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Mogra *(Jasminum officinale)*

Rajnigandha *(Polianthes tuberosa)*

Night blooming Jasmine *(Cestrum nocturnum)*

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Dr. I.P. Pandey, Coordinator
Universities' Journal of Phyto-Chemistry and Ayurvedic Heights,
1-Inder Road, Dehradun-248001 (Uttarakhand), India
E-mail: dr.sfarooq.him@gmail.com;editor@ujpah.in
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Editorial

Pharma export growth in formulations could decline to 10-12% annually over the next 5 years compared to an average growth of 19% seen in the last decade.

Many of Pharma firms derive a large portion of their revenue from generic formulation sales to U.S, which is the largest generics market. The industry has posted a growth of of about 19% in the last decade, the report said, primarily because of the exports of generics medicines.

Exports of generics have been the growth engine of industry for a long while now, but the script is changing because the value of drugs going off patent is declining even as pricing pressure are increasing.

The new drug applications (NDAs) approved by the USFDA reveals that Indian companies got approvals for just 26 products between January 2006 and June 2015- a fraction of 840 garnered by global pharmaceutical companies.

Cancer in our country is spreading rapidly.MD Anderson in Houston is dedicated to accelerating the end of cancer and identified many new traditionally undruggable cancer targets which could offer potentially fresh approach to treating cancer.Drugs from Ayurvedic text book should also be done as cancer is reported as Ardhbudh.

Collaborations amongst Cancer Research organisations in our country is also a must to boost the R&D in cance treatments.

By 2050,10 million people could die each year unless new antibiotics are developed. Drug resistant super bugs account for an estimated 700,000 deaths worldwide today but that number could rise to 10 million with in the next few decades unless new antibiotics are developed.

Keeping in view of the above problems facing by the pharma industry and the ailing humanity I must say that the best way is the nature's way. Promotion of indigenous system of medicines through research using most advanced equipments by young scientists is a now indispensible.

Iam happy about the relationship I have in person with the members of the board of UJPAH where each one of them voluntarily supportive for the cause of encouraging the young scientists working on Indian Herbal system.

Similarly, I congratulate the young scientists ,departments Heads of universities and others officials who deserve gratitude for the support rendered in our endeavor by sending students and staff members for making this mission successful. In the end, my special thanks to Dr.Rajendra Dobhal ,DG,UCOST who always stood for the cause like my younger brother.

Dr. S. Farooq
Chief Editor

Active Constituents of Essential Oil Inhibit Growth and β-lactamase Production in Drug Resistant Pathogenic Bacteria

Mohammad Shavez Khan[1], Manisha Gupta[1], Abdullah Safar Althubiani[2], Mohammad Sajjad Ahmad Khan[1] and Iqbal Ahmad[1*]

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

[2]Department of Biology, Faculty of Applied Science, Umm Al–Qura University, Makkah, 21955, Saudi Arabia

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

Abstract- Development of multidrug resistant in pathogenic bacteria and slow discovery of new antibiotics has triggered interest in search of new anti-infective compounds from medicinal plants. β-lactamases are the groups of hydrolytic enzymes responsible for resistance to widely used β-lactam antibiotics. In this study we report antibacterial activity of four active compounds of aromatic plants namely Eugenol, Citral, Geraniol and Thymol, exhibited to MDR strains of *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. These phytocompounds showed varying level of antibacterial activity with zone of growth inhibition varying from 10.28 ± 0.34 to 22.83 ± 0.76 mm. *Klebsiella sp.* were relatively less sensitive than *E. coli* and *S. aureus*. MIC data also revealed the similar pattern of sensitivity against phytocompounds. Overall, eugenol was found more effective compared to other compounds. Further, effect of these compounds on β-lactamase production by *test bacteria* was assessed and found that at sub-MIC concentration, eugenol (0.4%v/v) could inhibit the production of β-lactamase in more than one strain tested. The findings revealed that in addition to antibacterial activity, essential oil's phytocompounds at non-inhibitory concentrations (sub-MICs) demonstrate anti-resistance activity by inhibiting the synthesis of β-lactamase. Further investigation is needed to explore the efficacy of phytocompounds against different types of β-lactamases and other resistance mechanism in bacteria.

Keywords: β-lactamases, Essential oil, Eugenol, *Klebsiella pneumoniae*, Phytocompounds,

Introduction

The continuous increase in new and modified modes of antimicrobial resistance in pathogenic microbes leads to serious public health problems and imposes a global risk (Molton et al., 2013). Unprecedented selection pressure and slow progress in the development of new antimicrobials gradually results in increase prevalence of multidrug resistance pathogenic bacteria including microbial resistance to β-lactam antibiotics (Ahmad and Aqil, 2007). Bacteria develops resistance by mutation and resistance gene acquisition. Different mechanism of resistance are present in bacterial cell. Inactivation of antibiotic by producing hydrolytic enzymes e.g. β-lactamases, is the most common mechanism against β-lactam antibiotics (Dale-Skinner and Bonev, 2008). β-lactamase production is also under control of transmissible plasmids and hence aggravation of antimicrobial resistance among the bacterial populations has been a major concern (Bush, 2002, Orhan et al., 2011; Maheshwari et al., 2016a, Maheshwari et al., 2016b).

Targeting β-lactamase with enzyme inhibitors along with combinational therapy of β-lactam drugs constitute the most common and successful chemotherapeutic approach (Rolinson, 1991). The most common clinically relevant β-lactamase inhibitors like clavulanic acid, tazobactum and sulbactum are successfully used against β-lactamase mediated resistance in combination with β-lactam antibiotics (Bebrone et al., 2010). However, these first generation inhibitors are

effective only against certain class of enzyme and further modification in the enzyme structure resulted into evolution of extended spectrum β-lactamases that leads to the enhance probability of development of β-lactamase-inhibitor resistance (Georgopapadakou, 2004). Increase in emergence of β-lactamase variants that are able to resist the action of commonly used inhibitors led to the immediate need of searching novel sources for the development of effective extended spectrum inhibitors. Novel approaches to combat drug resistant bacteria have been proposed including intelligent designing of test system for new drug discovery from natural products (Ahmad et al., 2008).

Plants and associated natural products have been always considered as repository of phytocompounds having broad range of bioactivity along with low toxicity when compared to synthetic molecules/drugs (Akkiraju et al., 2015). Essential oils and their constituents obtained from aromatic plants, known for their multiple biological activities including antibacterial, antifungal, antiviral, antitoxigenic, antiparasitic and insecticidal properties (Bakkali et al., 2008). Essential oils are characterized by two or three major components at high concentrations compared to others components which are present in trace amounts. The main group is composed of terpenes and terpenoids (geraniol, thymol, carvacrol etc.) and the other of aromatic (eugenol, menthol etc.) and aliphatic constituents, all characterized by low molecular weight. These major constituent of essential oils are widely studied for their biological activities and traditional uses (Ahmad et al., 2008, Bakkali et al., 2008). However, information on their uses against multidrug resistant and antidrug resistant properties is scanty.

Considering the role of major components of essential oil in determining the biological properties of essential oil and their use as traditional medicine against infectious diseases, in this study, we explore the effect of four active constituents of essential oils on drug resistant bacteria and their potential to inhibit bacterial resistance mechanism, which may provide new effective alternative to control drug resistance problem in pathogenic bacteria.

Material and Methods

Microorganism used and their antibacterial drug sensitivity profile

The test organism used include *E. coli* UP 2566, (Central Drug Research Institute, Lucknow, India) and our laboratory collection of different test drug resistant bacteria isolates was originally provided by Prof. Abida Malik, Department of Microbiology, JNMC, AMU, Aligarh. These bacteria were tested for antibiotic sensitivity by disc diffusion method (Bauer et al., 1966). Antibiotic disc were purchased from Hi-Media Laboratory Ltd., Mumbai (India).

Essential oil's compounds

The pure compounds, Citral, Eugenol, and Thymol were procured from Hi-Media Laboratory Ltd., Mumbai (India) and Geraniol was kindly provided by The Himalaya Drug Company, Dehradun (India).

Detection of antibacterial activity of compounds and MIC determination

Antibacterial activity of essential oil's constituents was determined by disc diffusion method as described by Bauer et al., 1966. Briefly, sterile disc impregnated with 6 ul of test compound was placed onto Nutrient agar (Hi-Media lab. Ltd., Mumbai) plates previously spread by test microbial culture. Plates were incubated at 37 ^{o}C for 24 h. After incubation plates were scored for antimicrobial activity by measuring the diameter of zone of inhibition. Minimum inhibitory concentration (MIC) of test compounds against bacterial strains was determined by tube broth dilution method using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth (Eloff, 1998) as describe elsewhere (Aqil et al., 2005).

