammation of the heart muscle, RESPIRATORY: lung function abnormalities and NEUROLOGIC & PSYCHIATRIC effects like Alzheimer, depression, anxiety and changes in mood.

India encountered COVID-19 with corona vaccines successfully and by the grace of Almighty no such side effects was noticed as was noticed by the treatment of cancer (multiple myeloma) from Thalidomide medicine given to the pregnant ladies they gave birth to a limbless children, Maxaform a house hold remedy for dysentery gave complete blindness if continuously used for 14 days of prescribed dose. Similarly pain killers give irreversible kidney damage. Now toxicity test are must that's why out of 10,000 products only one receives approval. This issue of UJPAH is based on therapeutic effects of such herbs which are scientifically proved and being used for a very long time under Indian indigenous system.

In India we call them medicinal herbs, in USA dietary supplements and in Japan called kampo and in Russia called Travy (Травы). Traditional Medicines by which ever name they may be called these herbs are being used worldwide in one way or the other. We should promote Indian herbal medicines manufactured under AYUSH. I am happy that this issue deals with the Herbs as the solution to such effects.

I am grateful to the UJPAH board members to make this issue a memorable for science fraternity of the Uttarakhand and to all those Scientists, Research scholars, Students and teachers who contributed for bringing out this issue and the people of science at large. Before concluding, I express my special gratitude to our chief guests, special and other guest present and the Management of Graphic Era University specially Prof. Amit Gupta, Life sciences who have provided all the guidance and help to provide venue and hospitality for holding this seminar. In the end I thank to Prof. (Dr.) Kamal Ghanshala, the president of the Graphic Era University.

Dr. S. Farooq
Chief Editor

Contents

Phytochemical Study and Antioxidant Activities of the Coastal Asteraceae *Achillea Maritima*

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Abstract – This work was focused on the phytochemical study and antioxidant activities of hexane, dichloromethane, ethyl acetate, butanol and aqueous extracts, from the aerial parts of *Achillea maritima*.

The antioxidant activity of *Achillea maritima* extracts was evaluated using the two methods DPPH and FIC. The DPPH activity revealed that ethyl acetate and butanol extracts have an important antioxidant activity at 0.12 and 0.06 mg/mL for the aerial parts of the plant with a percentage of inhibition ranging from 88.29 to 89.72 %, respectively. The results obtained showed that the ethyl acetate extract exhibited a significantly higher ferrous ion chelation activity (83.1%) than the other extracts compared to the EDTA positive control (100%). Dichloromethane and it aqueous extract showed an average capacity for chelation of Fe^{2+} (48.88 % and 45.81 %, respectively). However, the hexane and butanol extracts have a low chelating power (9.5% and 11.3%, respectively).

Keywords: *Achillea maritima*, Organic extracts, Antioxidant activities

Introduction

The human body has a complex system of natural antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a cardiovascular disease (Singh and Jialal, 2006), cancer (Kinnula and Crapo, 2004), neural disorders (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000), mild cognitive impairment (Guidi et al., 2006), Parkinson's disease (Boltonet al., 2000), alcohol induced liver disease (Arteel, 2003).

Plants are potential sources of natural antioxidants. It produces various anti-oxidative compounds to counteract reactive oxygen species in order to survive (Lu and Foo, 1995).

The genus Achillea is widespread all over the world and many species of this genus have been used as traditional herbal medicine.

Among these species, we found *Achillea maritima* (Asteraceae) which is an all-white-cottony plant found on the sands of the Atlantic and Mediterranean coasts.

Therefore, the aim of this study is to evaluate the antioxidative activity of Moroccan Asteraceae *Achillea maritima extracts*, using the two methods DPPH and FIC.

Material and methods

Sampling: *Achillea maritima* was collected at south of El-Jadida city, Morocco (33°14'35"N - 8°32'39"W). The samples were dried at room temperature, desiccated in the open air and protected from the sun's rays, then crushed in order to increase their surface area and facilitate solvent extraction.

Extraction: The samples of the aerial part (200 g) were extracted separately with ethanol (500 mL) for 24 h and the operation was repeated three times in succession. For each part of the plant, the organic phases were combined, dried with Na_2SO_4, filtered and evaporated under reduced pressure. The residue obtained for was solubilized using (200 mL) of distilled water at 100 °C. The mixture was transferred to a separatory funnel and left at room temperature for cooling, and then it was extracted (3x100 mL) with hexane.

The same operations were carried out, increasing the polarity, with the other organic solvents namely dichloromethane, ethyl acetate and butanol (Figure 1).

The four organic phases recovered were dried with anhydrous Na_2SO_4, and then they are filtered, concentrated under reduced pressure.

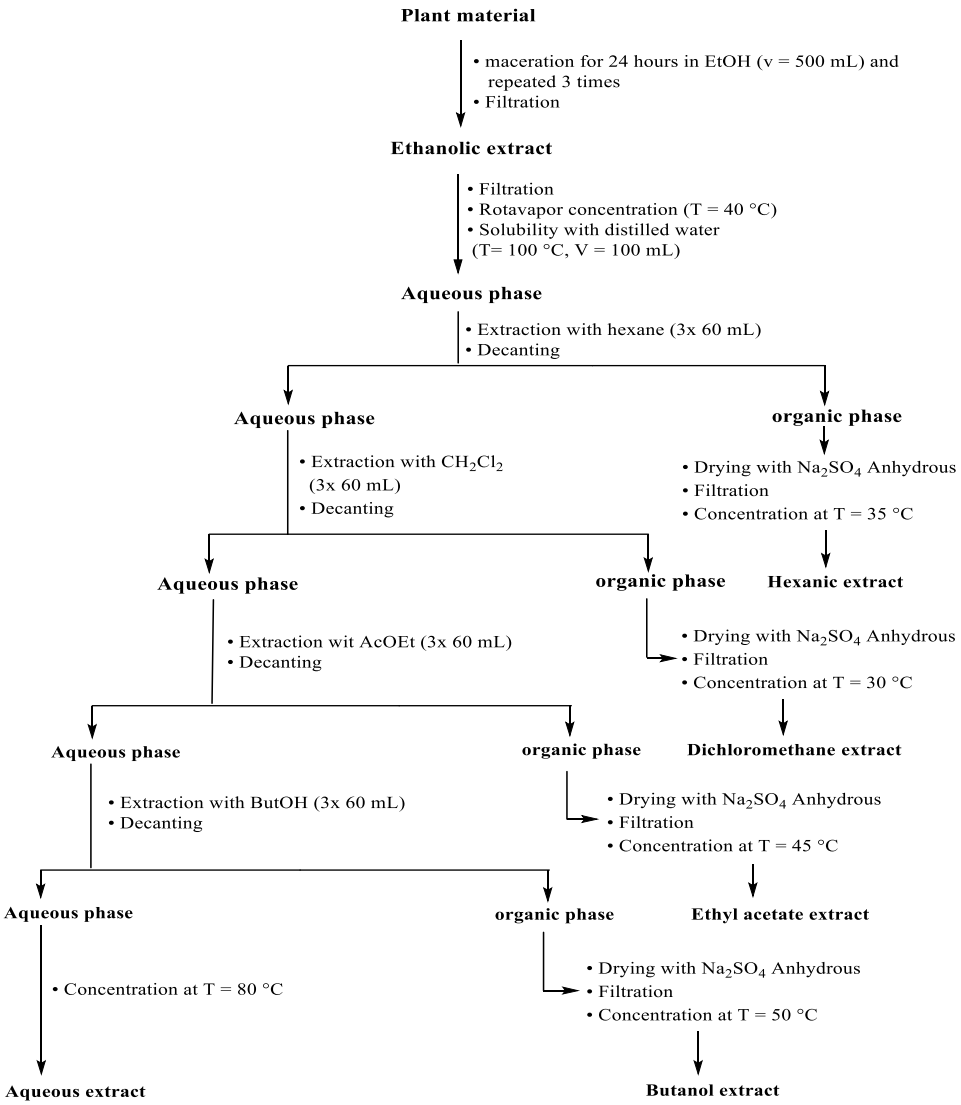

Figure – 1 Extraction protocol to the aerial part of *Achillea maritime*

Antioxidant activities

DPPH radical scavenging activity: The antioxidant activity each extractof *Achillea maritima*was established as diphenyl picrylhydrazyl (DPPH) free-radical scavenging according to the method described (Blois, 1958) with slight modification. DPPH (0.06 mM) was dissolved in methanol and added to each extract (with different concentration). The samples were incubated in the dark at room temperature for 30 min. After which the absorbance was measured at 517 nm using a spectrophotometer (UV-Visible Metashe 5200 HPC). The results were compared to a negative control (all reagents except the test extract) and positive controls (BHT and ascorbic acid). The percentage of DPPH radical scavenging was calculated with the following equation: DPPH scavenging activity (%) = [(Ac-As) /Ac] × 100 where Ac is the absorbance of the negative control (methanol with DPPH solution) and as is the absorbance of the sample.

Ferrous Ion-Chelating ability: The iron ion-chelating activity was determined using the method of (Dinis *et al.* 1994), 2.75 m of distilled water was added to1.0 ml of *Achillea maritima*extracts (with different concentration) after which the solution was mixed with 0.05 ml $FeCl_2$ (2.0 mM), 0.2 ml ferrozine (5.0 mM) and. The mixture was shaken vigorously and incubated for 10 min. at room temperature in the dark. The absorbance of the iron ions-ferrozine complex was measured at 562 nm. The ability of each extracts to chelate iron ions was calculated using the following equation: Chelating activity (%) = [1-(Asample – Ablank) / Acontrol] × 100% EDTA was used as the positive control, $FeCl_2$ solution substituted by distilled water was used as a blank, and the sample substituted by distilled water was used as a negative control.

Results and Discussion

DPPH radical scavenging activity: The *In vitro* scavenging ability of *Achillea maritima* extracts compared to the BHT and ascorbic acid are illustrated in Figure 2. From the obtained results, it was shown that ethyl acetate and butanol extracts have an important antioxidant activity at 0.12 and 0.06 mg/mL for the aerial parts of *Achillea maritima* with a percentage of inhibition ranging from 88.29 to 89.72 %, respectively. The values of EC_{50} obtained in DPPH assay for plant extracts are shown together with that of BHT (Table 1). The butanol extracts exhibited the highest radical scavenging activity with low EC_{50} value (0.06 mg/ml) followed by the acetate extract (EC50 = 0.1 mg/mL).

The difference in the radical scavenging activity of *Achillea maritima* could be associated with the nature of theirphenolic compounds. Accordingly, the strong antioxidant property of ethyl acetate and butanol extracts would be associated with their phenols, including the flavonoids which are responsible for their antioxidant effects and also to their free radical scavenging abilities (Zhang and Björn, 2009).

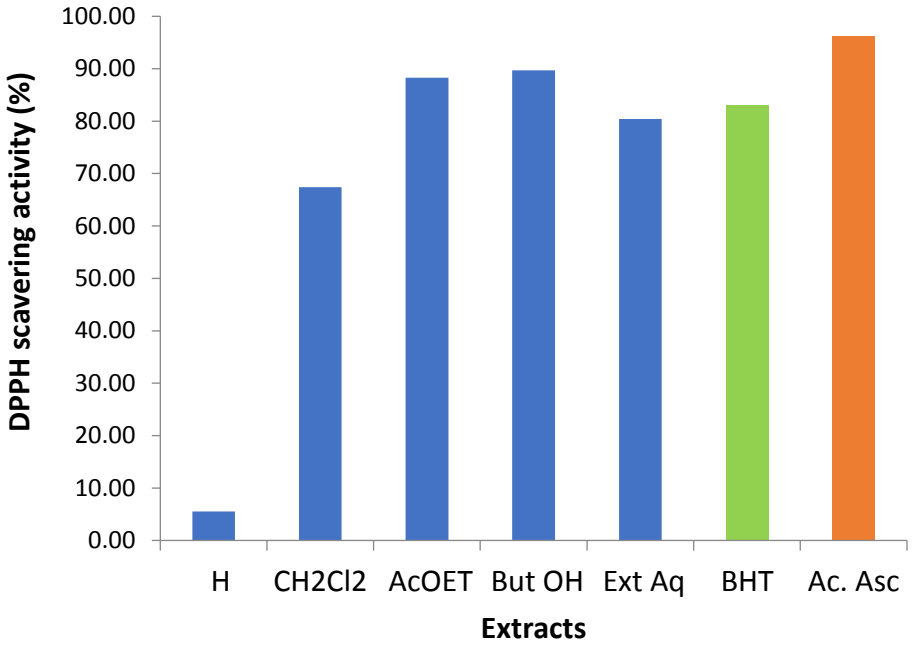

Figure – 2DPPH radical-scavenging activity of hexane (H), dichloromethane (CH_2Cl_2), ethyl acetate (AcOET), butanol (ButOH) and aqueous (Aq Ext) extracts from aerial part of *Achillea maritima* at0.12 mg/mL.

Table – 1DPPH radical scavenging activity (expressed as efficient concentration, EC50) for *Achillea maritima* compared to the BHT.

Extract	DPPH scavenging activity (EC50, mg/ml)
H	16.110
CH_2Cl_2	1.652
AcOET	0.100
ButOH	0.060
Aq	0.370
BHT	0.383

Ferrous Ion-Chelating activity (FIC)

The five organic extracts from aerial part of *Achillea maritima* were tested for their ferrous ion chelating activities. The obtained results showed interesting ferrous ion-chelating ability. Thus, ethyl acetate extract exhibited a significantly higher ferrous ion chelation activity (83.1%) with the EC50 value of 0.03 mg/mL (Table 2) than the other extracts compared to the EDTA positive control (100%). While for the dichloromethane and aqueous extracts showed an average capacity for chelation of Fe^{2+} (48.88 % and 45.81 %, respectively). However, the hexane and butanol extracts have a low chelating power (9.5% and 11.3%, respectively) (Figure 3). It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion(Gordon, 1990). According to (Movahedian *et al.*, 2016), the antioxidant properties of phenolic compounds can be mediated by chelating trace metals involved in free radical production.