Determination of β-lactamase production in bacterial isolates

β-lactamase production in bacterial isolates was detected by rapid idometric method described by Catlin, (1975). The test bacterial isolates were grown overnight on nutrient agar plates amended with 25 µg/ml ampicillin. Several colonies of test isolates were taken from plates to make a dense suspension in 0.1 ml of penicillin solution in sugar tubes. After 30 min, 2 drops of starch solution and 1 drop of iodine reagent were added. Rapid decolourization of blue color indicates presence of β-lactamase.

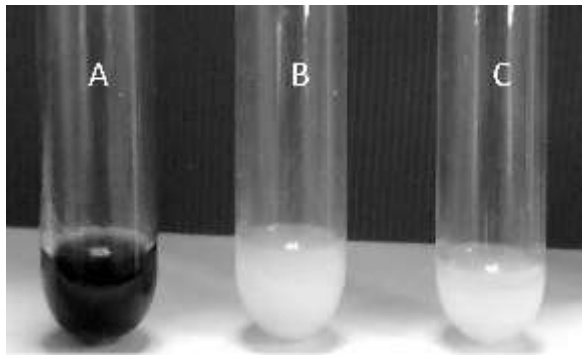

Figure-1 β-Lactamase production by *Klebsiella pneumoniae.*
A = No production of β-lactamase(Negative Control)
B & C= Positive for β-lactamase production

Inhibition of β-lactamase production by phytocompounds

Inhibition of β-lactamase production was evaluated at sub-MIC concentration of test compounds against β-lactamase producing isolates by rapid idometric method as described above with little modification.. The bacterial culture was grown on nutrient agar plate supplemented with 25 µg/ml ampicillin and different concentrations of phytocompounds below its MIC value. Growth obtained were

Table-1 β-Lactamase Production and Antibiotic Resistance Pattern Among Test Bacterial Strains

Bacterial strains (Code)	β-Lactamase production	Drug resistance pattern
Klebsiella sp. (KL3)	+	Ac, Am, C, Ca, Cn, Cu, Do, K, M, R, T, Va
Klebsiella sp. (KL4)	+	Ac, Am, C, Cn, Do, K, M, Nf, R, S, T, Va
Klebsiella sp. (KL5)	+	Ac, Am, C, Do, K, M, Nf, R, S, T, Va
Klebsiella sp. (KL6)	+	Ac, Am, C, Ca, Cn, Cu, Do, M, Na, Nf, R, S, T, Va
E. coli (EC1)	+	Ac, Am, C, Ca, Cn, Cu, Do, K, M, Na, R, S, T, Va
E. coli (EC2)	-	Am, Cn, M, Na, S, T, Va
E. coli (EC62)	-	M, R, T, Va
E. coli (UP2566)	-	Cn, Cu, M, Nx, R, S, Va
Staphylococcus aureus (SA03)	-	M, Na, S, Va
S. aureus (SA07)	-	Ca, Cn, M, Na, Nx, Va
S. aureus (SA18)	-	Ac, Am, Ca, Cu, Cn, K, M, Na, Nx, R, S, Va
S. aureus (SA22)	-	Ac, Ca, M. Na
S. aureus (SA27)	+	Ac, Am, Ca, Cu, Cn, Do, M, Na, R, S, T, Va

Ac-Amoxyclav (30µg), Am-Amoxicillin (30µg), C- Chloramphenicol (30µg), Ca- Ceftazidime (30µg), Cn- Cephoxitin (30µg), Cu-Cefuroxime (30µg), Do-Doxycycline hydrochloride (30µg), I-Imipenem (10µg), K-Kanamycin (30µg), M-Methicillin (30µg), Na-Nalidixic acid (30µg), Nf-Nitrofurantoin (30µg), Nx-Norfloxacin (10µg), R-Rifampicin (30µg), S-Streptomycin (25µg), T-Tetracycline (30µg), Va-Vancomycin (30µg).

analysed for the production of β-lactamase. The concentration of phytocompounds inhibited enzyme production but not growth was considered as positive.

Results and Discussion

The results of antibacterial drug sensitivity of test strains presented in table-1 which demonstrated the resistance profile of *S. aureus*, *Klebsiella sp*. and *E. coli*. Multiple drug resistance (MDR) nature was common to all test isolates. Among selected MDR bacterial isolates *Klebsiella sp*. was found β-lactamase producer when ampicillin used as substrate (Fig. 1), while single isolates from *S. aureus* and *E. coli* were also β-lactamase positive. Production of β-lactamases is a common mechanism in *Klebsiella* isolates. However, strains of *E. coli* are also common producer of β-lactamases as reported by several workers (Alam et al., 2013; Maheshwari et al., 2016a). It is possible to detect other mechanism of resistance in these bacteria by detecting resistance genes by PCR (Hu et al., 2013 Mahaeshwari et al., 2016b).

All the four phytocompounds, citral, eugenol, geraniol and thymol showed broad spectrum antibacterial activity against test bacterial isolates as evident from their sensitivity behavior (Table-2). Eugenol inhibited growth of maximum test bacteria (93.75%) activity followed by thymol (87.50%) and citral (81.25%) and geraniol (68.75%). The efficacy of these compounds in term of growth inhibition was maximum against gram positive bacteria (*S. aureus*) followed by MDR *E. coli* and less active against MDR *Klebsiella sp*. Eugenol showed minimum MIC

Table-2 Antibacterial Activity and MIC of Essential Oil's Phytocompounds Against Test Bacterial Strains

Test bacterial strains	Phytocompounds							
	Citral		Eugenol		Geraniol		Thymol	
	Inhibition zone (mm)	MIC (%v/v)	Inhibition zone (mm)	MIC (%v/v)	Inhibition zone (mm)	MIC (%v/v)	Inhibition zone (mm)	MIC (%v/v)
KL3	-	>3.2	-	>3.2	-	>3.2	11.66 ± 0.94	1.6
KL4	-	>3.2	12.33 ± 0.47	0.8	-	>3.2	-	>3.2
KL5	10.66 ± 0.47	3.2	13.33 ± 0.94	0.8	-	>3.2	-	>3.2
KL6	-	>3.2	12.00 ± 0.82	0.8	-	>3.2	11.66 ± 1.25	1.6
EC1	13.52 ± 0.95	3.2	12.83 ± 0.76	0.8	12.49 ± 0.55	0.8	10.28 ± 0.34	1.6
EC2	14.65 ± 0.88	3.2	14.73 ± 0.92	0.4	12.03 ± 0.64	1.6	10.62 ± 0.72	1.6
EC62	22.83 ± 0.76	1.6	12.52 ± 0.76	0.8	12.83 ± 0.84	1.6	11.63 ± 0.61	0.8
UP2566	13.53 ± 0.64	0.8	13.00 ± 0.44	0.8	12.53 ± 0.84	3.2	12.03 ± 0.76	1.6
SA03	16.65 ± 0.88	0.8	17.65 ± 0.80	0.8	11.65 ± 0.59	0.8	11.65 ± 0.66	1.6
SA07	14.51 ± 0.31	0.8	14.00 ± 0.82	0.8	12.35 ± 0.92	3.2	13.35 ± 0.48	1.6
SA18	12.45 ± 0.37	1.6	11.65 ± 0.74	0.4	12.05 ± 0.99	3.2	13.35 ± 0.81	3.2
SA22	13.65 ± 0.89	1.6	13.66 ± 0.44	0.4	10.65 ± 0.45	3.2	13.25 ± 0.72	1.6
SA27	18.55 ± 0.98	3.2	15.65 ± 0.77	0.8	13.85 ± 0.38	1.6	23.65 ± 0.48	1.6

(-) Indicates no zone of inhibition, MIC: Minimum Inhibitory Concentration
Experiment performed in triplicates and data is presented as mean ± SD

Table-3 Inhibition of β-Lactamase Production by Phytocompounds of Essential Oil

Phytocompounds	Concentration (%v/v)	β-Lactamase producing isolates					
		KL3	KL4	KL5	KL6	EC1	SA27
Citral	0.4	+	+	+	+	+	+
	0.8	+	+	+	+	+	+
	1.6	+	+	-	+	+	+
Eugenol	0.2	+	+	+	+	+	+
	0.4	+	-	-	+	-	+
Geraniol	0.4	+	+	+	+	+	+
	0.8	+	+	+	+	+	+
Thymol	0.4	+	+	+	+	+	+

+ = Enzyme production (no inhibition)

- = Enzyme Inhibition

values ranging from 0.4-1.6 µg/ml followed by thymol, citral and geraniol (Table-2).

In-vitro efficacy of these phytocompounds against MDR bacteria are promising against *S. aureus* and *E.coli* indicating that resistance mechanisms present in bacteria could not protect the bacteria from action of essential oil demonstrating the efficacy of these compounds against above mentioned pathogenic bacteria. Similar observations on activity of essential oils and certain phytocompounds were also reported by other workers (Ahmad et al., 2008, Orhan et al., 2011). Sub-MIC concentrations of phyto-constiuents were evaluated for inhibition of β-lactamase production in β-lactamase producing bacterial isolates. Eugenol at 0.4% v/v and citral at 1.6% v/v showed inhibition, while geraniol and thymol remain ineffective against β-lactamase enzyme production at tested concentrations (Table-3).

Inhibition of enzyme activity is widely known by various β-lactamase inhibitors (Drawz and Bonomo, 2010). However, inhibition of β-lactamase synthesis has not been exploited as possible anti-resistance strategy. Preliminary investigation of this study indicated that phytocompounds can interfere in the synthesis of antibiotic hydrolyzing enzymes (β-lactamases). Eugenol and citral exhibited such effect; however, further investigation will reveal the mechanism of inhibition and possible intervention in drug resistance ability of bacteria.

Acknowledgment

We are thankful to Dr. S. Farooq, Director, The Himalya Drug Company (Dehradun) for his continuous support and encouragement for research on medicinal plants and Ayurvedic medicine.

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Hepatoprotective Activity of Musli Safed (*Chlorophytumarudinaceun* Baker) in Albino Rats

Mohd Nadeem*, Mohd Urooj, Habibur Rahman and Shariq Ali Khan
Pharmacology Research Unit, Regional Research Institute of
Unani Medicine, Aligarh-202002 U.P., India
*E-mail: nadeemmohd81@yahoo.com

Abstract- Musli Safed *(Chlorophytumarundinaceum* Baker) powder in triturated form was evaluated for its hepatoprotective activity against carbontetrachloride CCl_4 (1ml/kg s.c) induced liver damage in albino rats. The hepatoprotective activity was evaluated at dose levels of 1g/kg and 3gm/kg orally. The SGOT, SGT, Serum Alkaline phosphatase and Serum Acid phosphatase was evaluated for assessment of hepatoprotective activity. The drug preparation of Musli safed brought down significantly the CCl_4 induced elevated level of SGOT, SGT, Serum Alkaline phosphatase and Serum Acid phosphatase.

Keywords: Musli Safed, CCl4, Hepatoprotective Activity

Introduction

Known as Musli Safed in Unani, *Chlorophytumarudinaceun* Baker belongs to the family Liliaceae. The plant is well known for its aphrodisiac property. It is reported to be used in jaundice, asthama, piles, joint pain, leucorrhoea, gonorrhea, chronic diarrhea and in fever. It enhances the production of sperm, purifies blood and strengthens the body. It alleviates the body heat, removes bile imbalance and increases the urine output[1,2]. It is reported to have a tonic effect[3,4].

Hepatoprotective agents such as corticosteroids, antiviral drugs and immunosuppressive compounds used in modern system of medicine have their characteristic adverse effects on human body which makes the quest for safe and effective medicine for liver diseases imperative. Moreover evolution of a safe and effective hepatoprotective agent is the need of the hour. Since Musli Safedis reported to be used in jaundice in patients, it was in this context that it was chosen for evaluation for hepatoprotective activity.

Chemical Constituents of Musli Safed

It is a rich source of over 25 alkaloids, vitamins, minerals, proteins, carbohydrates, steroid and polysaccharides. The demand of safedmusli is all over world which has made it famous by such names as "Indian Viagra", "Roots of gold", "Herbal Viagra", "The wonder crop" etc. The most noted a benefit of Safedmusli (Chlorophytum arundinaceum) is its use in treating sexual deficiencies. It has been used for years to treat libido and infertility problems. Major biochemical constituents of *Chlorophytum arundinaceum*are carbohydrates 42%, protein 10%, fibres 20 - 30%, saponins 2 -17% and alkaloids 15 - 25%. Primarily saponins and alkaloids impart medicinal value[5].

Methodology

Animals

The study was carried out in Swiss albino rats (100-150gm) of either sex. The animals were procured from Mr. Rahat Hussain Enterprises Biological Suppliers Babri Mandi Aligarh. They were acclimatized to the conditions for one week before experimental study. The animals were maintained in a standard environmental condition at a room temperature of (25±2 degree Celsius) with 12 Hrs light/Dark cycles, humidity (50-55%), and had free access to food pellets.

Procurement and Preparation of Drug

MusliSafed roots were procured from Dawakhana, A.K. Tibbiya College, AMU, Aligarh. The roots were powdered and soaked in a measured quantity of water overnight. The soaked

Table-1 Effect of MusliSafed Pretreatment on Biochemical Parameters in Carbontetrachloride induced Liver Damage in Albino rats

PARAMETERS → GROUPS↓	SGOT Units/ml	SGPT Units/ml	SALP KAU	Total Serum Acid phosphatase KAU	Prostatic Fraction Of Serum Acid Phosphatase KAU
Normal Control (n=5)	140.80±11.82	41.50±6.26	80.53±10.97	8.30±1.36	3.99±0.61
CCl_4 1ml/kg s.c (n=5)	191.00**±10.64	77.25**±9.04	133.85**±11.89	21.24**±3.05	9.99**±1.43
MoosliSufaid 1g/kg p.o + CCl_4 1ml/kg s.c (n=5)	165.50±11.90	55.00±7.54	106.01±13.27	15.31±2.76	6.42±1.65
MoosliSufaid 3g/kg p.o + CCl_4 1ml/kg s.c (n=5)	155.00*±9.00	49.75*±6.09	93.76*±12.20	11.60*±2.36	5.54*±0.96

Values are Mean ± Se
n= NO OF RATS IN A GROUP
P*<0.05, P<,0.01,P***<0.001**

powder was triturated in a pestle mortar and administered orally to experimental animals.

Evaluation of Hepatoprotective Activity

Albino rats of either sex were divided in four groups of five each. Group I served as control, while group III and Group IV animals were given Musli Safed preparation at doses of 1g/kg and 3g/kg for nine consecutive days. Group II animals served as CCl_4 control and were administered distilled water for the first seven days. Thereafter on the on the 8th and 9th day all the animals in Group II, III and IV were injected CCl_4 (1ml/kg). On the 10th day blood samples from animals of all four groups were collected from retro-orbital plexus for serum enzymatic estimations. The parameters estimated were SGOT, SGPT, Serum alkaline phosphatase and Serum acid phosphatase. Method of Reitman and Frankel[6] was used for SGOT, SGPT estimations while serum alkaline and acid phosphatases were estimated by the method of Kind and King[7].

Statistical Analysis

Students 't'test was employed for determination of statistical significance of data of all the studies conducted.

Results and Discussion

The findings of the results revealed that there was significant decrease in CCl_4 induced elevation of SGOT, SGPT, Serum alkaline phosphatase and Serum acid phosphatase at the dose of 3g/kg by Musli Safed (Table-1). The hepatoprotective activity of the Musli Safed may be attributed either to its free radical scavenging[8] activity or due to prevention of generation of free radical or reactive electrophilic metabolites.

Conclusion

It may be concluded from the above findings that Musli Safed has hpatoprotective activity at higher dose level. Further studies are required to evaluate its mechanism of action and separation of active ingredient responsible for hepatoprotective activity.