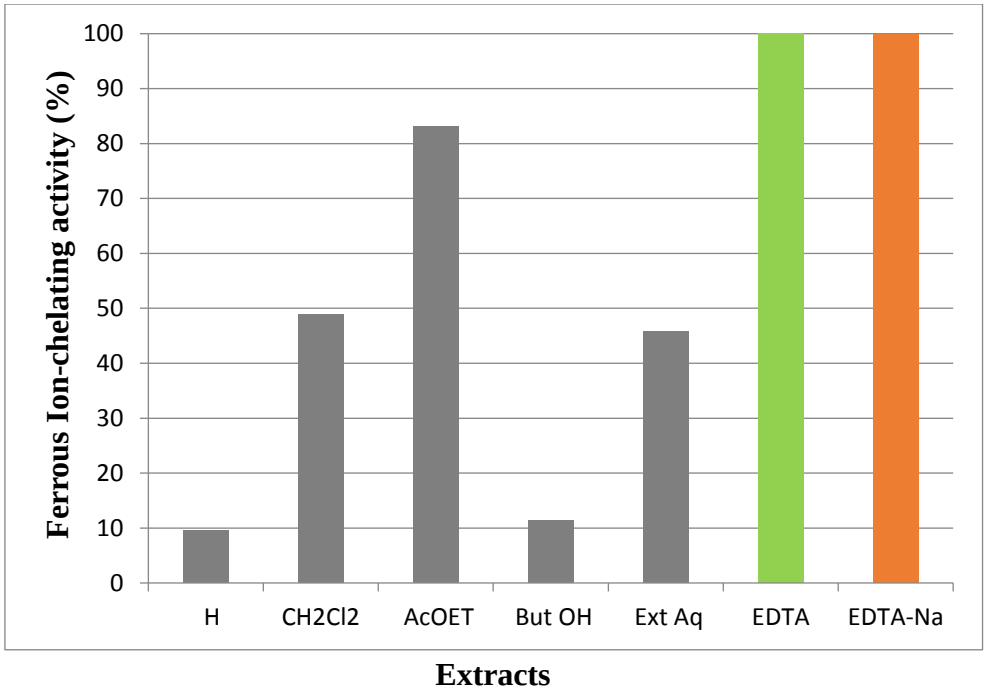

Figure - 3 Ferrous ion chelating activity of hexane (H), dichloromethane (CH_2Cl_2), ethyl acetate (AcOET), butanol (ButOH) and aqueous (Aq Ext) extracts from aerial part of *Achillea maritima* at0.25 mg/mL

Table – 2 Fe^{2+} chelating ability (expressed as efficient concentration, EC50) for *Achillea maritima*

Extract	Fe^{2+} chelating ability (EC50, mg/ml)
H	2.545
CH_2Cl_2	0.283
AcOET	0.033
ButOH	1.152
Aq	0.243
BHT	2.545

Conclusion

In this study, five organic extracts (hexane, dichloromethane, ethyl acetate, butanol and aqueous) from aerial parts of *Achillea maritima,* collected from the Atlantic coasts of Morocco, were tested for their potential antioxidantactivities. The antioxidant activities showed that ethyl acetate and butanol extracts have an important antioxidant activity at 0.12 and 0.06 mg/mL for the aerial parts of *Achillea maritima* with a percentage of inhibition ranging from 88.29 to 89.72 %, respectively. However, ethyl acetate extract exhibited a significantly higher ferrous ion chelation activity (83.1%) with the EC50 value of 0.03 mg/mL.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Effects of Sucrose and Myo-inositol on In Vitro Shoot Multiplicationof Promising Interspecific F1 Hybrid of Eucalyptus (*Eucalyptus Tereticornis X Eucalyptus Grandis*)

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Abstract –Micropropagation technique is standardized for its multiplication, using nodal segments of 25-30 years old trees i.e. promising interspecific F1 hybrid of Eucalyptus (*Eucalyptus tereticornis X Eucalyptus grandis*). 0.1% Mercuric chloride solution for 10-15 minutes used for surface sterilization of nodal segments followed by 0.1% fungicide treatment for 1 minute and then washed 4-5 times with sterilized distilled water. These surface sterilized nodal segments were cultured on MS medium combination with auxin and cytokinin (NAA + BAP) for axillary bud proliferation. MS medium with combination of 1.5mg/l BAP + 0.1mg/l NAA gave optimum rate of axillary bud induction. The *in vitro* shoot were cultured on MS medium with different concentration of BAP (0.1–3.0 mg/l) alone or in combination with NAA (0.1-1.5mg/l) and supplemented with sucrose at 3% level was the best for the growth and development of shoots. These proliferated axillary shoots were excised and subcultured on MS + 1.0 mg/l BAP + 0.1mg/l NAA medium to proliferate *in vitro* shoots.

Keywords: Eucalyptus, Ms medium and Proliferation

Introduction

Tissueculture, amajor aspect of biotechnology has a lot of potential for rapid mass multiplication and large-scale clonal plant development. The Forest Research Institute, Dehradun has generated promising interspecific F_1 hybrids of Eucalyptus that have shown a hybrid vigor in diameter, height, and wood quality. FRI-6 is a hybrid of *E. tereticornis* and *E. grandis* that serves as a control (Venkatesh and Sharma, 1979). Because it contains *E. tereticornis* and *E. grandis* as parent species, this hybrid has a high economic value. The former has a rapid growth rate, best pulp quality, good stem form, and likes high rain fall places, whereas *E. tereticornis* is a drought tolerant species, hence this hybrid is quite likely to be suited for intermediate zones (Venkatesh and Sharma, 1979).

Material and Methods

Explants source and its culture: The source material for micropropagation was nodal segments with a single auxiliary bud. The nodal segments were rinsed in Certified (ICI ltd. India) solution for 4-5 minutes before being surface sterilized with 0.1percent Mercuric chloride solution (10-15 minutes) and then treated with 1.0 percent Bavistin for one minute. Other sterilants, such as $NaOCl_2$ (4%) and H_20_2

(20%), were also investigated for nodal segment sterilization. Surface sterilized nodal segments were washed with sterile distilled water three times. The axillary buds were surface sterilised and grown on Murashige and Skoog's (MS) medium combination with cytokinin (Kinetin and BAP). Before autoclaving the medium at 121^{o} C for 15 minutes, the pH was adjusted to 5.8. Cultures were kept at 25 ± 2 ^{o} C with 16 hours of illumination from white fluorescent tubes at a photon flux density of 2500 lux.

In vitro **multiplication of shoot cultures**Proliferation of axillary shoots was seen in axillary buds cultivated on liquid and semi-solid MS media treated with cytokinin. These axillary shoots were removed and subcultured on semi-solid and liquid MS media containing BAP (0.1-3.0 mg/l) alone or in combination with NAA (0.1mg/l–1.5mg/l). The purpose of this study to increase the number of shoots to find the highest rate of shoot multiplication, a variety of studies were carried out. *In vitro* multiplied shoots were subcultured in a propagule of 6-8 shoots for this. A minimum of 12 repetitions were taken for each experiment. After a 5-week gap, observations were recorded. The shoots produced were excised in propagules and subcultured every 4-5 weeks once the ideal shoot multiplication medium was established. At 25±2^{o}C, cultures were multiplied and kept under a photon flux density of 20-30 $\mu EM^{-2}S^{-1}$ during a 16-hour photoperiod. At the end of the experiment, the number of propagules cultivated and the number of propagules derived were compared.

Statistical Analysis All of the trials (experiments) were carried out three times. There are 12 duplicates ineach treatment. For data from a completelyrandomized design, statistical tools Excel 2.0 and GenStat 8.0 were used to evaluate the data reflecting the means of three experiments. During the study, the data collected for various parameters was subjected to one and two way analysis of variance (ANOVA). The significance of the data was determined using the F-test, and critical difference (C.D.) values of 5% were calculated for comparing differences in treatment means.

Results and Discussion

In vitro shoot multiplication

Effect of Plant Growth Regulators: The proliferated axillary *in vitro* shoots were excised from the mother explants and different hormonal concentration (0.1-3.0 mg/l BAP) used in MS medium for promoteshoot proliferation. These multiplied *in vitro* shoots were later dissected out into propagule (group of 6-7 shoots) and were subcultured on MS medium supplemented with different concentration of (0.1-3.0mg/l) BAP for further *in vitro* shoot multiplication. The best shoot multiplication rate was obtained in MS medium supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA. On this optimal medium the shoot multiplication of 4-5 folds in every 5 weeks subculture duration was obtained (Table-1). Optimum rate obtained in MS medium for the establishment of shoot cultures in Eucalyptus hybrids. In earlier reports, MS medium has been successfully used for shoot initiation and establishment of Eucalyptus F_1 hybrids cultures (Gupta *et al*., 1981, 1983; Kapoor and Chauhan 1992; Chang *et al.,* 1992; Bennett, 1994; Bisht *et al*., 2000a and 2000b; Joshi *et al*., 2003) and *Eucalyptus* F1 hybrids (Arya *et al*., 2009).

Table – 1 Hormonal interaction (BAP+NAA) on *in vitro* shoot multiplication in MS medium. Data recorded after 5 weeks.

Hormonal conc. (mg/l)	Average no. of shoots developed	Multiplication rate	Average no. of shoots length (cm)
0.1 NAA + 1.0 BAP	49.8 ± 1.9	7.12 ± 0.28	1.12 ± 0.03
0.5 NAA + 1.0 BAP	40.5 ± 1.8	5.79 ± 0.26	1.10 ± 0.04
1.0 NAA + 1.0 BAP	31.8 ± 2.00	4.55 ± 0.44	0.75 ± 0.08
1.5 NAA + 1.0 BAP	27.8 ± 1.7	3.98 ± 0.24	0.73 ± 0.09
Significance	***	***	***
CD	6.48	0.947	0.192

NS – non – Significant, *- Significance at 5%,
- Significance at 1%, *-Significance at 0.1%

Effect of sucrose: Because the photosynthetic power of cultures is restricted, carbon sources in the form of sugars must be introduced to the nutritional medium for development and proliferation of cultures *in vitro*. In this case, the growth and development of *in vitro* shoots were examined using varied concentrations of sucrose (1 percent - 6 percent) in MS media. Sucrose at 3% in MS medium produced the best results, with *in vitro* shoot multiplication of 6-7 folds (Table-2). The *in vitro* shoots did not multiply in a sucrose-free media, and the leaves and shoots turned pale green over time. The findings of this study are consistent with the reports of many researchers that used 3 percent sucrose as a carbohydrate source for shoot proliferation in several Eucalyptus species. (Gupta *et al.*, 1981; Gupta and Mascarenhas, 1983; Kapoor and Chauhan, 1992; Bisht 2000a and 2000b). However, there are many reports on successful multiplication of Bamboo shoots with 2% sucrose (Nadgir *et al.*, 1984; Nadgauda *et al.*, 1990; Saxena, 1990; Joshi and Nadgauda, 1997; Yasodha *et al*., 1997). Sharma *et al*., 2013 had reported that increased level of sucrose at 3-4% did not effect shoot number but caused albinism. In this study, at high levels of sucrose (5-6%) no such instance of albinism were noted but the shoot multiplication rate declined. Similarly at 1% sucrose thin shoots and leaves were developed which were not suitable for further subculturing.

Effect of myo-inositol: The effect of myo-inositol on the rate of *in vitro* shoot multiplication was investigated. In comparison to the other concentrations tested, MS medium supplemented with 100mg/l gave the best multiplication rate. *In vitro* shoot multiplication was lowered in MS medium without myo-inositol. Excessive myo-inositol use in MS medium (150 mg/l and above) not only reduced *in vitro* shoot multiplication rate but also had a negative effect on in vitro shoots. As a result, 100mg/l myo-inositol was added to the medium in all trials as the optimal necessary for the growth and multiplication rate of *in vitro* shoots (Table-3).

Table – 2 Effect of different concentration of sucrose on *in vitro* shoot multiplication. Shoots cultured on 1.0 mg/l BAP + MS medium. Data recorded after 5 weeks.

Sucrose concentration	Multiplication rate	Average number of shoots produced	Average shoot length (cm)
0 %	2.57 ± 0.19	12.83 ± 0.94	2.00 ± 0.05
1 %	4.35 ± 0.33	21.75 ± 1.64	2.10 ± 0.05
2 %	7.03 ± 0.30	35.17 ± 1.52	2.40 ± 0.06
3 %	10.53 ± 0.42	52.67 ± 2.08	3.20 ± 0.06
4 %	9.35 ± 0.39	46.75 ± 1.95	2.80 ± 0.07
5 %	8.13 ± 0.38	40.67 ± 1.90	2.20 ± 0.06
6 %	6.55 ± 0.38	32.75 ± 1.87	1.80 ± 0.05
Significance	***	***	**
CD	0.97	4.88	0.174

NS – non – Significant, *- Significance at 5%,**- Significance at 1% *** -Significance at 0.1%± Values represent the Standard deviation

Table – 3 Effect of myo-inositol on shoot multiplication rate. Shoots inoculated on MS medium + 1.0 mg/l BAP. Data recorded after 5 weeks.

Myo-inositol concentrations	Multiplication rate	Shoots produced (Average number)	Average shoot length (cm)
Control	2.00 ± 0.20	10.00 ± 0.10	1.80 ± 0.07
50 mg /l	5.12 ± 0.27	25.58 ± 1.36	2.20 ± 0.07
100 mg/l	9.92 ± 0.43	49.58 ± 2.13	3.10 ± 0.05
150 mg /l	8.50 ± 0.37	42.50 ± 1.84	2.80 ± 0.06
200 mg/l	7.32 ± 0.42	36.58 ± 2.12	2.50 ± 0.06
Significance	***	**	***
CD	0.97	4.94	0.18

NS – non – Significant, *- Significance at 5%, **- Significance at 1%, ***-Significance at 0.1%± Values represent the Standard deviation

Conclusion

Tissue culture technique utilized to standardized *in vitro* shoot multiplication, using nodal segments of 25-30 years old trees of interspecific F1 hybrids of Eucalyptus. The conclusion of this paper is used 3% sucrose with 10mg/l myo-inositol was added to the MS medium supplemented with BAP produced the best results for in vitro shoot multiplication.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Antioxidant and Antitumor Activities of Leaf Extract of *Eclipta Alba*

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Abstract –Medicinal plants are nature's hidden and unexplored treasures (nature's pharmacy) for humanity since times immemorial. The plant *Eclipta Alba* has many medicinal value used in the traditional Ayurvedic and Unani System. *Eclipta Alba* (L.) commonly known as bhringraj as well as false daisy, a species of plant in the family Asteraceae.This herb contains many bioactive components such as coumestans i.e. wedelolactone and dimethyl wedelolactone, flavonoids, stero-ids, etc. We have examined it antioxidant activity through DPPH & reducing power assay and we got in it total phenolic content, flavonoids and sterol as good antioxident agents. The antioxidant activity was assessed through DPPH and reducing power assay, which was explained in terms of effective concen- tration EC50/IC50.

Keyword: *Eclipta Alba*, Flavonoids, Antitumor, Antioxidants and Wedelolactone.

Introduction

The use of synthetic drugs causes many side effects as well as resistance in pathogenic microbes. Thus, there is a need to focus on developing herbal drugs. Among them, a very well-known plant is *E. Alba* (L.) Hassk. It belongs to the family Asteraceae and is native of India and its neighbouring countries.

Past ethnomedicinal literature revealed that plant and plant parts are highly of medicinal value but still these plants only used in limited areas like respiratory tract disorders (including asthma),gastro- intestinal disorders, fever, hair loss. Many types of research were done and found *E. Alba* was effective against many diseases, but still, there is little dark areawhich remainsto be enlightened to the world[1]. According to Saint Vallalaar, *Eclipta* is number one herb in hierarchy and has been reported to possess antimicrobial, anti-cancer and antioxidant properties and promote hair follicle growth (Soni and Soni, 2017).Active ingredients of *Eclipta Alba*are as follows- coumestans i.e wedeloactone,alkaloids i.e, Ecliptalbine, flavonoids, glycosides, polyacetylenes and triterpenoids. The leaves containstigma-sterol, α-terthienylmethanol, wedelolact-one, demethylwedelolactone and dimethyl wedelolactone-7-glucoside[2].