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Antifungal and Free Radical Scavenging Efficacy of *Hymenodictyon excelsum* Bark

Garima Mishra, Nishat Anjum, Y.C. Tripathi* and A.K. Pandey
Chemistry Division, Forest Research Institute, Dehradun, 248006, Uttarakhand, India
*E-mail: tripathiyc@gmail.com

Abstract- There is wide spread interest in drugs derived from plants as green medicine is believed to be safe and dependable, compared with costly synthetic drugs that have adverse effects. The study was thus aimed to evaluate the antifungal and antioxidant efficacies of the bark of *Hymenodictyon excelsum. Antifungal activity of methanolic extract of H. excelsum bark was assessed against* some important and frequently occurring pathogenic fungi viz., *Alternaria alternate, Aspergillus flavus*, *Cladosporium cladosporidies, Drechslera halodes* and *Fusarium moniliforme by* agar-well diffusion method whereas antioxidant activity was evaluated using a modified 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. In the antifungal assay, bark extract at concentration of 40mg/ml showed growth inhibition almost at par with synthetic fungicide Carbendazim and maximum 50 mg/ml concentration of extract even higher than the positive control. DPPH free radical scavenging assay showed that the methanol extract of *H. excelsum* bark showed effective free radical scavenging at higher concentrations. Percentage inhibition of 95.36 and 55.94 is exhibited by the highest dose (100 µg/ml) of the ascorbic acid, and bark extract respectively. The observed antifungal and antioxidant potency of *H. excelsum* bark which may be due to presence of chemical constituents, confirmed the use of the plant in traditional medicine. The results of the study provide reasonable basis for further investigation of the plant in the quest of potential natural bioactive compounds.

Keywords: *Hymenodictyon excelsum, Bark, Antifungal activity, Antioxidant activity*

Introduction

Medicinal plants have been an important therapeutic aid for various ailments since millennia. Plants have served as the richest source of raw materials for traditional as well as modern medicine all over the world. India is one of the nations with the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Many of the indigenous medicinal plants have been used in traditional system of medicine in various forms (Amin et al., 2013). Drugs of plant origin remain an important source to combat serious diseases, especially in developing countries. Approximately 60-80% of the world's populations still depend on traditional medicines for the treatment of common illnesses (WHO, 2002). Currently, naturally derived products play an important role as a source of medicine and medicinal plants are universally considered as important sources of new chemical substances with potential therapeutic effects. Many pharmaceutical agents have been discovered by screening natural products from plants, based on ethnopharmacological data which provides a substantially increased chance of finding active plants relative to a random approach (Saslis-Lagoudakis et al., 2012). There are several reports regarding the various pharmacological efficacies of extracts, enriched fractions and bioactive phytochemicals obtained from plants. Some of the plant derived bioactive compounds are preferred for their therapeutic purposes either as a single entity or in combination. Of recent times, most of the industries are focusing on the use of natural biomolecules for drug production.

Hymenodictyon is a genus of flowering plants under family *Rubiaceae* (Coffee family). The

genus comprises about 30 species of trees and shrubs distributed mostly in tropical and sub-tropical parts of Asia and Africa. The species belonging to the genus are having oppositely arranged serrated leaves, small, clustered flowers and many seeded capsules. Some of the species are commercially useful for tanning and dyeing purposes. *Hymenodictyon excelsum* **(Roxb.) Wall.** commonly known as Bhorsal, Kukurkat, Bhaulan, Bauranga, Pottaka, Kusan and Aligango in trade, is a medium to tall growing deciduous tree distributed throughout Oceania and Southeast Asia. It occurs in India, Burma, Java, Bangladesh, Indonesia, Malaysia, Philippines and Thailand. *It is also* found in the Himalayan region of India (**Gurung, 2002;** Razafimandimbison and Bermer, 2006). In traditional system of medicine, the bark of *H. excelsum*is has been used as an astringent and febrifuge and for treatment of fever and tumors, while the leaves are used to treat ulcers, sialitis, sore throat, tonsillitis and inflammatory conditions (Nareeboon et al., 2009).

Phytochemical investigation of the plant have reported tannin, toxic alkaloid, hymenodictine, aesculin, an apioglucoside of scopoletin and hymexelsin in the stem bark (Rao et al., 1988) whereas anthraquinones, rubiadin and its methyl ether, lucidin, nordamnacanthal, damnacanthal, 2-benzylzanthopurpurin, anthragallol, soranjidiol and morindone have been isolated from roots (Rastogi and Mehrotra, 1990). Acetylenic fatty acids, triglyceride and triterpenes were isolated from the leaves of the plant (Nareeboon et al., 2009). Some of the compounds namely aesculin, aesculetin, morindone, nordamnacanthal, damnacnathal isolated from the plant are reported to inhibit Glycogen Synthase Kinase 3 Beta (gsk3beta) (Karthik et al., 2014). The plant has been reported to have antioxidant and anti-inflammatory properties (Kar et al., 2013). However, pharmacological investigations of the plant have so far been very limited. The present study was thus aimed to investigate the antifungal and free radical scavenging activities of *H. excelsum* bark extract for assessing its potential application in plant based medications to control diseases caused by pathogenic fungi and free radicals.

Material and Methods

Plant Material

The barks of *Hymenodictyon excelsum* were collected from the Botanical Garden of Forest Research Institute, Dehradun Uttarakhand, India. The specimen were identified and authenticated from Botany Division FRI and voucher specimen was preserved in the Chemistry Division, FRI for future reference. Cleaned and shade dried barks were chopped by an electronic grinder to coarse powder and used for further studies.

Extraction of Plant Material

The powdered plant material (200g) was extracted with 1000 ml methanol (CH_3OH) by using a Soxhlet apparatus for 8 h. The methanol extract so obtained were filtered with Whatman filter paper (No.1) and the filtrates were concentrated using rotary evaporator. Then the extracts were evaporated to dryness over water bath and solvent free extracts of respective parts were obtained (Nostro et al., 2000). The dried extracts were stored in labelled sterile specimen bottles at 20^0C until assayed.

Determination of Antifungal activity

Test Fungi

For antifungal evaluation of *H. exceisum* bark, some important and frequently occurring pathogenic fungi viz., *Alternaria alternata* (AA), *Aspergillus flavus* (AF), *Cladosporium cladosporidies* (CC), *Drechslera halodes* (DH) and *Fusarium moniliforme* (FM) were selected. These fungi were isolated from the infected seeds by Standard Blotter Method (ISTA, 1999) and identified based on growth characteristic, mycelial morphology, spore morphology and other important characters using standard protocol (Barnett and Hunter, 2000; Mukadam, 2006). Pure cultures of each of the selected fungal species were made separately and maintained at

5^0C on PDA slants. These pure cultures were used for antifungal assay.

Preparation of Test Solutions

Test solutions of a series of concentrations viz., 5, 10, 20, 30, 40 and 50 mg/ml were prepared from methanolic extracts of *H. excelsum* bark by dissolving the extract in Dimethyl sulfoxide (DMSO). All test solutions were kept in refrigerator at 4^0C for future use.

Preparation of Fungal Inoculums

For antifungal assay cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 1-5x10^5 cfu/ml.

Preparation of Media

The medium was prepared by dissolving Potato dextrose agar (PDA) media (HiMedia) in distilled water and autoclaving at 121^0C for 15 minutes. 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm diameter) and allowed to solidify, which were used for antifungal assy.

Antifungal Activity Assay

Antifungal activity of methanolic extract *H. excelsum* bark was determined using agar-well diffusion method (OECD, 2001). Spore suspensions (0.2ml) were applied on the surface of the presterilized and autoclaved PDA petridishes and spread by using a sterile glass spreader. Wells of 6mm diameter were made in centre of each of the PDA petriplates with the help of sterilized cork borer. The wells were filled with test solutions of bark extract as prepared above with three replications for each treatment. Carbendazim (2mg/ml) and DMSO were served as positive and negative control respectively for each of the three extracts. All the petridishes including treatments and controls were allowed to diffuse at room temperature for 2 hours and then incubated at room temperature (28 ± 2^0C) for 72 hours. After incubation, the antifungal activity of extracts was determined by measuring the diameter (mm) of inhibition zones.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined through the broth dilution method (Gatsing et al., 2010; Chandur, 2013). Fungi were first grown in the potato dextrose broth for 24 hrs and then the inoculums were diluted for five times (10^{-5}dilution) to control its vigorous growth. Then each test tube was added with 1.8 ml of potato dextrose broth and different concentrations (1-10 mg/ml) bark extracts followed by inoculation of 0.2 ml of respective fungi and kept at 28˚C for 48 hrs. The tubes were examined for visual turbidity. Lowest concentrations of the extracts showing no turbidity (without microbial growth) were considered as the minimal inhibitory concentration.