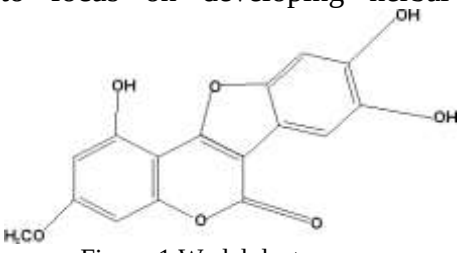
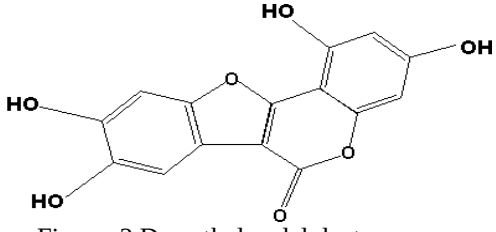


Figure-1 Wedelolactone



Figure–2 Demethylwedelolactone

Emerging research evidence has suggested that the medicinal plants containing a wide variety of natural antioxidants, such as phenolic acids, sterols,flavonoids are of great value in preventing the onset and/or progression of many human diseases[3] (Halliwell et al. 1992).

Material and Methods

Ethics Statement: All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee (IAEC) was obtained as per the prescribed guidelines.

Plant Material:*Eclipta alba* was collected and the sample was verified by Dr. K. R. Arya, Principal Scientist, Botany Division, CSIR-Central Drug Research Institute Lucknow (UP), India.

Preparation of *Eclipta Alba* extract

*Eclipta Alba*was dried in an oven at 40 °C for 5 days and then blended in an electric blender. The powder was kept within 80% alcohol and left at room temperature for overnight soaking. The crude content was filtered through 125 mm Whatman qualitative filter paper under sterile condition. This procedure was repeated 5 times and then solvent (alcoholic extract of *Eclipta Alba*), thus collected, were evaporated to dryness under reduced pressure using a rotary evaporator below 50 °C. The residue was further subjected to dryness by incubating them for 8 days at 37°C. The extract was stored at 4 °C until use. The yield of the extract was 12.5% (w/w).

Phytochemistry: *Eclipta Alba* (L.) contains wide range of active principles which include coumestans, alkaloids, flavonoids, glycosides, triterpenoids. The leaves contain stigmasterol, β-terthienyl-methanol, wedelolactone, demethylwede-lolactone and demethylwedelolactone-7-glucoside. The roots give hentria- contanol and heptacosanol. The roots contain polyacetylene substituted thiophene. The aerial part contains phytosterol, β-amyrinin the n-hexane extract and luteolin-7-glucoside, β-gluco- side of phytosterol, a glucoside of a triter- penic acid and wedelolactone. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid occur in this plant[4].

Sterols and Flavonoids: Sterols seen in *E. Alba* are phytosterol, β-glucoside of phytosterol, daucosterol and stigmasterol-3-oglucoside in the entire plant body. Flavonoids like apigenin, luteolin and luteolin-7-glucoside[4].

Total Phenolic Content: Total phenolic contents in the extracts were determined spectrophotometrically by the Folin Ciocalteau method 29. Dried extracts were reconstituted in distilled water (1 mg/ml). Folin-Ciocalteau reagent (0.5 ml) was added to the extract solution (0.5 ml), and the total volume was adjusted to 8.5 ml with distilled water.

The tubes were kept at room temperature for 10 min, and thereafter 1.5 ml of sodium carbonate (20%) was added. The tubes were incubated in a water bath at 40 ºC for 20 min; the intensity of the blue colour developed was measured by recording the absorbance at 755 nm using a UV-visible spectrophotometer (Varian, CARY-300 Bio). The reagent blank was also prepared using distilled water. For quantification of the total phenolic in the extract, a standard calibration curve was prepared using Gallic acid[1].

Pharmacological Evaluation

Antimicrobial Effects: Extracts of *Eclipta Alba*is used for relieving infections. It fights against all micro organisms which cause boils, infections and inflammations.

Anticancer Properties: Extraction of *Eclipta Alba* is useful in inhibiting the growth of cancer cells.

Insecticide Properties: The extracts of *Eclipta Alba* are effective as an insecticide and provide an opportunity to chemical pesticides. Pest control can be performed in an ecofriendly way. *Eclipta Alba* juice is used to make hair oil which is powerful in controlling several problems related to hair like dandruff, hair falling and hair thinning

etc. The juice of the leaves is used to make Kajal which is useful in relieving ailments related to eyes. In Siddha medicine it is used to relieve several diseases. Popular liver tonic with the name Liv.52 contains this herb. A black dye that is obtained from the plant is used for tattooing and hair dyeing[5].

Antioxidant Activity

The antioxidant effects of *E. prostrata (Syn.E. Alba)* were evaluated in Charles River Sprague-Dawley rats. The extract at 50mg/kg and 100 mg/kg dose significantly reduced the oxidative biomarkers such as serum lipid peroxide, serum hydroxyl radical levels. In another study, the in vitro antioxidant activity was evaluated based on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay. An IC_{50} value of extract was determined to be 45.68µg/mL for the whole plant as compared to the IC_{50} of 3.26µg/mL of standard ascorbic acid. When evaluated using hydrogen peroxide scavenging assay, the extract showed potent activity with the IC_{50} values of 1.34 µg/mL as compared to ascorbic acid (IC_{50}: 1.03 µg/mL)[6]. The antioxidants present in the extract of E.*prostrata* showed the reduction of ferricyanide complex (Fe^{+3}) to ferrous form (Fe^{+2}) in a dose-dependent manner. The highest reducing ability (75.59%) for the whole plant of *E. prostrata* was reported at 250 µg/mL concentration. The IC_{50} value for reducing ability of the extract was 100µg/mL[7]. The studies are mostly conducted using in vitro methods and detailed mechanism is yet to be established.

Results and Discussions

The outcome or result demonstrated that the whole quantity of these alkaloids was considerably different among the solvents, but the ratio pattern of the alkaloid content was established to be helpful in classifying the samples. The total phenol content is maximum in P.ether (92 ± 3) followed by acetone and ethanol 88 ± 3 and 68 ± 2 respectively.

Eclipta prostrate (L.) (Syn. *E.Alba)* is widely used as traditional medicine in various countries especially for skin, liver and stomach problems, and for promoting hair growth. Various compounds such as coumestan derivatives, steroidal, phenolic acids and flavonoids were isolated and identified from the extracts. Similarly, various biological activity evaluations were performed for extracts and isolated compounds such as antioxidative, anti-microbial, Insecticidal and anticancer. Many of these activities were performed based on in-vitro methods and mechanisms of action are not explored in detail using animal models. Properly designed clinical studies are necessary to evaluate the safety and efficacy *E. Alba* in future.

Conclusion

The leaf of *Eclipta Alba* showed significant antioxidant and antitumor activities in the model tested.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Phytochemical Investigation of EtOH Extract of Flowers of *Senecio Chrysanthemoide,* Asteraceae

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Abstract - From the ethanolic extract of flowers of *Senecio chrysanthemoide*one flavone diglycosideone xanthone glycoside have been isolated. The structures of the isolated compounds were identifiedwith the help of chemical and spectral studies.
Key words: *Senecio Chrysanthemoide*, Asteraceae, Flavone Glycosides and Xanthone.

Introduction

Senecio species are used in folk medicine for the treatment of cuts, wounds and as antiemetic, anti-inflammatory, antimicrobialand vasodilator. The parts mostly used are leaves, stem and flowers[1],the pyrrolizidine alkaloids and the furanosesquiterpenoids are the most important constituents of this genus and thought to be responsible for all of pharmacological activities[2-5].The genus *Senecio*, belongs to the tribe Senecioneae, is the largest and most complex genus in the family of the Asteraceae (Compositae) includes more than 1500 species with a worldwide distribution[6].The chemical constituents of the genus *Senecio* include notably sesquiterpenoids, monoterpenoids[7-8], diterpenoids[9], triterpenoids[10], phenolic and flavonoid compounds[11-16], essential oils[17] and pyrrolizidine alkaloids[5]. In India the genus *Senecio* comprises 43 species including *S. chrysanthemoide* which grows endemically in all over India[18].The present paper deals with isolation and characterization of flavone di-glucoside and 3-acetoxyxanthone from the alcoholic extract of the flowers of *S. chrysanth- emoide.*

Material and Methods

Mps. Uncorrected, Column chromatography was carried out on silica gel (60-120) mesh. Merck, eluting solvent ($CHCl_3$MeOH). U.V. was taken in MeOH. ^{1}H-NMR spectra were taken using TMS as internal standard and $CDCl_3$ and CD_3OD as solvents, all the signals are expressed as values downfield from TMS.

Collection of Plant Material

The Flowers of *S. chrysanthemoides* were collected in flowering stage from in Tunganath Chopta, Rudraprayag U.K. India. The plant was identified by expert Taxonomists Department of Botany HNB Garhwal University Srinagar Garhwal. The voucher specimen (H.N.24446) is available in the herbarium of Plant Identification

Laboratory department of Botany HNB Garhwal University Srinagar Garhwal.

Extraction and Isolation

The air-dried and coarsely powered flowers of the plant were defatted with light petroleum in a soxhlet. The defatted mass was exhaustively extracted repeat- edly with 90% aqueous EtOH, until the extractive became colorless. All the extracts were mixed and concentrated under reduced pressure using rotatory vacuum evaporator.The concentrated extract was adsorbed on silica gel and fractionated through column chromato-graphy using the solvent system chloroform: methanol (97:3). The polarity of solvent was gradually increased by addition of methanol. Repeated column chromatography afforded compounds 1 and 2 together with β-sitosterol and kaem- ferol.

Results and Discussion

The ethanolic extract of flowers of *S. chrysanthemoides* on repeated column chromatography over silica gel afforded compounds 1 and compound 2 together with β - sitosterol and kaempferol. The structure of β – sitosterol and kaempferol was confirmed by their comparison with an authentic sample (TLC) and reported data of the compound[19]. The structure of compound 1 was identified as α-L arabinopyranosyl (1→3) β-D glucopyrano-syl (1→3)β – hydroxy oleane -12- ene 28 methyl acetate and compound 2 as xanthone 3-Acetoxy-1-hydroxy-6-methoxy 8-O-β-D glucopyranosyl (1→3) α–L rhamnopyranosyl with the help of chemi-cal and spectral studies.

Compound 1

It was obtained as crystalline solid from MeOH

Melting Point : 220-222 ^{0} C

Molecular Formula : $C_{30}H_{28}O_{16}$

Molecular Weight : 644

^{1}H-NMR (CDCl$_3$,100MHz,δppm):2.66 (d, J=1.6 Hz), 3.49 (s), 3.25 (s), 7.70 (d, J=8.4 Hz), 6.38 (d, J=7.5 Hz),7.02 (d, J=7.5 Hz), 7.29 (d, J= 1.5 Hz), 5.45 (d, J=6.8 Hz), 5.49 (d, J=7.5 Hz),5.42 (d, J=6.8 Hz,C-1' anomeric proton),4.98 (d, J=5.8 Hz ,C-1"), 3.65-4.72 (sugar multiplets)

^{13}C-NMR (CDCl$_3$,150MHz, δ ppm):79.3 (C-2), 43.0 (C-3), 190.1(C-4) 162.8 (C-5), 110 (C-6), 163.5 (C-7), 99.7 (C-8),127.9 (C 9),115.7(C-10), 123.9 (C-11), 113.6 (C-12),141.1 (C-13), 147.0 (C-14)**,** 41(-OCH_3), 40.2 (-OCH_3), 20.9 (-OCH_3)

Arabinopyranosyl: 101.9 (C-1'), 73.1 (C-2'), 76.0 (C-3'), 69.9 (C-4'), 76.0(C-5'), 69.4 (C-6')

Glucopyranosyl: 103.1 (C-1"), 73.1 (C-2"), 75.5 (C-3"), 69.3 (C-4") 75.0 (C- 5"), 60.3 (C-6")

The compound 1 was found to be positive for coloration with methanolic $FeCl_3$, Molish test and Shinoda test (Mg/HCl) there by indicating flavonoid nature of compound. IR spectra of compound displayed absorption bands at 3302, 1665, 1622, 1505 showed presence of hydroxyl and carbonyl functions in the compound. The molecular formula of compound was calculated as $C_{30}H_{28}O_{16}$ which correspond the molecular weight 644 amu ,due to the presence of molecular ion peak at m/z 644 $[m]^+$, 645$[M+H]^+$, 675$[M+CH_3OH]$. Other fragment ion peaks appeared at m/z 452$[(M+H)^+$–(OCH_3+162)] and 273$[(M+H)^+$-(OCH_3+O H+2×162)]$^+$. The ^{1}HNMR spectrum of compound displayed two doublets at δ 6.38 (d, J = 7.5 Hz , C-12) and δ 7.02 (d, J = 7.5Hz, C-13) and one doublet of 1.6 Hz coupling constant appeared at δ 2.66 (C-3) assigned for flavonoid proton. A

doublet of 8.4 Hz at δ 7.70 ascribed to C-6 of flavonoid. The position of two, 3H proton singlet at δ 3.49 and δ 3.25 assigned for two OCH3. The ^{1}H signals appeared at δ 3.60-5.4 ascribes the sugear moiety in the molecule. The position of two doublets at δ 5.42 (d, J= 6.8 Hz) and δ 4.98 (J=7.5 Hz) represents two anomeric sugars. The presence of two sugars were further in agreement with the FAB-MS data displayed peaks at m/z 452 and 273 arose by the loss of two sugar molecule from the molecular ion peak.All These values were in agreement with its^{13}C NMR data. The coupling constant value of anomeric sugar showed β configuration in both the sugar molecule. Compound when hydrolyzed with 7% methanolic HCl furnished two glucose molecules (from PC and TLC). Methylation, methanolysis and partial hydrolytic studies revealed the position of both the sugar in different carbon. All these data were in agreement with the reported data of flavonoidal glycoside[20].Hence compound was identified as **Flavon 5, 4'dimethox 8 methyl 7-O-β-D glucopyranoside-5'-β D-glucopyranoside. (**Figure-1)

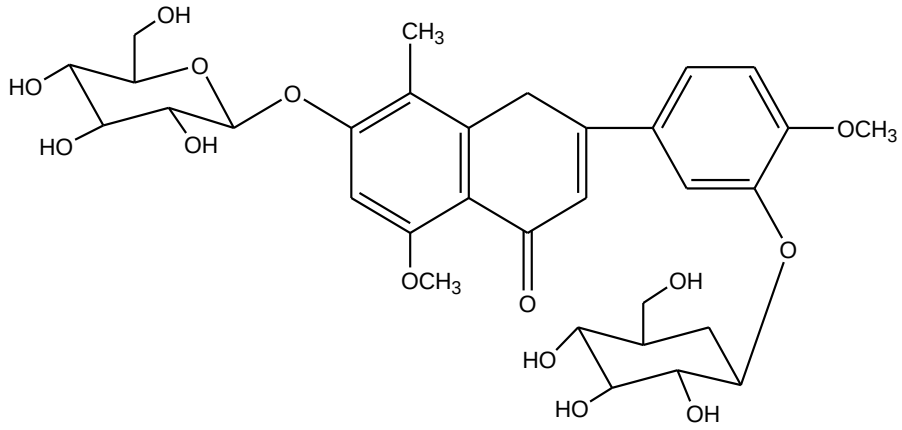

Figure – 1

Compound 2

It was obtainted as colourless crystalline solid from MeOH

M.P. : 196-198^{0} C
Molecular formula : $C_{28}H_{30}O_{15}$
Molecular weight : 606
I.R. (ν_{max}^{KBr}) cm^{-1} : 2995, 3000, 1640
FAB MS (m/z) : 606[M]$^{+}$, 607 [M+H]$^{+}$, 460 [M+ H-Rham]$^{+}$298 [MH–(rha+glu)]$^{+}$, 255, 209
^{1}HNMR (CDCl$_3$100MHz,δppm): 6.83(d,J= 8.4Hz), 7.68(s), 7.24(s), 6.62(d, J=8.4Hz), 2.48 (s), 2.65(s),9.06 (s)

It gave green coloration with $FeCl_3$ and also responded positive test with Molish reagent there by indicating phenolic nature of the compound[21]. IR spectrum of compound showed characteristic bands at 2995, 3000 and 1640 cm^{-1} for phenolic hydroxy and carbonyl groups. ^{1}H - NMR

Glycone:5.43 (d,J=7.8Hz), 5.2(s), 3.5-4.9 (Sugarproton).
^{13}C NMR (CDCl$_3$150MHz, δ ppm)
:Aglycone163.9 (C- 1), 117.6 (C- 2),1473 (C-3),122.21(C-4),125.4(C-5),145(C-6),128.6(C-7),163.3(C-8),191.4(C-9), 18.4(C-10),138.7(C-11), 116.3 (C-12), 115.7(C-13), 115.4(C-14), 176.0 (C=O), 43.3(CH3),56.3 (OMe)
Glycone:103.0 (C-1'),73.6 (C-2'),79.6 (C-3'), 63.1 (C-4'),76.3 (C-5'), 61.2(C-6').
Glycone:101.9 (C-1"),73.4 (C-2''),77.4(C-3''),69.9(C-4"),60.9(C-5"),18.5(C-6').

spectrum of the compound displayed two 1H proton singlets at δ 7.24 and δ 7.68 for C-5 and C-4 hydrogen, which confirmed the presence of xanthone skeleton in compound. Two doublets of 8.4 Hz coupling constant appeared at δ 6.83 and δ 6.62 showing an ortho coupling. Two up field sharp singlets at δ 2.48 and δ 2.65 indicated the presence of two methoxy group in compound, whereas a weak singlet at δ 9.06 were assigned for hydroxyl group. Two anomeric proton resonated at δ 5.43 (d, J = 7.8 Hz) and δ 5.2 (s) with other sugar peaks appeared between δ 3.1-4.9 assigned for 10 sugar protons. The molecular weight of compound was deduced as 606 amu which corresponding the molecular formula $C_{28}H_{30}O_{15,}$ due to the presence of molecular ion peak at m/z 607 $[M+H]^+$The presence ofthese different groups were in agreement with the mass fragmen- tation of a compound as shown by its FAB-MS which furnished peaks at m/z 460 $[M+H\text{-}Rham]^+$, 298$[M+H–(Rham+Glu)]^+$, 255 $[M–(2Glu+COCH_3)]$ and 209$[M-(2gly+C OCH_3+OCH_3)]$. The structure of glycone was further supported by its hydrolysis studies. Compound was hydro- lyzed with 7% methanolic HCl for about 8 hours. It furnished an aglycone identified as 3-Acetoxy 1-hydroxy 6-methoxyxanth- one from its reported data. The neutralized hydrolysate gave two sugars identified as glucose (PC, Rf value 0.18) and rhamnose (PC, Rf value 0.37). Compound 2 on partial hydrolysis yield one rhamnose (PC, Rfvalue 0.37) and one Mono glycosidic aglycone. The prosapogenin on further HOH yield one xanthone as an aglycone (TLC) and glucose (PC, RF) showed the sequential loss of sugars.

The point of glycosidation was established by the ^{13}C-NMR data of sugars which was fixed at C-3 of glucose with C-1 of rhamnose.The configuration was found to be β in glucose and α in rhamnose by the J value of anomeric sugar in its ^{1}H-NMR spectrum. These all values were compared with the reported data of xanthone glycosides[20].Thus on the basis of spectral studies compound 2 was identified as **xanthone 3-Acetoxy-1-hydroxy-6-meth-oxy 8-O–β–D glucopyranosyl (1→3) α–L rhamnopyranoside**. (Figure-2)

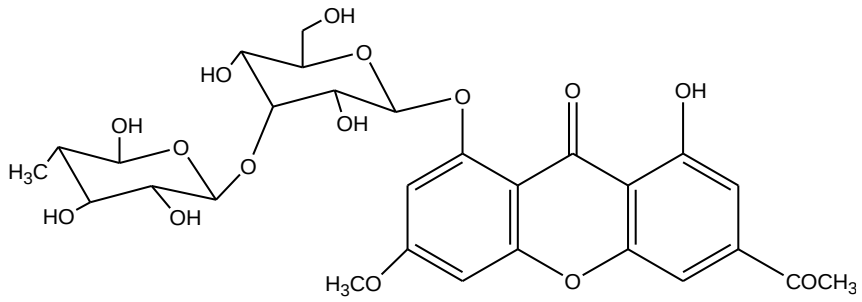

Figure – 2

Acknowledgement

Authors are thankful to IIT New Delhi for NMR spectra and CDRI Lucknow for Mass spectrum of compounds.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research

are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Phytochemical Analysis of Leaves of *Ardisia solanacea* Roxb.

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Abstract – The ethylacetate extract of air-dried and powdered leavesof *Ardisia solanacea* was subjected to repeated coloumn chromatography (CC) over silicagel eluted with choloroform and methanol ($CHCl_3$: MeOH; 100:0→1:1) afforded various fractions which on repeated column chromatography over silica-geleluted with different solvents yielded β-sitosterol, gallic acid, Quercetin, Myricetin and a new alkylphenolic compound identified as (-)-5-(1,2-Dihyd-roxypentyl) benzene-1,3-diol.
Identification of these compounds were made on the basis of analysis of their physical and spectroscopic data and chemical methods.

Key words: *Ardisia solanacea*, Ethyl acetate extract, Alkylphenol, Flavanoids.

Introduction

The *Ardisia* is the largest genus of *Myrsinaceae* family, consisting of approximately 500 species of evergreen shrubs and trees found throughout the subtropical and tropical regions of the world[1]. *Ardisia* species were used in traditional medicines inimprovement of liver cancer, swelling, rheumatism, earache, cough, fever, diarrhea, inflammation, respiratory tract infection, traumatic injury, broken bone, pain, snake and insect bite, birth complications and to improve general blood circulation[2]. Diverse types of compounds have been isolated from this genus, such as polyphenols, triterpenoid saponins, coumarins, quinones, flavonoids and alkylphenols[3]. *Ardisia* species possess very important biological activities like anti- oxidant, analgesic, utero-contraction, anti-platelet, cytotoxic, anti-inflammatory, cAMP inhibiting, anti-feedant, anti-thrombin, hepatoprotective, anti-tumour, antibiotic, antiviral, anti-allergic and anti-HIV activities[3].
Ardisia solanacea Roxb., have been reported to possess stimulant and carminative properties and used as anti-acetlycholine, in internal injury, stomachache especially after childbirth, as a febrifuge, in diarrhoea and in rheumatism[4-5]. Triterpenoids, alcohols, bauerenol, α-amyrin and β-amyrin have been reported so far from the leaves of*A. solanacea*[6]. In the present study the phytochemical analysis of ethyl acetate extract of air dried leaves of *A. Solanacea* was carried out using column chromatography over Si-gel using various solvents afforded β-sitosterol, gallic acid, Quercetin, Myricetin and a new alkylphenolic compound identified as (-)-5-(1,2Dihydroxypentyl)benzene1,3diol(1).
Identification of these compounds was made by physical and spectroscopic techniques.

Material and Methods

CC was carried out over silica gel (60-120 mesh BDH) using gradient elution with different solvent systems in order of increasing polarity. TLC was carried out on Silica-gel (E-Merck and BDH) coated on a thin glass plate (0.25 mm thickness containing 13% $CaSO_4$ as binder). Spots on TLC were detected by spraying with 5%

H_2SO_4 followed by heating at 100^0C, 5% methanolic KOH, Benedict's reagent, iodine vapours, UV and alcoholic $FeCl_3$ solution. Melting points were recorded in BOETIUS microscopic m.p. apparatus. The UV-spectra (λ_{max}, nm) were recorded in Systronic spectrophotometer using MeOH as solvent. The IR-spectra (ν_{max}, cm^{-1}) were recorded using KBr palettes on FT-IR-8100 Shimadzu spectrophotometer and optical rotations were recorded on JASCO DIP-140 digital polarimeter in methanol. NMR spectra were recorded in BRUKER DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrophotometer with DMSO-d_6 and CD_3OD solvents. Chemical shifts are given in ppm scale with TMS as an internal standard. Mass spectra were recorded in JEOLD-300 (EI/CI) spectro- meter.

Plant Material: The leaves of *Ardisia solanacea* were collected from Laxman Siddh Harawala (Dehradun), Uttarkhand, India, in September 2017. The plant species was identified by Dr. Sumer Chand, Department of Systematic Botany, Forest Research Institute, Dehradun, U.K. A voucher specimen (H.R N0.101) was deposited in the Department of Botany Govt.P.G. College, Uttarkashi, U.K., India.

Extraction and Isolation: The air-dried and powdered leaves (3.5kg) of *A. solanacea* were exhaustively defatted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass was extracted with 80% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was successively partitioned with ethyl acetate and n-butanol. The ethyl acetate and n-butenol layer was separated out and concentrated under reduced pressure to give EtOAc extract (42.5g) and n-BuOH extract (20.5g).

The EtOAc extract was found to have more concentration of the phyto constituents as monitored by TLS, therefore, it was subjected to repeated coloumn chromatography (CC) over Si-gel eluted with $CHCl_3$:MeOH (100:0→1:1) afforded various fractions. The like fractions (monitored by TLC) were mixed together. Fraction **I**, on repeated CC over Si-gel using gradient elution with C_6H_6: EtOAc yielded compound β-sitosterol (51 mg), gallic acid (46mg). Fraction **II**, on repeated CC over Si-gel using gradient elution with $CHCl_3$: MeOH yielded gallic acid (23mg), and an alkylphenol identified as (-)-5-(1,2-dihydroxypentyl)benzene-1,3-diol (**1**)(113mg). Fraction **III**, on repeated CC over Si-gel eluted with gradient elution $CHCl_3$: MeOH, afforded quercetin (51mg) and myricetin (63mg).

Results and Discussion

β-sitosterol(1)
White amorphous solid
M.p. 135-137^0C
$[\alpha]_D^{25}$: -36^0 (c=0.1, $CHCl_3$)
IR (ν_{max}^{KBr}):3340, 2970, 2959, 2920, 1463 cm^{-1}.

Gallic acid (2) White crystalline solid
M.P. 160-162ºC
IR (ν_{max}^{KBr}): cm^{-1} 3492, 2900-2650, 1715 cm^{-1} (-C=O)
^{1}H-NMR(400 MHz, DMSO-d_6): δ 6.93 (2H, *s*, H-2 and H-6), 8.73 (1H, *brs*; C_4-OH), 9.41 (2H, C_3-OH and C_5-OH) and 12.74 (1H, *s*, -COOH)
^{13}C-NMR (100 MHz, DMSO-d_6): δ 121.07 (C-1), 109.45 (C-2,C-6), 144.86 (C-3,C-5), 139.51 (C-4), and 167.64 (-COOH).

(-)-5-(1,2-dihydroxypentyl)benzene-1,3-diol (**3**)
Brown powder
M.p.287-289°C
$[\alpha]_D^{25}$ -15.4 (c=1.0 MeOH)
IR (ν_{max}^{KBr}): cm^{-1} 3600, 2918, 1609, 1585, 1505
HREI-MS: m/z (212) 212.1213 $[M]^+$, $C_{11}H_{16}O_4$ (calc. for 212.1217);
^{1}H-NMR (400 MHz, CD_3OD):δ 6.16 (1H, *t*, *J*=2.2 Hz, H-2), 6.33 (2H, *d*, *J*=2.2 Hz, H-4, 6), 4.35 (1H, *d*, *J*=5.4 Hz, H-1'), 3.63 (1H, *m*, H-2'), 1.52 (1H, *m*, H-3'$_a$), 1.33

(1H, *m*, H-3'$_b$), 1.29 (2H, *m*, H-4') and 0.89 (3H, *t*, *J*=7.0 Hz, H-5')

^{13}C-NMR (100 MHz, CD_3OD): δ159.17 (C-1, 3), 102.47 (C-2), 106.69 (C-4, 6), 145.90 (C-5), 78.49 (C-1'), 75.83 (C-2'), 35.27 (C-3'), 19.83 (C-4'), and 14.35 (C-5').

Quercetin (4)

Yellow crystalline solid

M.p. 308-309°C

IR (ν_{max}^{KBr}): cm^{-1} 3289, 3122, 2991, 1660, 1584, 1545, 1457, 1553, 1287, 1205, etc.

HREI-MS: m/z 302.0731 [M]$^+$, (calc. for $C_{15}H_{10}O_7$; 302.0724)

^{1}H-NMR (400 MHz, DMSO):δ 6.19 (1H, *d*, *J*=2.0 Hz, H-6), 6.40 (1H, *d*, *J*=2.0 Hz, H-8), 7.52 (1H, *d*, *J*=2.0 Hz, H-12), 6.81 (1H, *d*, *J*=8.5 Hz, H-15), 7.67 (1H, *dd*, *J*=8.5, 2.0 Hz, H-16), 12.63 (H, *brs*,C_5-OH), 10.85 (1H, *brs*, C_7-OH), 9.72 (1H, *brs*, C_{13}-OH), 9.14 (2H, *brs*, $C_{14,3}$-OH)

^{13}C-NMR (100 MHz, DMSO):δ148.01 (C-2), 136.46 (C-3), 177.47 (C-4), 161.21 (C-5), 98.64 (C-6), 164.10 (C-7), 93.47 (C-8), 158.01 (C-9), 104.20 (C-10), 123.99 (C-11), 115.56 (C-12), 148.81 (C-13), 144.81 (C-14), 116.21 (C-15), 121.67 (C-16).

Myricetin (5)

Yellow powder

M.p. 343-344°C

lR (ν_{max}^{KBr}): cm^{-1} 3390, 2920, 1625, 1600, 1500, 1455, 1368, 1280, 1220, etc.

HREI-MS: m/z 318.0709 [M]$^+$; (calc. for $C_{15}H_{10}O_8$; 318.0713)

^{1}H-NMR (400 MHz, CD_3OD):δ 6.11 (1H, *d*, *J*=2.0 Hz, H-6), 6.32 (1H, *d*, *J*=2.0 Hz, H-8), 7.23 (1H, *s*, H-12, 16)

^{13}C-NMR (100 MHz, CD_3OD):δ148.21 (C-2), 136.23 (C-3), 176.69 (C-4), 161.87 (C-5), 99.62 (C-6), 165.62 (C-7), 94.45 (C-8), 158.22 (C-9), 104.50 (C-10), 122.43 (C-11), 109.87 (C-12, 16), 146.47 (C-13, 15), 137.67 (C-14).