Determination of Antioxidant Activity

The antioxidant activity of *H. excelsum* bark was evaluated using a modified 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Sharma and Bhat, 2009). The free radical inhibition potential of any compound depends on its ability to donate hydrogen or free electrons. DPPH molecule is a stable free radical with typical purple colour that decays in the presence of an antioxidant, which can donate an electron to it. The change in absorbancy is spectrometrically measured at 517nm. Stock solutions of the methanolic extract of bark and standard ascorbic acid were prepared in ethanol with concentrations ranging from 100 to 1.56 µg/ml. To the diluted sample (0.1 ml), 2.9 ml of 0.004% DPPH solution was added. Then the contents were mixed properly and incubated in the dark for 20 min at $28\text{-}30^0$C to complete the reaction. The changes in absorbance was measured at 517nm on UV/V in double beam spectrophotometer (Chemito 1700).The absorbance was determined at 517 nm. The percentage DPPH radical scavenging activity was calculated by the following equation.

$$\text{Radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

The results of each extract was expressed IC_{50} (50% inhibiting concentration value), that is concentration of the extract that causes 50 % loss of the DPPH activity (scavenge the initial DPPH concentration by 50%). IC_{50} value was expressed in µg/ml. Lower IC_{50} value is indicative of better DPPH radical scavenging capacity.

Results and Discussion

The antifungal activity of *H. excelsum* bark extract determined by the agar-well diffusion method is shown in Table-1.

However, 30 mg/ml test concentration of extract is considerably effective on growth inhibition of all the test fungi. Test concentration of 40mg/ml showed growth inhibition almost at par with synthetic fungicide, Carbendazim (positive control). Further, growth inhibition results in all the five fungi is the highest at 50 mg/ml concentration of extract and are found to be higher than the positive control (Table-1). The mean radial growth inhibition of test fungi *Alternaria alternata*, *Aspergillus flavus*, *Cladosporium cladosporidies, Drechslera halodes* and *Fusarium moniliforme* with various concentrations of extracts of all the three parts of the plant ranged between 3.52- 42.31, 5.23- 45.26, 4.59-43.51, 5.29-44.93 and 6.26-45.53 mm respectively.

Table-1 Antifungal Activity of *H. excelsum B*ark *E*xtract Against Fungi

Conc. (mg/ml)	Zone of Inhibition in mm				
	AA	AF	CC	DH	FM
5	3.52±0.21	5.23±0.53	4.59±0.33	5.29±0.41	6.26±0.47
10	8.60±0.15	10.87±0.31	8.96±0.25	9.89±0.33	10.51±0.31
20	18.23±0.33	21.33±0.51	20.51±0.13	16.66±0.43	17.53±0.33
30	27.53±0.23	31.87±0.31	29.23±0.20	30.69±0.31	32.66±0.43
40	35.65±0.08	38.65±0.15	35.65±0.15	35.65±0.08	35.65±0.08
50	42.31±0.19	45.26±0.31	43.51±0.13	44.93±0.53	45.53±0.31
+ Control	32.00±0.07	36.63±0.18	33.53±0.41	35.46±0.71	33.67±0.71
– Control	–	–	–	–	–

Values are given in mean±SD for three replicates. AA=*Alternaria alternata*, AF=*Aspergillus flavus,* CC= *Cladosporium cladosporidies,* DH= *Drechslera halodes,* FM=*Fusarium moniliforme*

Minimum inhibitory concentrations (MIC) *H. excelsum* bark extract for all the test fungi were found in the range of 3.25 – 4.50 mg/ml. It is confirmed by the results that bark extracts of *H. excelsum* have very low MIC values against the test fungi.

Fungal growth inhibition results presented clearly indicated that the methanolic extract of *H. excelsum* bark exhibited varying degrees of antifungal activity against all the five test fungi. From the result, it is also evident that growth inhibition of all the fungi increased with increase in test concentration of extracts. Of the different test concentrations, it is observed that inhibition of radial growth at concentration of 5, 10 and 20 mg/ml is less than that of positive control.

Antioxidant Activity

The results of DPPH radical scavenging assay of *H. excelsum* bark extract are shown in Table-2. It is evident from the values presented in the Table 2 that both bark extract and standard, ascorbic acid showed DPPH radical scavenging in a dose dependant manner.

The methanol extract of *H. excelsum* bark showed effective free radical scavenging at higher concentrations. Percentage inhibition of 95.36

Table-2 Antioxidant Activities of Bark Extract and Standard

Concentrations (µg/ml)	Percent inhibition of DPPH Radical	
	Bark Extract	Ascorbic Acid
100	55.94	95.36
50	26.32	92.87
25	23.45	90.59
12.5	06.63	51.85
6.25	03.28	30.93
3.125	02.36	14.23
1.5612	00.87	11.36
IC_{50} (µg/ml)	34.95	9.45

and 55.94 is exhibited by the highest dose (100 µg/ml) of the ascorbic acid, and bark extract respectively. Inhibition of DPPH radicals above 50% is considered as significant for antioxidant properties of any compounds (Sanchez-Moreno et al., 1998). IC_{50} of the bark extract and standard were 34.95 and 9.45 µg/ml. The presence of phenolic compounds could be one of the main reasons for the antioxidant activity. Oxidative stress has been associated with a variety of pathologic conditions in humans. The scavenging activity of the extract used in the present study reflects the antioxidant properties but concentrations which brought this change, are high as compared to standard reference used. This further suggests that scavenging activity of DPPH radical is present in few compounds of the extract used and there is need to purify and characterize the individual components and test them for their antioxidant properties both *in vitro* and *in vivo.*

Phytochemical screening of *H. exccelsum* bark extracts have reported the presence of alkaloids, flavonoids, terpenoids, saponins, phenolic compounds and tannins (Chakraborty et al., 2016). The antifungal and antioxidant potency of *H. excelsum* bark may be due to presence of chemical constituents of complex molecular structure and diverse action mechanisms.

Conclusion

The results obtained from the present study are in agreement to a certain degree with the traditional therapeutic uses of the plant. The results of the antifungal and antioxidant assays confirmed the use of the plant in traditional medicines and could form a good basis for selection of the plant for further investigation in the quest of potential natural bioactive compounds.

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Preliminary Screening and Phytochemical Investigations of Different Solvent Extracts of *Citrus aurantium* Peel, Fruit and Seeds

*[1]S. C. Sati, [1]Manisha D Sati and [2]Shikha Saxena
[1]Department of Chemistry HNB Garhwal University Srinagar, Uttarakhand, India
[2]Department of Chemistry, DAV PG College, Dehradun, India
*E-mail: sati_2009@rediffmail.com

Abstract- Plants and their secondary metabolite constituents have a long history of use in modern 'western' medicine and in certain systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine. In some cases, the active principles of plant-derived products have been isolated and characterized, and their mechanisms of action are understood (e.g., ephedrine alkaloids in some species of Ephedra). For many, however, including virtually all of the most common products in the marketplace, such information is incomplete or unavailable. This is in large part due to the complexity of herbal and botanical preparations; they are not pure compounds. It is also a function of the traditionally-held belief that the synergistic combination of several active principles in some herbal preparations is responsible for their beneficial effects. The objective of the present study was to investigate the presence of various phytochemicals from the EtOH, aqueous and $CHCl_3$ extracts, Citrus peel, fruit and seeds.

Introduction

The herbal and medicinal value of plants appears in all early records of human activity, from the Chinese 5000 years ago, to the herbalists, apothecaries, pharmacists, and physicians of all succeeding generations, to modern use of herbs, their extracts, and synthetic products to treat minor ailments and diseases today.

Citrus aurantium (bitter orange) is a plant belonging to the family *Rutaceae*, The most important biologically active constituents of the C. aurantium fruits are phenethylamine alkaloids octopamine, synephrine, tyramine, N-methyltyramine and hordenine. It is rich in vitamin C, flavonoids and volatile oil. Synephrine is a primary synthesis compound with pharmacological activities such as vasoconstriction, elevation of blood pressure and relaxation of bronchial muscle, whose fruit extracts have been used for the treatment of various diseases such as gastrointestinal disorders, insomnia, headaches, cardiovascular diseases, cancer, antiseptic, anti-oxidant, antispasmodic, aromatic, astringent, carminative, digestive, sedative, stimulant, stomachic and tonic. In recent era, there is a great thrust on screening of herbal extracts and formulations for antiobesity action. The present investigation was designed to investigate the presence of various phytochemicals in the three different extracts of citrus aurantium peel, whole fruit and seeds.