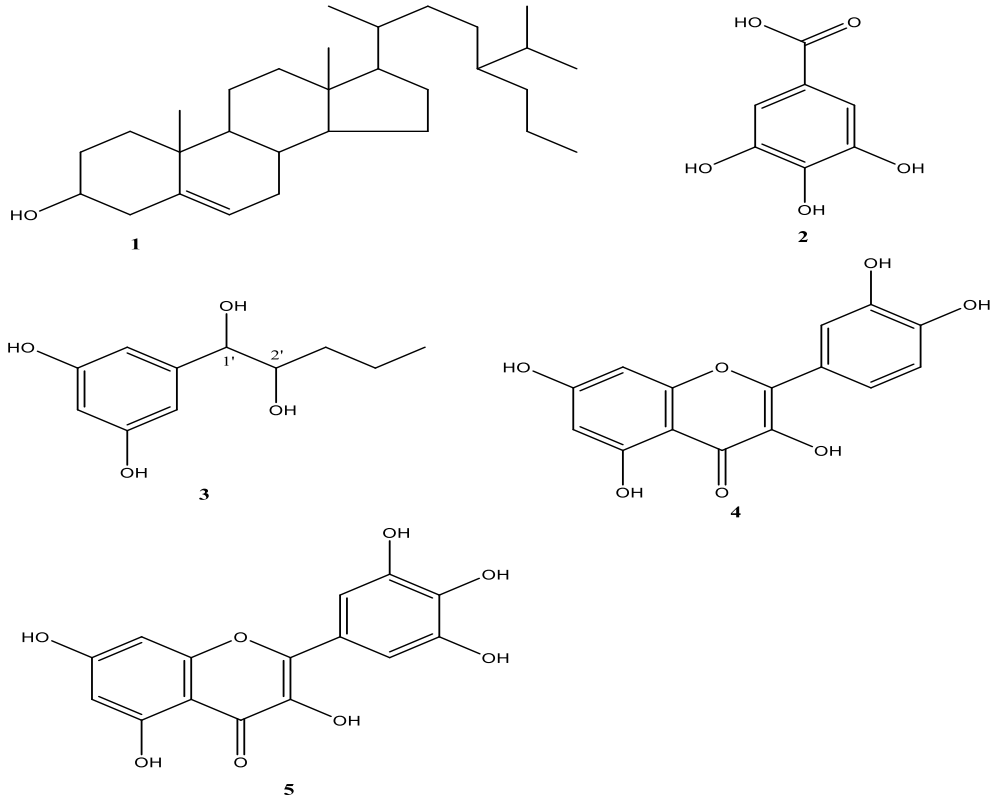

Figure – 1, 2, 3, 4, 5 Isolated Compounds from *Ardisia solanacea*.

The ethyl acetate extract of air dried leaves of *A. solanacea* on repeated CC over Si-gel afforded a new alkylphenol identified as (-)-5-(1,2-dihydroxypentyl)benzene-1,3-diol (**3**) along with β-sitosterol (**1**), gallic acid (**2**), Quercetin (**4**), Myricetin (**5**). The

identification of β-sitosterol[7], gallic acid[8], Quercetin[9] and Myricetin[10-11] was made by direct comparison of their spectral data with the reported data. These compounds were previously isolated from *Ardesia* species[12-18]. Compound **3**was isolated first time from leaves of *A. solanacea* to the best of my knowledge.

Compound**3** obtained as brown amorphous powder. It responded positive to ferric chloride test which indicated the phenolic nature of the compound. The molecular formula of the compound was determined to be $C_{11}H_{16}O_4$ from its HREIMS which showed molecular ion peak at m/z 212.1213 (clac. for 212.1217). Its IR spectrum displayed presence of hydroxyl group at 3600 cm^{-1} and phenyl ring at 2918, 1609 and 1585 cm^{-1}. The ^1H NMR spectrum of compound **3** displayed presence of two meta-coupled doublets which indicated the presence of 1,3,5-trisubstituted aromatic ring in the molecule. The chemiscal shift values of these meta coupled doublets at δ 6.16 (1H, *t*, *J*=2.2 Hz, H-2) and 6.33 (2H, *d*, *J*=2.2 Hz, H-4, 6) in the aromatic region indicated the presence of resorcinol moeity[19]. In aliphatic region the ^1H-NMR spectrum showed presence of two signal for methine protons at δ 4.35 (1H, *d*, *J*=5.4 Hz, H-1') and 3.64 (1H, *m*, H-2'). The downfield chemical shifts of these protons indicated that hydroxyl group was present at C-2' and C-3' position. Which was confirmed from downfield chemical shift of carbon atom at δ 78.49 (C-1') and 75.83 (C-2') The ^1H-NMR spectrum also displayed signals due to one methyl group at δ 0.89 (3H, *t*, *J*=7.0 Hz, H-5'), and two methylene groups at δ 1.52 (1H, *m*, H-3'$_a$), 1.33 (1H, *m*, H-3'$_b$), and 1.29 (2H, *m*, H-4'). These NMR data indicated the presence of 1,2-dihydroxypentyl side chain in the molecule. The non equivalence of methylene protons (H-3') indicated the presence of chiral centre adjacent to this group.

The ^{13}C NMR spectrum displayed presence eleven carbon atoms whereas DEPT spectrum displayed presence of one methyl, two methylene, four methine (one of double intensity) and two quaterary carbon (one of double intensity atoms). The two equivelent aromatic carbon atoms (C-1, 3) resonated at δ 159.17indicated that the phenolic group was persent at C-1 and C-3 position of phenyl ring which was corroborated with the oxygen carrying carbon of resorcinol. The value of coupling constant (*J* = 5.4 Hz) between H-1' and H-2' protons suggestted that threo configuration of compound **3**.

Conclusion

On the basis of above discussed spectral evidences the structure of compound **3** was determined to be (-)-5-(1,2-dihydroxy-4-methyl-pentyl)benzene-1,3-diol. It was further cofirmed by comparison of spectral data with reported values[20].

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Antibacterial Activity of Methanolic Extract of *Tagetes Erecta* (Marigold)

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Abstract – Infectious diseases have always been one of the important concerns of human and have continuously attracted the attention of a large number of various medical and laboratory professionals. On the other hand, treatment with antibiotics has other problems such as drug resistance and side effects, so the use of new herbal medicines with fewer side effects can be a great help in treating these types of infections. The objective of this study was to investigate the antibacterial activity of marigold (*Tagetes erecta*) methanolic extract on five reference strains including *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus* and *Streptococcus pyogenes*. Then, their antimicrobial effects were investigated using agar well diffusion and tubular dilution methods. The extract from the flowers of *T. erecta*showed a great potential antibacterial activity against both
Gram negative and Gram positive bacteria.
Keywords:*T. erecta*, Pathogens, Agar Well diffusion assay.

Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects[1].India possesses almost 8% of the estimated biodiversity of the world with around 0.126% million species[2]. The World Health Organization (WHO) estimate edthat approximately 80% of world population relies mainly on traditional medicines, mostly plant drugs in their health care. Today, Ayurveda coexists with modern system of medicine, and is still widely used and practiced. About 30% of the currently used therapeuticsis of natural origin[3]. In the indigenous health care deliverysystem, numerous plant species and natural products derived from plants are to treat diseases of infectious origin[4].
Plants are used medicinally in different countries and are a source of many potent and powerful drugs[5]. Traditionally, plants have been used to treat many diseases, especially infectious diseases, including diarrhea, fever and cold, as well as birth control and oral hygiene through the world[6].
On the other hand, the emergence of resistant strains among Gram-negative bacilli and Gram-positive cocci such as *Pseudomonas, Klebsiella, Enterobacter, Staphylococcus* and *Enterococcus* has resulted in some problems in the treatment of infections caused by these bacteria[7]. Plant-derived antimicrobials eliminate bacteria with different mechanisms from antibiotics which this is clinically important in the treatment of infections caused by resistant microbial strains[8].
Many studies have been conducted on extracts prepared from plants that have been collected randomly or in one of the above methods. These studies have further focused on the evaluation of antimicrobial activity, anti-worm activity, anti-viral

activity, cytotoxic and mutagenic activities as well as general pharmacological activities[9-14].

Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and climatic phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites[15]. Nowadays, scientific studies have returned to ‘’Natural’’ products[16]. Researches have focused intensively on finding pharma- ceutical equivalents of traditional uses of medicinal plants, as well as research on the discovery of new antimicrobial com- pounds from a wide variety of plants[17-18].

Tagetes species, contains about 56 species belonging to the Asteraceae family, popularly known as marigold, are grown as ornamental plants and thrive in varied agroclimates. Bioactive extracts of different *Tagetes* parts exhibit nematocidal, bactericidal, fungicidal and insecticidal activity. Nematocidal activity of roots is attributed to thienyls while the biocidal components of the essential oil from flowers and leaves are terpenoids. Also carotenoid pigments from *Tagetes* are useful in food coloring.

Fig. 1: *T. erecta*(Marigold) grown in garden

Extracts of *Tagetes* sp. has vast amount of orange-yellow carotenoids[18]. *Tagetes erecta* L., known as marigold, is a single or perennial plant and can spread from tropical to temperate climate under wide conditions. *T.erecta* extracts are ingredient of medicinal drugs used to treat common cold, inflammation, bowel and stomach illnesses, skin infections, cough, cold and, wound[19]. Phytochemical studies of itsdifferent parts have resulted in the isolation of various chemical constituents suchas thiophenes, flavonoids, carote- noids and triterpeniods. The plant *T.erecta* has been shown to contain quercetagetin, a glucoside of querceta- getin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxybenzoate, quercetin, thienyl and ethyl gallate[19-26]. The flavonoid -Patulitrin is one of the potential elements for its anti-bacterial activity[27].

The study aimed at to isolate the antibacterial substance from the fresh flowers of *T. erecta* by using extraction method using methanol as the solvent and to assess its antibacterial activity against some human pathogens.

Materialand Methods

Collection of T. erecta flowers: The fresh flowers from T. erecta was collected from the garden, were dried in an oven at 35 °C for 48 h.

Extraction in solvent- Methanol: An extraction procedure was used to isolate the antibacterial substance from the flowers. Firstly, 5 g of the dried materials were extracted by maceration within 75 ml of a 4:1 ratio of methanol: water mixture under the dark conditions and at room temperature for 24 h. This step was repeated three times during 72 h. The extracts from each step were collected, filtered, and concentrated at 40 ℃ using a rotary evaporator to give a final dry weight of 300 mg. The extracts were stored at -86 °C and resuspended with 1 ml of 80% methanol before antimicrobial testing.

Test organisms: The test organisms used in this study was taken from National Institute of Chemical Laboratory (NCIM), Pune. The organisms used in the study were

Escherichiacoli, Klebsiella pneumo- niae, Staphylococcus aureus, Bacillus cereus and *Streptococcus pyogenes.*

Propagation and maintenance of test organisms: The bacterial test organisms were streaked on the Nutrient Agar slants and were incubated overnight at 37˚C.

Antimicrobial activity: The testing of the bacterial cultures for the inhibitory effect of *T. erecta* was performed by using agar well diffusion method.

Agar Well Diffusion Assay (Zone of Inhibition Evaluation): Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 0.5

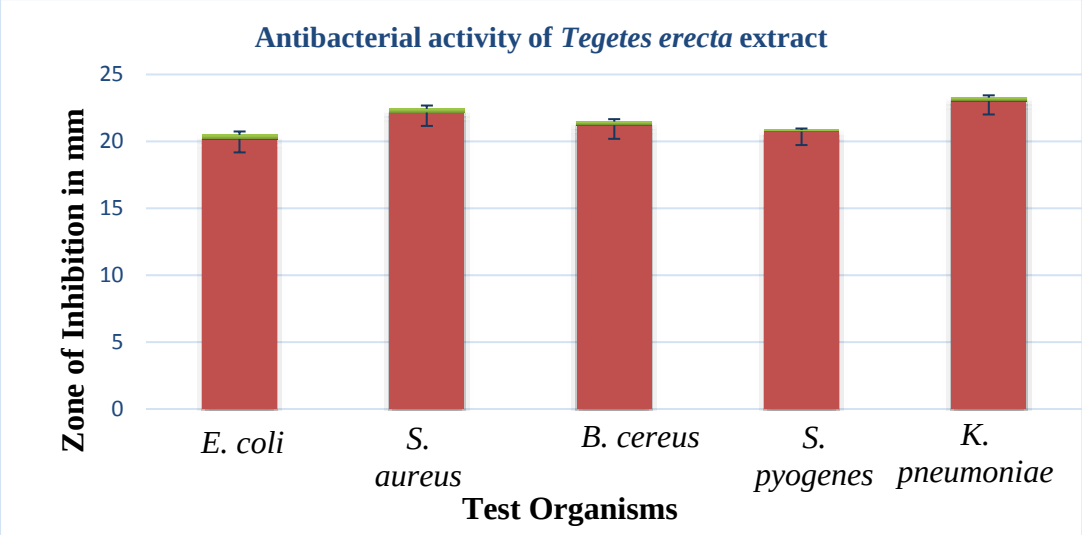
Mc Farland density of bacterial and fungal culture was adjusted using normal saline (0.85% NaCl) using densitometer to get bacterial and fungal population of 1.0 x 10^8 cfu/ml. 100µl of each of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA (Mueller-Hinton agar), mixed well and poured into sterile Petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 100 µl of respective essential oil were pipette out into the well in assay plates. Bacterial plates were incubated overnight at 37ºC. Following incubation, petriplates were observed for the inhibition zones, diameters of which were measured by using Vernier Calipers.

Results and Discussion

Table-1 Antibacterial activity of Tagetes erecta extract

S. No.	Test Organisms	Zone of Inhibition in mm
1	*Escherichia coli*	20.36 ± 0.26
2	*Staphylococcus aureus*	22.33 ± 0.24
3	*Bacillus cereus*	21.35 ± 0.23
4	*Streptococcus pyogenes*	20.80 ± 0.11
5	*Klebsiella pneumoniae*	23.15 ± 0.21

The values of three determinations are expressed as Mean ± S.D.



Graph-1 Representation of antibacterial activity of *T.erecta* methanolic extract

Antibacterial activity of the methanolic extract of *T. erecta* (10 mg/100ml) was performed. Inhibition is maximum for *Klebsiella pneumoniae* (23.15 ± 0.21mm) and minimum for *Escherichia coli* (20.36 ± 0.26mm) Results are presented in Table1 and Graph 1. The results showed a variant sensitivity of *T. erecta* methanolic extract against Gram negative and Gram positive bacteria. Thus, proving *T. erecta* to be most potent plant for new drug discovery.

Hence, for all human ailments, herbal medicines are available in our surrounding itself. *T. erecta* (Marigolds) are naturalized to many warm climate areas all over the world. They are also used in perfumery[28]. Several recent papers reported that antibacterial activity is due to flavonoids[29]. The flavonoids are also toxic to insects, which further modify the alkaloids and incorporate them into their own defense secretion[30]. Plants selected for anti microbial activity have shown appreciable results due to the presence of tannins, flavonoids and sterols[31].

Conclusion

In conclusion, the study has showed that the flowers of *T. erecta* have properties that can inhibit the growth of bacterial pathogens and there should be need for the use of this plant and its derivatives for the purpose of antimicrobial activity. To overcome anti biotic resistance an immense boosting should be panelized. The herbal anti microbial plants should be more high-lighted in the world of medicine.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Screening of Antibacterial Potential and Phytochemical Analysis of Medicinal Plant *Barleria Prionitis*.

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Abstract – Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, phenols, terpenoids, steroids, saponins, tannins etc. and getting these chemicals out into the herbal remedy depends upon the solubility of these compounds in various solvents. In the present study aqueous ethanol diethyl ether acetone and methanol extracts of *Barleria prionitis* leaves were investigated for phyto- chemical and antimicrobial activity. The microorganisms employed were Staphylo- coccus aureus *Escherichia coli*, and *Pseudomonas aeruginosa* and *Bacillus subtilis*. The susceptibility of bacterial strains against the all extracts was determined using the disk diffusion method. The findings showed that potential antibacterial properties of the extracts against the organisms tested. The most susceptible microorganisms were S. *aureus*, while the least susceptible was *E. coli*. Aqueous extracts had no activity against the test bacteria the leaves of plant were found abundant with biologically active phytochemicals.

Keywords: *Barleria prionitis*, Phyto-chemical, Antimicrobial.

Introduction

Nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world. (Al-Essaet al., 1998). Antibiotics is one of the most serious public health problems, especially in developing countries where infectious diseases still represent a major cause of human mortality (World Health Organization, 2014). *Barleriaprionitis* is a shrub in the family Acanthaceae, native to Island and Main land Southeast Asia, China, the Indian Subcontinent, the Arabian Peninsula and northeastern Africa. It used not only as an ornamental but also as a hedge and extensively as a component of folk medicines to treat whooping cough and tuberculosis. (Malik, 2021). One of the oldest forms of medical practice is the use of plants for therapeutic purposes; teas, syrups, tinctures, among others have been used as medicines and in many cases come to be the sole therapeutic resource of certain communities and ethnic groups (Amorozo, 2002; Oliveira et al., 2012). Thus, knowledge about the therapeutic potential of plants is of great scientific and medical interest, as an effective alternative to the battle against resistant micro- organisms (dos Santos et al., 2015). Herbal treatment is still used for many health problems.The current investigation was carried out to screen the antibacterial activityand phytochemical analysis of medicinal plant *Barleriaprionitis* leaves used for herbal treatment by local communities against some pathogenic

bacterial strains.

Material and Methods

Collection of plant material: The leaves of *B. prionitis*were collected from the Botanical Garden, Department of Botany, D.A.V (PG) College, Muzaffarnagar, UP, India and identified by the HOD of the department, Dr. Sanjeev Kumar. The plant leaves were washed with running tap water. The leaves were shade dried at room temperature for 14 days and blender to get fine powders. The powders were stored in airtight container at room temperature for further studies.

Preparation of plant leaves extracts: The powered leaves of the plant *Barleria Prionitis* were homogenised, and about 30g of the sample from each variety was extracted separately with 300mL (10%) of aqueous and four different solvents: methanol, acetone, di ethyl ether and ethanol for 48 h in an orbital shaker at 100 rpm and room temperature. The extracts were filtered and filtrates were concentrated in a rotary evaporator at 45°C. Extracts were kept at 4 °C until the further analyses.

Bacterial cultures: Four standard human enteric pathogenic bacteria viz. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* were procured from the Laboratory of the Department. All the pathogens were sub cultured on nutrient agar slants and preserved at 4ºC for further study.

Evaluation of antibacterial activity of extracts of *Barleriaprionitis*:Antibacterial assay was carried out by agar disk diffusion method. (Bauer A, 1959). One ml of standardized suspensions of the microorganisms was depositedin Petri dishes (diameter 90 mm) and 20 ml of nutrient Agar at 50ºC was added. After solidification, aliquots of 10% of the ethanol extract of filtered sample were appliedto paper disks (6mm in diameter, Whatman No.1), which resulted in disks containing 180-200µg oftheextract. After evaporation of the loading solvent,each disk was placed at the centre of the Petridishes containing previously inoculated Nutrient agar medium platesand incubated at 37ºC for 24 h. At the end of theincubation time, the diameter of microbial growthinhibition zone was measured in millimeter (mm).

Phytochemical screening of leaves of *Barleria Prionitis*: Chemical tests were carried out on the aqueous extract ofBarleriaprionitisusing standard to identify the constituents as described bySofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984), (Omoya and Akharaiyi, 2012), (Jyothi-prabha and Venkatachalam, 2016). (Harborne and Williams, 2000)

Screening for alkaloids: To 5 ml each of the spice extracts, 5 ml of aqueous hydrochloric acid was added on a steam bath at 60°C for 5 min. The spice extract was filtered with a 3 layered muslin cloth. In one ml of the filtrate, few drops of Draggendoff's reagent were added. Appearance of Blue black turbidity was positive for alkaloids.

Screening for steroids: 1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening for tannins: 5 ml each of the extracts was stirred separately with 100 ml distilled water and filtered. One millilitre ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

Screening for terpenoids: 5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for flavonoids: 5 ml of diluted ammonia solution was added to aqueous

extract followed by the addition of 1 ml concentrated H_2SO_4. Appearance of yellow colour indicated the presence of flavonoids.

Screening for saponins: 5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence of the presence of the saponins.

Test for coumarins: Two ml of every extract is treated with 3 ml of 10% NaOH. A yellow colouration determined in every extract indicated the presence of coumarins.

Screening for anthraquinones: 5ml of every extract resolution is hydrolysed with diluted targeted H_2SO_4 extracted with benzene. 1ml of dilute ammonia is additional thereto. Pink coloration steered the positive response for anthraquinones.

Screening of Phenols: 2ml of plant extract, 2ml of distilled water followed by 10 % FeCl3 solution was added. Bluish black colour indicates the presence of phenol.

Results and Discussion

From the ancient time, analysis of biologically active natural yields fromplants has attracted several natural product researchers. Natural products play a very important role within the field of recentmedication analysis and development due to its low toxicity, simple handiness, and low price. The latest analysis investigation has as certained that the bioactive and inhibitorpotentials of those plants are attributed to the presence ofphenols, flavonoids, alkaloids, terpenoids, saponin, and tannins (Agbor *et al*., 2011). Hence, thiswork principally focuses on phytochemical screening in the leaves of *B. prionitis*. The results are summarized and mentioned below.The current study was carried out on the *B.prionitis* unconcealed the presence of medicinally active constituents. The results investigated and summarized in Table-1. The phytochemical screening in the leaves of *B. prionitis* showed that the abundant presence of tannin, saponins, steroids, flavonoids, terpenoids, anthraquinones, phenol, and coumarins in aqueous extracts. Terpenoids are concerned with medication and antineo plastic functions thus employment of the plant to treat burns, skin diseases, and bug stings (Bown Deni*et al*., 1995), Flavonoids are also present in all the extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Salah N,1995; Rio DA,1997]. Tannin rich medicinal plants are used as healing agents in a number of diseases Doughari JH (2012). Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemo- therapeutic agents, anaesthetics and Central Nervous Stimulants (Noble RL 1990; Madziga HA, 2010).

Table – 1 Phytochemical screening of different extracts of leaves of*Barleriaprionitis.*

S.No.	Phytochemical	Aqueous	Ethanol	Acetone	Di ethyl ether	Methanol
1	Alkaloids	+	+++	++	+	+++
2	Steroids	-	+	+	+	++
3	Terpenoids	++	+++	+++	+	+++
4	Tannins	+	+++	+++	+	+++
5	Flavonoids	+	++	++	+	+++
6	Saponin	++	++	++	+	+
7	Anthraquinone	++	++	++	+	+
8	Coumarin	+	++	++	+	++
9	Phenols	++	++	++	+	++

(- = absence, + = presence)

Evaluation of antibacterial activity of extracts:

This study was conducted to evaluate the efficacy of ethanol, methanol, aqueous di-ether ether and acetone extracts of *Barleria prionitis* against selected human bacteria pathogens namely Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis.The ethanolic extract of *Barleriaprionitis*

significantly inhibited the growth of all the bacterial pathogens, whereas the aqueous extract did not inhibit the growth of any bacteria. However, ethanolic extract at 100% concentration posed more lethal effect followed 50% concentration. Table-2 showed antibacterial activity of ethanol, methanol, di ethyl ether, acetone and aqueous extracts of *Barleriaprionitis* (leaves). Aqueous extracts had no activity against the test bacteria.The result of antibacterial activity of ethanol, methanol and aqueous extracts of *Barleriaprionitis* leaves showed that ethanol extracts had good activity against *Staphylococcus aureus and Bacillus subtilis*, this study is in agreement with a study by Omokhua et al., (2008) who also reported that crude extract of neem plant was very effective against Staphyloccous aureus and *E. coli* (Saba et al., 2011). These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000). *E. coli* was less susceptible to all the plant extracts. This study is in conformity with the study of Saradha jyothi (Sofowara, 1993) which showed that neem plant leaf posse good antibacterial activity, The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals (Talaly and Talaly, 2001).

Table – 2 Showing the antibacterial potential of different extracts of leaves of*Barleriaprionitis* against various bacterial strain: (Inhibition zone in mm)

S.No.	Bacterial Culture Name	Aqueous	Ethanol	Acetone	Di ethyl ether	Methanol
		Inhibition zone in (mm±SD)				
1	***E.Coli***	-	2.2±0.22	2.6±0.19	4.0±0.27	2.9±0.28
2	***Pseudomonas aruginosa***	-	4.6±0.27	5.5±0.28	4.3±0.23	5.4±0.33
3	***Staphylococcus aureus***	-	8.1±0.17	8.3±0.56	6.8±0.18	7.9±0.37
4	***Bacillus subtillis***	-	7.7±0.24	7.8±0.36	7.6±0.28	8.2±0.33

Conclusion

Determination of the natural phyto chemicals and antimicrobial compounds can facilitate to develop of new drug candidates for antimicrobial medical aid. *Barleriaprionitis* is used regionally for herbal drugs however, nonetheless to be totally explored. Our results suggest that *Barleriaprionitis* can serve as potential source of bioactive healthy compounds and their consumption could be useful in the prevention of diseases. It is also suggested that aqueous extracts were autoclave-sterilized before use as autoclaving is reported to cause less damage to the antibacterial activities of the
aqueous extract However, further studies ought to be administered on this plant so as to isolate, identify, characterize and elucidate the structure of the bioactive
compounds and verify their mechanism of action.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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In Vitro Antimicrobial Activity of *Helicteres Isora* Extracts Against Gastrointestinal Pathogen

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Abstract – The problem of drug resistance to human pathogenic bacteria and rising of infectious diseases has been reported from all over the world. This increasing prevalence of multidrug resistant strains of microorganisms and reduced susceptibility to antibiotics raises an urgent need to search for new sources of antimicrobial agents. Human infections, particularly those involving the skin and mucosal surfaces constitute a serious problem, especially in tropical and subtropical developing countries. (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, and Salmonella sp.* were observed to be the most frequent pathogens cause gastrointestinal infection. MRSA gained much attention in the past decade, as it is a major cause of hospital-acquired infections. In this study, different extracts of *Helicteres isora*, *was* screened for the antimicrobial activity against the multidrug resistant pathogens. Results are encouraging indicating the potential antibacterial activity against the test organisms and further validate the traditional use of *H. isora* in abdominal cramps and gastrointestinal infections.

Key word: *In Vitro* Antimicrobial Activity-*Helicteres isora,* Organic solvents extracts-Drug resistant bacteria

Introduction

Infectious disease are the world's leading cause of premature deaths, killing almost 50 000 people every day. Infections due to variety of bacterial etiologic agents such as pathogenic *Escherichia coli, Salmonella* spp., *and Staphylococcus aureus* are most common. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh et al 1992 and Mulligen et al; 1993). With the continuous use of antibiotics microorganism have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hyper sensitivity, immune suppressant and allergic reactions (Lopez et al.2001 and Idsoet al.1968).This has created immense clinical problems in the treatment of infectious diseases (Davis 1994). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important recourse to combat serious diseases in the world. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field.

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity (Shelef 1983; Zaika 1988; Beuchat and Golden 1989 and Juven et al.1994). In recent times, the search for potent antibacterial agents has been shifted to plants. Most plants are medicinally useful

in treating disease in the body and in most of cases the antimicrobial efficacy value attributed to some plants is beyond belief. Claims of effective therapy for the treatment of dysentery, diarrhea, respiratory disorders, skin diseases, syphilis, fever, leprosy, eye diseases and kidney and urinary disorders by traditional herbalist in India have prompted our interest in the scientific investigation of such herbal medications (Mukherjee, 1953; Chopra et al; 1956; Kritikar and Basu, 1980; Anonymous, 1986 and Nadkarni,1989).Conservative estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists. There are about 120 plant-based drugs prescribed worldwide and they come from just 95 plant species. Approximately 250,000 species of flowering plants and only 5000 have had their pharmaceutical potential assessed. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics (Kunin 1993). Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable (Anonymous 1986). There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, basic health needs in the developing countries. The WHO reported that 80% of world populations rely chiefly on traditional medicines/herbs for primary healthcare have steadily increased world wide in the recent years.

Helicteres isora belongs to family Sterculiacae is a sub-deciduous shrub or small tree of having spreading habit with stem 1-5 inches in diameter, reaching a height of 5-15 feet. The species is native to Asia and Australia1.It occurs, throughout India, from Jamuna eastwards to Nepal, Bihar and Bengal and southern India and Andaman Islands. It occurs as undergrowth, especially as a secondary growth in forests. The fruits are astringent, acrid, refrigerant, demulcent, constipating, stomachic, vermifuge, vulnerary, haemo-static and urinary astringent. They are useful in vitiated conditions of pitta ophthalmitis, colic, flatulence, diarrhea, dysentery, verminosis, wounds, ulcers, hemorrhages, epistaxis and diabetes 6. The fruit extract of possess weak anti-HIV (I) activity. The fruits were also found to possess significant antispasmodic activity. **Promotes relief from abdominal spasm & pain**. Promotes wellbeing from intestinal infestations & loose motions. Supports as antioxidant and blood purifier. (Chunekar KC, 2010; Sharma PV. 2012 and Satake et al, 1999)Keeping in view this study is designed to evaluate the antimicrobial activity of *Helicteres isora***.**

Material and Methods

Collection of plant materials: *Helicteres isora*fruits were collected from the The Himalaya Drug Company Dehradun India. The collected plant material was identified by the department of Pharmaco., The Himalaya Wellness Company Dehradun. Fruits were washed under the running tap water 2-3 times and once with sterile distilled water and dried under shade and then homogenized to fine powder and stored in air tight container till further use.