Material and Methods

Collection of Plant Material

The fruits of *Citrus aurantium* were collected from Mandal, near Gopeshwar Chamoli and specimens were identified with Taxonomists, Department of Botany, HNB Garhwal University Srinagar, Garhwal Uttarakhand, India.

Preparation of Ethanolic Extract and Chloroform Extract

The peel, whole fruit and seeds of *Citrus aurantium* was dried in hot air oven at 30 -50 ^{0}C

for a week. The dried plant material was powdered using mixer grinder, and subjected to soxhlet extraction with 90% EtOH and $CHCl_3$for 24 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerator. The condensed extracts were used for preliminary screening of phytoconstituents.

Preparation of Aqueous Extract

The peel, whole fruit and seeds powder was boiled in distilled water for 30 minutes, the filtrate was placed at room temperature overnight. The filtrate was evaporated to dryness in hot air oven and stored in refrigerator. The condensed extracts were used for preliminary screening of secondary metabolites

Phytochemical Analysis of Different Extracts

Different extracts were tested for the presence of active principles using standard procedures.

Test for Steroids and Triterpenoids

Liebermann Burchard Test: Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Test for Glycosides

Keller Killiani Test: Test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Con. sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Bromine Water Test: Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Foam Test: Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Alkaloids

Hager's Test: Test solution was treated with few drops of Hager's reagent (saturated picric acid solution).Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Flavonoids

Ferric Chloride Test: Test solution when treated with few drops of ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline Reagent Test: Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Test for Tannins

Gelatin Test: Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Test for Proteins

Biuret Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Test for Free Amino Acids

Ninhydrin Test: Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Carbohydrate

Benedict's Test: Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Vitamin C

DNPH Test: Test solution was treated with Dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Results and Discussion

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The various extracts of peel, whole fruit and seeds of *Citrus aurantium* have revealed the presence of Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Carbohydrate and Vitamin C. Proteins and free amino acids were found to be absent in all the extracts. Saponins and alkaloids were present only in the whole fruit and seeds extract while flavonoids were found to be present only in the peel and whole fruit extract. From this analysis, ethanolic extract of whole fruit was found to have more constituents compared to peel and seeds extracts. The results of preliminary phytochemical analysis are shown in Table. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical

Table- Preliminary Phytochemical Analysis

TESTS	Fruit peel extract			Whole fruit extract			Seed extract		
	EtOH	Aq.	$CHCl_3$	EtOH	Aq.	$CHCl_3$	EtOH	Aq.	$CHCl_3$
Triterpenoids and steroids									
LB Test	+	+	-	+	_	_	+	+	+
Glycosides									
Kellerkilliani Test	+	+	+	-	+	+	+	-	-
Bromine Water	-	+	-	-	+	+	+	-	-
Saponins									
Foam Test	-	-	-	+	+	-	+	+	-
Alkaloids									
Hager's Test	-	-	-	+	-	-	+	-	+
Flavonoids									
Ferric Cloride Test	+	+	-	+	+	-	-	-	-
AlkalineReagent Test	+	+	-	+	+	-	-	-	-
Tannins									
Gelatin Test	+	-	-	+	+	-	+	-	-
Proteins									
BiuretTest	.	.	.	.	.	.	.	.	.
Free amino acids									
Ninhydrin Test	.	.	.	.	.	.	.	.	.
Carbohydrate									
Benedict's Test	+	+	-	+	-	-	-	+	+
Vitamine C									
DNPH Test	-	+	-	+	-	+	-	+	+

compounds. The phytochemical screening in the present study, has revealed the presence of triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate and vitamin C in the peel extract; triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate and vitamin C in the whole fruit extract and triterpenoids, steroids, glycosides, saponins, alkaloids, tannins, carbohydrate and vitamin C in the seeds extract. Further, the presence of different phytoconstituents in the three different extracts may be responsible for the therapeutic properties of citrus. Flavonoids and tannins are phenolic compounds and that the plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of fruit. These phytochemicals and other chemical constituents of medicinal plants account for their medicinal value. The presence of saponins in whole fruit and seeds extract and glycosides in all the extracts might play a role in the cardioprotective potential of Citrus. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

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Effect of Carbohydrates and pH on *in vitro* Shoot Multiplication of Promising Interspecific F1 Hybrid of Eucalyptus (*Eucalyptus tereticornis X Eucalyptus grandis*)

Barkha Kamal[1]*, Nidhi S. Belwal [1], I.D. Arya[2] and Vikas Singh Jadon[1]
[1]Sardar Bhagwan Singh PG Institute of Biomedical Sciences and Research
Department of Biochemistry and Biotechnology, Balawala, Dehradun, India
[2]Arid Forest Research Institute, Jodhpur, India
*E-mail: barkhabiotech2005@gmail.com

Abstract- The tissue culture technology is standardized for its multiplication, using axillary buds of 25-30 years old plants. The axillary buds were surface sterilized with 0.1% mercuric chloride solution for 10-15 minutes, followed by 0.1% Bavistin treatment for 1 minute and subsequently washed 3-4 times with sterilized distilled water. These surface sterilized axillary buds were cultured on MS medium supplemented with cytokinin and auxin (BAP + NAA). Axillary bud break was achieved in 5 weeks. MS medium supplemented with 1.5mg/l BAP + 0.1mg/l NAA proved to be the best hormonal combination for induction of axillary bud which resulted in the development of 1-3 axillary shoots. The proliferated shoots 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 essential for the development and growth of shoots. These proliferated axillary shoots were excised and subcultured on MS + 1.0 mg/l BAP + 0.1mg/l NAA medium to increase the number of shoots. During the present investigation, it was found that good shoot multiplication occurred in the pH range of 5.5 to 6.0. Shoot multiplication of FRI-6 was maximum when the pH of the medium was 5.8.

Introduction

Tissue culture, an important aspect of biotechnology has a great potential for rapid and mass multiplication of clonal production of plants. Promising interspecific F_1 hybrids of Eucalyptus developed in India by Forest Research Institute, Dehradun has displayed a very high degree of vigour both in diameter, height and wood quality. FRI- 6 is a control hybrid of *E. tereticornis X E. grandis* (Venkatesh and Sharma, 1979). This hybrid is of immense economic interest because it involves *E. tereticornis* and *E. grandis* as two parent species. The former shows faster growth rate, good stem form, provide best quality of pulp and prefers high rain fall areas while, *E. tereticornis* is drought tolerant species and thus it is very likely that this hybrid may be suited for intermediary zones (Venkatesh and Sharma, 1979).

Material and Methods

Explant source and its culture

Nodal segments with single axillary bud were used as the source material for micropropagation. The axillary buds were first washed with Cetrimide (ICI ltd. India) solution for 5 min and thereafter surface sterilized with 0.1% Mercuric chloride solution (10-15 min) followed by 1.0% Bavistin treatment for one minute. Other sterilant like $NaOCl_2$ (4%) and H_20_2 (20%) were also tested for sterilization of nodal segments. Surface sterilized nodal segments rinsed with 3-4 times sterile distilled water. The surface sterilized axillary buds were cultured on semi-solid Murashige and Skoog's (MS) medium supplemented with cytokinin (BAP and Kinetin). The pH of the medium was adjusted to 5.8 prior to autoclaving the medium at 121° C for 15 min. Cultures were maintained at 25 ± 2 ° C with 16 hrs illumination with the photon flux density of 2500 lux, form white fluorescent tubes.

Establishment and multiplication of shoot cultures

Axillary bud cultured on liquid and semi-solid MS medium supplemented with cytokinin, resulted in axillary shoots proliferation. These axillary shoots were excised and subcultured on fresh liquid as well as semi-solid MS medium supplemented with BAP (0.1-3.0 mg/l) alone or in combination with NAA (0.1 mg/l 1.5 mg/l) for further shoot multiplication. Different set of experiments were conducted to obtain the maximum shoot multiplication rate. For this, multiplied shoots were subcultured in a propagule consisting of 6-8 shoots. For each experiment, a minimum of 12 replicates were taken. Observations were recorded after an interval of 5 weeks. Once the optimal shoot multiplication medium was established, the shoots produced were excised in propagules and subcultured every 4-5 weeks. Cultures were multiplied and maintained under 20-30 μEM^{-2} S^{-1} photon flux density for 16 hrs. photoperiod at 25±2°C. The number of propagules cultivated and number of propagules derived at the end of subculture was regarded as the rate of multiplication.