Preparation of solvent extraction: The method of Alade and Irobi,(1993) was adopted for preparation of plant extracts with little modifications.The dried 25 g powdered fruit soaked separately in 100 ml Hexane, methanol, and aqueous .Each solvents were kept in separate flasks with powdered sample were kept in a rotating shaker for 3 days. The extracts were filtered through whatman Filter paper No.1 and the extracts were reduced to half of its original volume. The organic solvents were concentrated in vacuum using rotary

evaporator, while aqueous extract was dried using water bath.

Culture media: The media used for antibacterial test was Soyabean casein digest agar/broth of Hi Media Pvt. Ltd. Bombay, India.

Inoculum: The bacteria were inoculated into soyabean casein digest agar /broth and inoculated and incubated at 37 °C for 4 h and the suspension was checked to provide approximately 10^5 CFU/ml.

Microorganisms: The antibacterial activity of the extract was tested individually on G+ve and G-ve bacterial strains .All bacterial strains were obtained from Microbiologics Cooper avenue north, st. cloud, MN 56303.The G+ve strain used was *Staphylococcus aureus ATCC 6538 and* G-vebacterial strains were *E. coli ATCC 6538;* and *Salmonella spp. NCTC 6017.*

Determination of antibacterial activity: The agar well diffusion method (Perez et al; 1990) was modified. Soyabean casein digest agar (SCDA) was used for bacterial cultures. The culture medium is inoculated with the microorganisms suspended in Soyabean casein digest broth. A total of 8mm diameter wells were punched into agar and filled with plant extracts and solvent blank s(distilled water, hexane and methanol as the case may be).Standard antibiotic was simultaneously used as positive control. The plates were then incubated at 37°C for 18 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed.Wells were filled with 0.1 ml of 20 mg/ml concentration of each sample (2 mg/well). Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm.

Results and Discussion

The antimicrobial activities of the extracts were evaluated against 3 test microorganisms including one G+ve bacteria, three G-ve bacteria. Their potency was assessed by diameter of zone of inhibition. Among all the tested extracts hexane extract was found to have maximum zone of 21mm against *Staphylococcus aureus* (Table-1 Plate-1) followed by *E.coli* (18mm), and *Salmonella Spp.*(16mm).

The significant antimicrobial effect of *Helicteres isora* against all the pathogen confirmed that the compound present in the crude extract are responsible for the effective antimicrobial activity.

Conclusion

The traditional therapeutic indications of *Helicteres isora* studied appear to have a fairly good degree of correlation with their antimicrobial activity. The herb *Helicteres isora* appear to have broad spectrum of action and it could be useful in antiseptic, disinfectant formulations and in chemo-therapy .The antibacterial activities of the herb is particularly note worthy, considering the importance of these organisms in gastrointestinal infections.

Table – 1 Antibacterial activity of different extract of *Helicteres isora*

Test organism	**Diameter of zone of inhibition (mm)**				
	Hexane extract	**Methanol extract**	**Aqueous extract**	**Acetone extract**	**Ciprofloxacin**
E. coli	18	16	NAD	17	28
Staph. aureus	21	19	16	18	30
Salmonella spp.	16	15	12	15	28

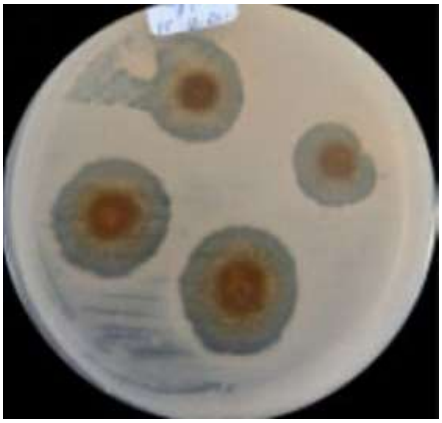

Plate-1 Antibacterial activity of fruit extracts of *Helicteres isora*

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this researcharecommonlyused products in research. There is no conflict of interest between authors and producers of the products.

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Pharmacognostic Evaluation and Antimicrobial Activity of SomeMedicinal Plants Extracts Commonly Used in Indian Traditional Medicine

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Abstract – Indian traditional medicines have been used to boost health since the time of immemorial and the achievement of contemporary medical science mainly depends on drugs initially obtained from natural resources. In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents. *Adhatoda vasica*, *Tinospora cordifolia, Glycyrrhiza glabra*, *Boerhavia diffusa, and Eclipta Alba* are ethnomedicinal plant. They are used in different diseases like breathing disorders, burning sensation, Cough, decrease in bone tissue, blood disorders, tuberculosis, as refrigerant, aphrodisiac, in insect bites, rheumatism, as tonic and in general debility. They are vital component of many Ayurvedic formulations. Despite the common utilization of these plants, convincing study required for reporting the pharmacognostic evaluation along with their antimicrobial activity.

Key words: *Adhatoda vasica*, *Tinospora cordifolia, Glycyrrhiza glabra*, *Boerhavia diffusa, and Eclipta Alba*, Pharmacognostic Evaluation, Physicochemical, Histochemical, Zone of Inhibition, Antimicrobial.

Introduction

A large percentage of the world's population depends upon natural products for medicine. Folk medicine and ecological awareness suggest that natural products are harmless[1]. Therefore trend is shifting from synthetic to herbal medicine, which has been called as 'Return to Nature'[2]. India, have a pluralistic healthcare system. Herbal drugs constitute a major share of all the formally recognized systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. Almost, 70% modern medicines in India are derived from natural products[3]. Natural products sustained to play a highly substantial role in the drug discovery and development process[4]. Medicinal plants play a crucial role not only as traditional medicines but also as trade commodities[5]. The role of information derived ethnomedicine and its utility for drug discovery purposes is important[6]. A lot of work has been done on ethnomedicinal plants in India but still some important plants are still to be scrutinized. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids,flavo- noids, glycosides, etc., which have been found *in vitro* to have pharmacological properties[7].

Adhatoda vasica: *Adhatoda vasica* also known as malabar nut tree is part of the Acanthaceae plant family. It is a small evergreen, sub herbaceous bush which grows commonly in open plains, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia[8]. It is a highly reputed plant used in Ayurvedic system of medicine for the treatment of various ailments of respiratory systems like bronchitis, asthma

and it is also used in the treatment of malaria, dysentery and diarrhea[10] and has many other medicinal applications[9-11], *Adhatoda vasica* Linn. Also has antiinflammatory, analgesic, diarrhoea, dysentery, antioxidant, hepato-protective, Sedative, antispasmodic, anthelmintic properties[12], Antimicrobial activity[13], Antidiabetic activity[14], Wound healing effect[15]. Infertility[16]. Antiulcer[17]. Antibacterial[18]. Antihistaminic effect, mode rate hypotensive activity, thrombo-poietic activity[19].

Tinospora cordifolia: *Tinosporacordi-folia* (Wild) Miers, (*Guduchi*) is one of the important dioecious plants belongs to the family Menispermaceae. In Ayurveda, it is designated as *Rasayana* drug recomm-ended to enhance general body resistance, promote longevity and as anti stress and adaptogen[20-21]. This significant plant is also mentioned in important Pharmaco-poeias[22-23]. Phytochemistryof *T. cordifolia* belongs to different classes such as alkaloids, diterpenoidlactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysacc-harides[22-24]. Threemajor groups of compounds; protoberberine alkaloids, terpenoids, and polysaccharides are considered as putative active constituents of this plant[25-26]. *T.cordifolia* is widely used in folk loric veterinary medicine and traditional Ayurvedic medicinein India for its, antiinflammatory, immune modulatory, antipyreticactivity, antioxidant, anti-diabetic, antiallergic and antiarthritic activities and various other medicinal properties[27-31].

Glycyrrhiza glabra:In *Ayurveda Yashti-madhu* is one of the important plant which is been referred in various texts with many therapeutically uses. *Glycyrrhizaglabra* Linn. a perennial herb with a thick rootstock passing below into long, straight, cylindrical, slightly tapering, smooth, flexible, slightly branched roots, about 1.25cm in diameter, red or orange-brown on the surface, pale yellow within, and giving off at the top long horizontal subterranean stolons. Stems several from the crown, 2-4 feet or more high, erect, stiff, solid, strongly striates, shortly pubescent, branched. Leaves alternate, spreading, large, stalked, with very minute deciduous stipules, impair-pinnate, leaflets opposite in 4-7 pairs and a terminal one[32-33].

***Boerhavia diffusa*:** Punarnava *(Boerhavia diffusa* Linn.) is a flowering plant that is commonly known as punarnava which means rejuvenating or renewing the body. Punarnava (Hogweed) literally means 'bring back to life' or 'renewer'. Among 40 species of Boerhaavia, 6 species are found in India, it is a perennial, spreading hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal[34-35]. It grows well on wastelands and in fields after the rainy season[36]. The whole plant and preferably the roots are effectively used to cure several diseases including Jaundice[37]. Punarnava corrects the digestive system, alleviates fluid retention and very useful in managing heart diseases. It is also used to treat the anemia, hernia and respiratory distress, liver problems, managing lipids and cholesterol in healthy limits[38].

Eclipta Alba: *Eclipta Alba* is commonly known as Bhringaraja or Maka belonging to the family Asteraceae/Compositae. The herb contain wedelolactone and demethyl-wedelolactone which possessing potent antihepatotoxic property[39]. Other promi-nent chemical constituents present are Ecliptal, Ecliptine, Ecliptalbine, α-Terthi-enylmethanol, β-amyrin, Sigmasterol, Polypeptides etc. The other pharmaco-logical activities shown by plants are Antiviral, Antibactarial, Spasmogenic, Hypotensive, Analgesic, Antioxidant etc[40].

In the current investigation carried out, pharmacognostic evaluation and screening of different extracts of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba* have been used against E. coli and S. Aureus in order to screen new sources of antimicrobial agents.

Material and Methods

Collection and Authentication of Plant Material: *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba* were collected from the medicinal plant store of Himalaya Wellness Company Faridabad, Haryana, India, were air dried, powdered and stored in air tight containers. Above these plants were authenticated and identified by Dr. Maya Ram Uniyal Senior vadhya of Himalaya Wellness Company Faridabad. Pharmacognostic evaluations were done as per WHO Guidelines[41].

Chemicals: All reagents and chemicals used for pharmacognostic evaluation and antimicrobial activity were of analytical grade.

Pharmacognostic Evalaution: The organoleptic studies were carried out by with sense organs using simple technique like shape, size, colour, odour, taste etc. Histochemical reactions were applied with concentrated hydrochloric acid and phloro-glucinol for identification of lignified elements, iodine solution for starch grains, Sudan red-III for cuticle layer and oil globules, Ruthenium red for mucilage and acetic acid for calcium oxalate crystals.Physicochemical parameters such as loss on drying, ash values, pH value in 1% solution, aqueous, and alcoholic extractive values were carried out according to the methods recommended by the World Health Organization.[19]

Preparation of Plant Extract: After collection of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba* samples, they were powdered. Powder materials were passed through sieve no. 40 and used for extractions. Weighed powder was extracted using hexane, chloroform, ethyl acetate, ethanol and aqueous solution in Soxhlet apparatus till exhausted. These extracts was evaporated at 40°C in rotary vacuum evaporator to dryness.[20] The extracts obtained from successive extraction *i.e.* Hexane extract (HE), Chloroform extract (CE), Ethyl acetate extract (EAE), Ethanol extract (EE) and residual Aqueous extract (AAE).

Test Micro-organisms and growth Media: The antibacterial activity of different extracts were studied against two bacterial strains, one Gram-positive (*Staphylococcus aureus* ATTC-6538,) and one Gram-negative (*Escherichia coli* A TC C 8739,) based on their pharmacological importance. Both the strains of micro-organism were obtained from Department of microbiology, Himalaya Wellness company Faridabad, Haryana. The strains of *Staphylococcus aureus* and *Escherichia coli* were maintained on nutrient broth at 37°C and suspension were stored in refrigerator till used**.** Commercially available Mueller-Hinton agar (MHA) (Hi-media, Mumbai) was prepared according to the instructions on the leaflet. Immediately after autoclaving the media, it was allowed to cool. Freshly prepared and cooled medium was poured into glass flat-bottomed petri-plates on a level, flat surface to give a uniform depth of approximately 4 mm. This corresponded to 30 ml for each plate with a diameter of 90 mm. The agar medium was allowed to cool at room temperature and unless the plates were used the same day otherwise these were stored in a refrigerator (2 to 8°C) for further use within seven days. Representative samples of each batch of plates were examined for sterility by incubating at 37°C for 24 hours or longer.[42]

Agar Well Diffusion Method for Determination of Zone of Inhibition (ZOI): Antibacterial activity was carried out using well diffusion method. The test cultures were spread with the help of spreader on the top of the solidified media and allowed to dry. The tests were conducted with 100mg/ml concentrations of these crude extracts per well with three replicates. Dimethyl Sulphoxide (DMSO) (Himedia Mumbai) was used as negative control. Streptomycin discs (10**µg**/disc) of 6 mm were used as positive control. The plates were incubated for 24 h at 37 ºC.

Zone of inhibition (ZOI) was recorded in millimetres and the experiment was repeated thrice.The inoculums were prepared by making a direct broth suspension of 24-hour agar plate. The suspension adjusted to match the 0.5 McFarland turbidity standards. Dried extracts were accurately weighed and dissolved in the DMSO to yield the 100mg/ml concentration, using sterile glassware. These were stored in refrigerator for further use. The wells were made in the incubated MHA media plates with the help of sterile cork borer (steel) of 6 mm and plates were labelled properly. 50 µl of the working solution of plant extract were loaded into the respective wells with the help of micropipette. The plates were incubated 24 h at 37C. The plates were then observed for the zone of inhibition (ZOI) produced by the anti- bacterial activity of different plant extracts. At the same time ZOI of both organism by different extracts were measured with the help of the ruler for the estimation of effectiveness of anti-bacterial substance and tabulated.

The plates were then incubated in the inverted position at 37°C for 24 h The diameters of the zones of complete inhibition as observed by the unaided eye are measured, including the diameter of the disc/well. Zones were measured to the nearest whole millimeter, using a ruler; these petri plates is held in non-reflecting background and illuminated with reflected light. The zone margin were taken area showing no obvious, visible growth which can be detected with the unaided eye. The same procedure was followed for each strain and extract[43].

Results

Pharmacognostic evaluation:

Pharmacognostic evaluation has been done with respect to Organoliptic properties, histochemical evaluation andphysico-chemical studies.Organoliptic evaluation which is done by sense organs is the simplest and quickest means to ascertain the identity and purity of a drug. Organoliptic characters as shape, size, colour, odour, taste etc. are evaluated. These features of *dhatoda vasica*, *Tinospora cordifolia*, *Glycyrrhiza glabra*, *Boerhavia diffusa*, *and Eclipta Alba* powder samples were observed. The details of results are presented in Table-1.

Table – 1 organoleptic evaluation of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba*

SNo.	Plant Name ▶ Parameter ▼	Observation				
		Adhatoda vasica	***Tinospora cordifolia***	***Glycyrrhiza glabra***	***Boerhavia diffusa***	***Eclipta alba***
1	**Colour**	Greenish brown	Brown	Yellowish	Brown	Brown
2	**Taste**	Bitter	Bitter	Sweet	Bitter	Characteristic
3	**Shape**	Branched herbs	Cylindrical	Cylindrical	Cylindrical	Branched herbs
4	**Odour**	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
5	**Size**	2-6 cm long pieces	2-8 cm long and 1-3 cm diameter	3-9 cm long and 2-4 cm diameter	2-8 cm long and 2-5 cm diameter	2-6 cm long pieces

Histochemical Characters

Powders study by using particular chemicals has been done. The results are presented in Table-2.

Table – 2 histochemical study of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa and Eclipta Alba* powder

S.No.	Reagent	Test	Nature of colour change	Observation				
				Adhatoda vasica	***Tinospora cordifolia***	***Glycyrrhiza glabra***	***Boerhavia diffusa***	***Eclipta alba***
1	Ruthenium red	Mucilage	Pink	-ev	+ev	+ev	+ev	-ev
2	Weak Iodine solution	starch	Blue	-ev	-ev	+ev	-ev	-ev

3	Sudan III	Fixed oil & fat	pink	+ev	+ev	+ev	+ev	+ev
4	Sulphuric acid (60%)	Calcium oxlate	Soluble, on standing show needles of calcium sulphate	+ev	-ev	-ev	-ev	+ev
5	Phloroglucinol-HCl	Lignins	Reddish brown to red rose	-ev	+ev	+ev	+ev	-ev
6	Millon's reagent	Protein	Yellow to brown	-ev	-ev	-ev	-ev	-ev

Physicochemical Analysis

The parameters which have been studied are moisture content, loss on drying, total ash, acidinsoluble ash, alcohol and water-soluble extractive values, foreign matter, and pH analysis. Ash values are useful to indicate presence of various impurities like carbonate, oxalate and silicate. The water soluble ash indicates amount of inorganic compound present in drugs whereas the acid insoluble ash indicate contamination with earthy material. Moisture content of Drugs should be at minimal level to discourage the growth of microorganisms during storage. Extractive values establish the amount of the active constituents. The extractions of any crude drug with a particular solvent yield a solution containing altered phytoconstituent. The compositions of these phytochemicals depend upon the nature of the plant and the solvent used. Results of physico- chemical analysis of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba*has been presented in Table-3.

Table – 3 physicochemical parameters of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba* powder

S.No.		**Observation**				
Physicochemical Parameter		***Adhatoda vasica***	***Tinospora cordifolia***	***Glycyrrhiza glabra***	***Boerhavia diffusa***	***Eclipta alba***
1	Total ash value	12.5 %	6.74 %	6.05 %	9.24 %	17.43 %
2	Acid-insoluble ash value	0.44 %	1.64 %	0.70 %	3.19 %	8.57 %
3	Water soluble ash value	1.08 %	1.54 %	0.84 %	2.11 %	1.47 %
4	LOD	4.21 %	4.63 %	4.44 %	3.29 %	4.12 %
5	pH 1% Solution	6.54	6.47	6.55	5.48	6.10
6	Foreign Matter	0.62 %	0.71 %	0.24 %	0.91 %	0.78 %
7	Alcohol soluble extractive value	13.27 %	4.18 %	17.37 %	2.76 %	14.43 %
8	Water soluble extractive value	28.10 %	13.40 %	25.19 %	10.34 %	25.02 %

Antimicrobial Activity

Results obtained in the present study revealed that tested extracts possess potential antibacterial activity against *E. coli,* and *S. aureus,* when tested by disc diffusion method the chloroform, Ethanol, Aqueous extracts showed most promising results. The maximum ZOI has been observed with chloroform extract 24 mm has been observed against *S. aureus,* and 20 mm *E. coli* and least with Hexane extract. Ethyl Acetate extract of *Boerhavia diffusa* exhibit highest activity against *S. aureus* of 20 mm and *Adhatoda vasica* extract haveleast against *E.coli* 13 mm. Ethanol extract of *Adhatoda vasica* showed maximum activity against *E. coli* 21 mm and least ethanol extract of *Glycyrrhiza glabra* 14 mm against *E. coli*. Hexane

extract of *Adhatoda vasica* and *Eclipta alba* showed equal and highest activity against *E. coli and S. aureus* as 10 mm and least activity of hexane extract of *Boerhavia diffusa* as for *E.coli*9 mm. Aqueous extract also have a good activity among all the extracts tested. The data pertaining to the antimicrobial potential of the plant extracts are presented in Table-4.

It is clear from the Table-4 that antibacterial activities of different extracts are showing promising results. *The growth inhibition zone measured ranged from 9 mm to 24 mm.* Trend of the activity of different extracts against *E.coli* and *S. aureus* is Chloroform> Ethanol > Aqueous > Ethyl acetate >Hexane. Maximum ZOI has been observed for Chloroform extract *i.e.* 24 mm and least ZOI for Hexane i.e. 9 mm.

Table – 4 zone of inhibition (in mm) of different extracts of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta alba* against E.coli (ATCC-8739) & *S. aureus (ATCC-6538)*

S.No.		Observation									
Plants ⇒ / Organisms ⇒ / Extracts ⇩		*Adhatoda vasica*		*Tinospora cordifolia*		*Glycyrrhiza glabra*		*Boerhavia diffusa*		*Eclipta alba*	
		E. coli (ATCC-8739) (Gram - ev)	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**
1	Aqueous + ve Control -ve Control	15 20 0	18 22 0	14 20 0	17 22 0	13 20 0	15 22 0	14 20 0	16 22 0	13 20 0	16 22 0
2	Hexane + ve Control -ve Control	10 20 0	12 22 0	11 20 0	13 22 0	12 20 0	14 22 0	09 20 0	13 22 0	10 20 0	12 22 0
3	Chloroform + ve Control -ve Control	20 20 0	24 22 0	17 20 0	21 22 0	19 20 0	23 22 0	18 20 0	22 22 0	15 20 0	19 22 0
4	Ethanol + ve Control -ve Control	17 20 0	21 22 0	16 20 0	18 22 0	14 20 0	17 22 0	16 20 0	18 22 0	15 20 0	19 22 0
5	Ethyl acetate + ve Control -ve Control	13 20 0	15 22 0	14 20 0	16 22 0	15 20 0	17 22 0	16 20 0	20 22 0	15 20 0	17 22 0

Discussion

Plants are rich in secondary metabolites like tannins, terpenoids, alkaloids and flavonoids and these secondary metabolites are responsible for antibacterial properties. The use of plants and its preparations to treat diseases is an ancient practice in world especially in developing countries like India where there is dependence on traditional medicine. Interest in plants with antibacterial properties has revitalized as a result of current problems associated with the use of antibiotics. The present studies aimed at the investigation of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta alba* ethnomedicinal plants *in vitro* antibacterial activity against Gram positive and Gram negative bacteria. The results presented here point out that these plants have a good choice for the development of new "leads". Hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta alba* extracts showed significant zone of inhibition against "Gram-positive" bacteria, *Staphylococcus aureus A*TCC 6538 and Gram-negative bacteria *and Escherichia coli* ATCC 8739.

This work shows that maximum ZOI has been observed in Chloroform extracts and least in hexane extracts. This means active components showing better antibacterial property are more lipophilic as compared to

non polar solvent. Phytochemicals such as alkaloid are generally reported in Chloroform extract.[44] Comparing results found in this study with those of the literature, we notice in a previous work on antimicrobial activity of some medicinal plants from Tunisia, that methanolic extracts of *C. monspeliensis* leaves have shown an interesting activity against *P. aeruginosa, S. aureus, E. faecalis* with inhibition zones diameters of 18.0, 20.0 and 15.0 mm, respectively.[45] Whereas, water-methanol extracts of fruit peels of pome-granate (*P. granatum*) have demons- trated a moderate activity when they were tested on *S. aureus, P. aeruginosa and K. pneumonia*e (13.0, 18.0 and 16.0 mm, respectively)[46]. This activity of pomegra-nate peels could be attributed to tannins, for which antimicrobial activity has been demonstrated.[47] The studies commenced here also suggest that presences of good antibacterial potency of the extracts are due to active compounds in these extracts. The results indicate that the tested crude extracts are potential source to be explored to identify new compounds. As these plants are used in Ayurvedic formulations the results also revealed the scientific basis of the traditional usage of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta Alba* and therefore received attention. This is supporting document to prove that these plants have therapeutic uses since ancient times. The use and exploration for drugs and dietary supplements derived from these plants have accelerated recently but much work has to be done.

Conclusion

Pharmacognostic evaluation plays an important role in quality control of the crude drug. The different characters observed in the *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa , and Eclipta alba* serve as base for the identification of right sample of the plant as drug and other studies. Five solvent extracts have been selected out of which Chloroform extracts, ethanol and water extracts have shown more promising results as compared to hexane and Ethyl acetate extracts. It can be concluded from this study that chloroform, ethanol and water are more suitable for further studies. Antibacterial leads seem to be more lipophilic in nature. The ZOI in chloroform extract is found to be even more as compared with standard drug Steptomycin against *S.aureus A*TCC 8739. The present study justified the claimed uses of *Adhatoda vasica, Tinospora cordifolia, Glycyrrhiza glabra, Boerhavia diffusa, and Eclipta alba* in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the prospective efficacy of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation for the potential discovery of new natural bioactive compounds.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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About Flowers on the cover page

❖ *Cichorium intybus* (Kasni)

Scientific name : Cichorium intybus
Higher classification : Cichoriinae
Rank : Genus
Family : Asteraceae
Subfamily : Cichorioideae
Kingdom : Plantae

Cichorium intybus is a genus of plants in the dandelion tribe within the sunflower family. The genus includes two cultivated species commonly known as chicory or endive, plus several wild species. Common chicory is a bushy perennial herb with blue or lavender flowers.

Traditional medicinal uses of *Cichoriumintybus*. According to the European monograph, traditional use of chicory roots includes the **relief of symptoms related to mild digestive disorders (such as feeling of abdominal fullness, flatulence, and slow digestion) and temporary loss of appetite**

❖ *Ocimum sanctum* (Tulsi)

Scientific name : Ocimum sanctum
Family : Lamiaceae
Order : Lamiales
Higher classification : Basil
Kingdom : Plantae
Rank : Species

Ocimum sanctum, commonly known as holy basil, tulsi or tulasi, is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

The Ocimum sanctum L. has been possess **antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardio-protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions**.

❖ *Withania somnifera* (Ashwagandha)

Scientific name : Withania *somnifera*
Higher classification : Nightshade
Order : Solanales
Rank : Genus
Family : Solanaceae
Kingdom : Plantae

Withania somnifera is a genus of flowering plants in the nightshade family, Solanaceae, with 23 species that are native to parts of North Africa, western Asia, south Asia, southern Europe, the Mediterranean, and the Canary Islands.

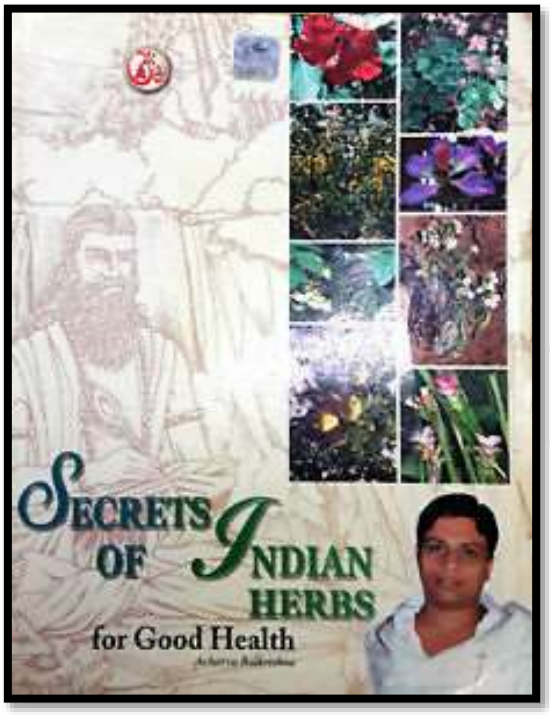
The overall medicinal properties of *Withaniasomnifera* make it a viable therapeutic agent for **addressing anxiety, cancer, microbial infection, immunomodulation, and neurodegenerative disorders**.

Terminalia arjuna (Arjuna)

Kingdom : Plantae
Division : Magnoliophyta
Class :Magnoliopsida
Order :Myrtales
Family :Combretaceae
Genus :Terminalia
Species :arjuna
Vernacular name : Arjuna

Terminalia arjuna is a medicinal plant of the genus Terminalia, widely used by Ayurvedic physicians for its curative properties in organic/functional heart problems including angina, hypertension and deposits in arteries. Its bark is astringent and is used in fevers and in fractures and contusions.
Bark styptic, tonic, febrifuge and anti-dysenteric; pulverised bark gives relief in symptomatic hypertension and acts as a diuretic in cirrhosis of liver. Fruits tonic and deobstruent. Juice of leaves used in earache. The cardio protective effects of Terminalia are thought to be caused by the antioxidant nature of several of the constituent flavonoids and oligomericproanthocyanidins, while positive inotropic effects may be caused by the saponin glycosides. In addition to its cardiac effects, Terminalia may also be protective against gastric ulcers.

Book Review



Secrets of Indian Herbs

For Good Health

Acharya Balkrishna

This review is about a book entitled 'Secrets of Indian Herbs for Good Health' authored by Acharya Balkrishna and published by Divya Prakashan, Patanjali yogapeeth, Mahrishi Dayanand Gram Uttarakhand. It describes one hundred and twenty five (125) herbs chapterwise. Each chapter of the book has been divided into five parts: Introduction, Appearance, Chemical Composition, Properties and Medicinal usages. The total covered pages are 420.

The chemical composition of herbs has been described but in an unscientific way without mentioning their chemical structure and specific bioactive constituents responsible for their therapeutic value. It is mentioned in the book that even a single herb is capable of curing number of diseases. It does not highlight specifically a herb or mixture of herbs for curing a particular ailment. The description in chapters totally lack in proper scientific presentation, assessment and interpretation.

The botanical names of the herbs should also have been mentioned along with their English, Hindi, Guajarati, Bengali, Punjabi, Arabic and Persian language names. It is, however a good collection on herbs but with a little more effort based on scientific explanatory notes, it could have been a better collected material.

Dr. I. P. Saxena
Editor, UJPAH

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  (Jul 12-13, 2022)
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- **ICEMC 2022: Ethnopharmacology and Medicinal Chemistry Conference,**
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  (Jul 19-20, 2022)
- **ICMAPS 2022: Medicinal and Aromatic Plant Sciences Conference,**
  Toronto
  (Jul 19-20, 2022)
- **ICPCP 2022: Pharmaceutical Chemistry and Pharmacology Conference,**
  Sydney
  (Aug 30-31, 2022)
- **ICPDDS 2022: Pharmacology and Drug Delivery Systems Conference,**
  Kuala Lumpur
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- **ICPPAA 2022: Phytochemistry, Pharmacognosy and Advanced Applications Conference,**
  Dubai
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- **ICEP 2022: Ethnopharmacology and Phytochemistry Conference,**
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- **ICPMP 2022: Phytochemistry and Medicinal Plants Conference,**
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