Statistical Analysis

All experiments were repeated thrice. Each treatment consists of 12 replicates. The data representing means of three experiments were analyzed with the help / use of statistical packages viz. Excel ver 2.0 and GenStat ver 8.0 for data of a completely randomized design. The data recorded for various parameters during the study were subjected to one and two way analysis of variance (ANOVA). The significance of the data was ascertained by F-test and the critical difference (C.D.) values at 5% computed, for comparing differences means of various treatments

Results and Discussion

***In vitro* shoot Multiplication**

Effect of Phytohormones

The proliferated axillary *in vitro* shoots were excised from the mother explants and cultured on semi-solid MS medium supplemented with 0.1-3.0 mg/l BAP for further *in vitro* shoot multiplication. These multiplied *in vitro* shoots were later dissected out into propagule (group of 6-7 shoots) and were subcultured on MS medium supplemented with 0.1-3.0mg/l BAP for further *in vitro* shoot multiplication (Table-1). 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 fold in every 5 weeks subculture duration was obtained (Table-2). MS medium proved to be the best medium for the establishment of shoot cultures in Eucalyptus hybrids. In earlier reports on Eucalyptus F_1 hybrids, 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 Effect of Cytokinin (BAP) on *in vitro* Shoot Multiplication. MS Medium Used. Data Recorded After 5 Weeks

Hormonal Concentration BAP (mg/l)	Average no. of shoots developed	Multiplication rate	Average no. of shoots length (cm)
Control	11.4 ± 0.93	1.63 ± 0.13	0.70 ± 0.06
0.1	10.6 ± 0.93	1.51 ± 0.13	0.78 ± 0.08
1.0	41.2 ± 1.71	5.89 ± 0.24	1.01 ± 0.08
2.0	34.0 ± 2.54	4.86 ± 0.36	0.76 ± 0.07
3.0	35.2 ± 2.32	5.03 ± 0.62	0.44 ± 0.04
Significance	***	***	***
CD	7.23	1.06	0.20

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

Table-2 Effect of Hormonal Interaction (BAP+NAA) on *in vitro S*hoot Multiplication. MS Medium Used. 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 Carbohydrate Source

For *in vitro* culture of shoots, carbon source in the forms of sugars has to be added to the nutritional medium for growth and proliferation of cultures as the photosynthetic capability of culture is limited. In present case, different concentration of sucrose (1%-6%) in MS medium was tested for the growth and development of *in vitro* shoots. Sucrose at 3 % in the MS medium gave the best results with 6-7 folds *in vitro* shoot multiplication (Table-3). On

Table -3 Effect of sucrose concentration on shoot multiplication rate. 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 -4 Effect of Myo-inositol on Shoot Multiplication Rate. Shoots Cultured on MS Medium + 1.0 mg/l BAP. Data Recorded After 5 Weeks.

Myo-inositol concentrations	Multiplication rate	Average number of shoots produced	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

Table -5 Effect of pH on Shoot Multiplication Rate. Shoots Cultured on MS Medium + 1.0 mg/l BAP. Data Recorded After 5 Weeks.

pH of Medium	Multiplication rate	Average number of shoots produced	Average shoot length (cm)
2.8	4.53 ± 0.21	22.67 ± 1.06	1.34 ± 0.13
3.8	4.47 ± 0.27	22.33 ± 1.36	2.27 ± 0.20
4.8	7.07 ± 0.34	35.33 ± 1.71	2.93 ± 0.07
5.8	10.50 ± 0.65	52.50 ± 2.27	3.22 ± 0.17
6.8	10.53 ± 0.92	52.67 ± 2.36	3.13 ± 0.13
7.8	7.10 ± 0.38	35.50 ± 1.89	2.93 ± 0.07
8.8	6.07 ± 0.25	30.33 ± 1.23	2.02 ± 0.17
9.8	5.77 ± 0.30	28.83 ± 1.51	1.91 ± 0.10
10.8	3.90 ± 0.25	19.50 ± 1.23	1.15 ± 0.14
Significance	***	***	***
CD	1.31	6.57	0.39

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

sucrose free medium the *in vitro* shoots did not multiply and with the passage of time leaves and shoots became pale green. The results of the present investigations are compatible with the reports of many workers who used 3% sucrose as a source of carbohydrate for shoot multiplication of different 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

Effect of myo-inositol was assessed on *in vitro* shoot multiplication rate. It was observed that MS medium supplemented with 100mg/l gave the best multiplication rate as compared to other concentrations tried. MS medium without myo-inositol showed reduced *in vitro* shoot multiplication. Excessive used of myo-inositol i.e. 150 mg/l and above in MS medium not only reduced the *in vitro* shoot multiplication rate but had a deleterious effect on *in vitro* shoots. Thus, in all the experiments 100mg/l myo-inositol was added into the medium as optimum required for the growth and multiplication rate of *in vitro* shoots (Table-4).

Effect of pH

Effect of different pH in the medium was assessed. Medium was varied from pH 3.8-7.8 in liquid and semisolid MS medium supplemented with 1.0 mg/l BAP +0.1mg/l NAA. Under acidic conditions (3.8-4.8) the *in vitro* shoots that developed were small and condensed. At increased pH levels (towards basic) *in vitro* shoot multiplication rate reduced. The best *in vitro* shoot multiplication was obtained on MS medium with pH 5.8 where *in vitro* shoot multiplication rate of 6-7 folds was obtained (Table-5). Thus, in all the subsequent experiments pH of the medium was adjusted to 5.8 prior to autoclaving. It has been reported that pH influenced the growth and development of *Ipomea* suspension culture mainly by effect on uptake or utilization of ammonia and nitrate (Martin and Rose, 1976). Though, the majority of plant tissues grow optimally at pH 5.0 to 5.5(Butenko, 1984) yet, the pH range is variable for individual plant tissues. During the present investigation it was found that good shoot multiplication occurred in the pH

range of 5.5 to 6.0. Shoot multiplication of FRI-6 was maximum when the pH of the medium was 5.8 these results compare favorably with those of other workers, (Bisht *et al.,* 2000a and Kapoor and Chauhan, 1992).

Moreover, pH of the medium also declines rapidly on subculture in the first few weeks. At pH higher than 5.8, precipitation of some minerals of the media may be a problem (Dalton *et al.,* 1983). Shoot length was better in acidic medium (2.8-4.8). Similar, results were reported in cultures derived from *Castanea sativa* seedlings in which low pH stimulated the elongation of shoots (Chevre *et al*., 1983).

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Pharmacognostic Evaluation of *Dendrobium macraei* Lindl.

Kundan Singh Bora* and Esha Vatsa
Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand, India
*E-mail: kundanresearch1381@gmail.com

Abstract- In the present study, an attempt has been made to highlight the importance of the plant Dendrobium macraei Lindl. (family-Orchidaceae) in the field of traditional medicines. It is commonly known as Swarna Jivanti. The plant is one of the important *Rasayana* drugs in Ayurveda. It is mainly found in Sikkim Himalayas, Bengal and Khasia mountains at altitude of 7000-8000 ft. The plant has been reported to be useful as cooling, alterative, astringent to the bowels, stimulant, nervine tonic, aphrodisiac, expectorant, in asthma, bronchitis, 'tridosha', throat troubles, fevers, burning sensations, biliousness, diseases of the eye and blood. *The current study emphasizes on* taxonomical details, macroscopic and microscopical studies and physico-chemical parameters. This study will help in authentication of plant material and will acts as a standardised parameter for future study.

Keywords: *Dendrobium macraei,* Pharmacognosy, Swarna Jivanti, Rasayana drug, Standardization.

Introduction

The plant Dendrobium macraei is the important botanical source of Ayurvedic drug Swarna Jivanti belonging to family, Orchidaceae. Various synonyms of *Dendroium macraei* viz. *Desmotrichum fimbriatum*, *Flickingeria macraei* (Lindl.)**,** *Flickingeria fimbriata, Ephemerantha macraei* (Lindl.)**,** *Callista macraei* (Lindl.), *Flickingeria rabanii* (Lindl.), *Dendrobium fimbriatum* (Bl.), *Dendrobium nodosum (*Dalz.)*, Flickingeria nodosa* (Dalz.) are also used as Swarna Jivanti. It is an epiphyte with creepy rhizome and pendulous stem (Santapau & Kapadia, 1966). The plant is sweet with a flavor, cooling, alterative, astringent to the bowels, tonic, aphrodisiac, expectorant, useful in asthma, bronchitis, throat troubles, fevers, burning sensations, biliousness, diseases of the eye and the blood. Plant is stimulant and tonic (Kirtikar & Basu, 1989). The plant is reported to contain alkaloids, carbohydrates, flavonoids, steroids, tannins and phenolic compounds. Jibantine, resinous principles α-and β-jibantic acid and diosgenin derivatives like denfigenin and defuscin as steroids are reported as chief constituents in *Dendrobium macraei* (Wealth of India, 1952). Jivanti is one of the important ***Rasayana*** drugs in Ayurveda and is used as an ingredient in formulations like jivantadya ghrita, jivantayadi rasa, jivantadyadi taila, Ashwagandhadi ghrita, anuthaila, chandanadi thail*a* which are used in treatment of tuberculosis, emaciation, fever, haemorrhage and various types of cardiac ailments (Sharma *et al.*, 2001; Yoganarasimhan, 2000). Therapeutically, Jivanti is used as alterative, aphrodisiac, astringent, cooling, diuretic and as a tonic in various types of debilities (Gupta *et al*., 1970).

Material and Methods

Plant Material

Plant material (*D. macraei* whole plant) was procured from Kankhal, Haridwar, Uttarakhand, India (Plate 1). The plant was identified and authenticated at the Herbarium of Council of Scientific and Industrial Research - National Institute of Science Communication and Information Resources (CSIR- NISCAIR), Delhi vide reference no. NISCAIR/RHMD/Consult/ 2015/2565-144.

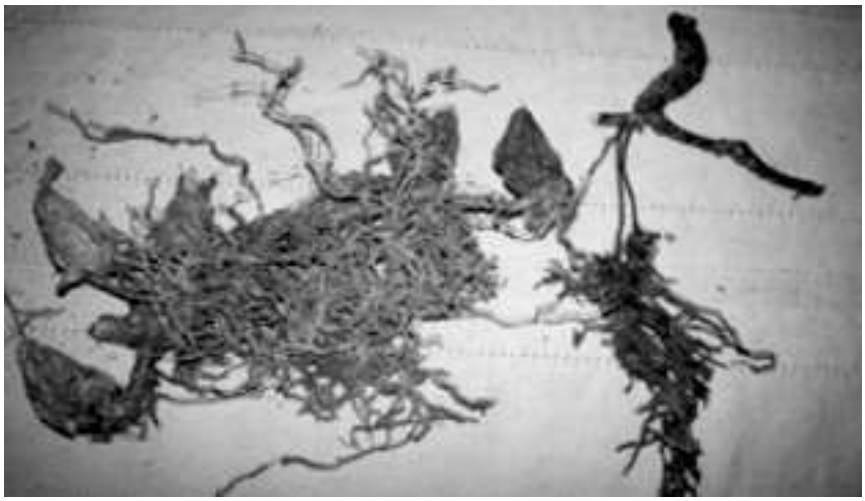

Plate-1. Whole plant of *D. macraei*

Pharmacognostic Evaluation

Organoleptic Studies

Organoleptic evaluation of drugs is based on color, odor, taste, size, shape and special features like texture and touch etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to impressions on organs of senses. Organoleptic evaluation of *D. macraei* plant was done on the basis of general and visual appearance of crude plant material.

Transverse Sections

Plant material is soaked in warm water along with 2 ml of glycerol to smoothen the surface of stem of plant material for transverse section cutting purpose. Transverse section was obtained by cutting along the radial plane of a cylindrical portion of the stem/root/ stolon and perpendicular to the long axis. Then, that section when observed under the microscope reveals the radial arrangement of tissues, concentric layers and vascular bundles. Safranine 5% solution in water is used for staining purpose of transverse sections of stem. For the section cutting of root/stem, a cylindrical portion which was almost straight was cut and both the edges of sample were cut off so as to make the surface of edges smooth. Then, the section of sample was cut. Sufficient and uniform sections of sample was cut and placed in a clean watch glass which contains the staining solution. After 2-3 minutes, the stained section was picked out with the help of a brush and transferred into plain water so that excess stain was washed away. Then, the prepared section was mounted with the help of a brush on a clean glass micro slide. One drop of glycerol- water (1:1) mixture was added over the section with the help of a dropper. Then, a clean cover slip was placed over the section with the help of a forcep and a needle. After that, the prepared slide was observed under microscope (Wallis, 2005).

Powder Microscopy

It was done by the dutch process. Firstly, 2 g of powdered drug was taken and 10% nitric acid solution (50 ml) was added and warmed for 2 min. Then, the solution was filtered and residue was obtained. It was washed with hot water and then filtered. Again the residue was taken and 10% sodium hydroxide solution (50 ml) was added, warmed for 2 min. and again the solution was filtered, residue washed with hot water and again

filtered. Finally, residue was taken for powder microscopy. Powder microscopical studies were carried out using various reagents such as Sudan red- III, iodine solution, ferric chloride solution, phloroglucinol + conc. hydrochloric acid solution. Photo-micrographs of transverse sections and powder microscopical features were carried out under compound microscope using Canon A1300 Power shot digital camera (16 mega pixels with 5× Zoom lens).

Physico-chemical Evaluation (Indian Pharmacopoeia, 2010; WHO, 1998).

Foreign Organic Matter

This organic matter in plant was determined by spreading 100 g of crude drug on clear smooth surface background by using magnifying lenses (10X). The experiment was done in triplicates.

Moisture Content

3 g of shade-dried drug was taken in a tared porcelain dish. The crude drug was heated at 105ºC in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade-dried drug material (WHO guidelines, 2002).

$$\% \text{ Moisture Content} = \frac{\text{Loss in weight of the sample on heating}}{\text{Weight of total amount of drug taken}} \times 100$$

Extractive value (WHO guidelines, 2002; WHO, 1998)

***Method*-1 *(Hot extraction*)**

4.0 g of coarsely powdered, accurately weighed air-dried material was placed in a glass-stoppered conical flask. 100 ml of water was added and weighed to obtain the total weight including the flask. Shook well and allowed to stand for 1 h. A reflux condenser was attached to the flask and gently boiled for 1h; cooled and weighed. Readjusted to the original total weight with the solvent specified in the test procedure for the plant material concerned. It was shaken well and filtered rapidly through a dry filter. 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105˚C for 6 h, cooled in a dessicator for 30 min, then weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

$$\% \text{ Extraction value} = \frac{[\text{final wt.} - \text{initial wt.}] \times 4}{\text{Wt. of drug}} \times 100$$

***Method*-2 (*Cold maceration*)**

4 g of coarsely powdered air-dried material, accurately weighed, was placed in a glass-stoppered conical flask. Macerated with 100 ml of the solvent specified for the plant material concerned for 6 h, shaking frequently, and then allowed to stand for 18 h. Filtered rapidly taking care not to lose any solvent, 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105˚C for 6 h, cooled in a dessicator for 30 min and weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

$$\% \text{ Extraction value} = \frac{[\text{final wt.} - \text{initial wt.}] \times 4}{\text{Wt. of drug}} \times 100$$

By following both the methods described above, various types of extractive values were calculated which are as follows.

- Water soluble extractive value
- Ethanol soluble extractive value
- Acetone soluble extractive value
- Chloroform soluble extractive value
- Petroleum ether soluble extractive value

Ash Value (Sharma *et al*., 2001; **WHO, 1998**)

Total Ash

2 g of powdered *D. macraei*, was incinerated in a crucible at a temperature 500- 600˚C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of total ash was calculated with reference to the air-dried drug.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of Acid Insoluble Ash

Ash, as obtained above, was boiled for 5 min with 25 ml of 70 g / L hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the (40 #) air-dried powered drug.

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of ash insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of Water Soluble Ash

Total ash was boiled for 5 min with 25 ml water and insoluble matter which was collected on an ash-less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450˚C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the (40 #) air-dried powered drug.

$$\%\text{Water soluble ash value} = \frac{\text{Weight of total ash - weight of water insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of Sulphated Ash

A silica or platinum crucible was heated to redness for 10 min, allowed to cool in a dessicator and weighed. Unless otherwise specified in the individual monograph, 1 g of the substance was transferred to the crucible under examination and the crucible and the contents was weighed accurately. Gently ignited at first until the substance was thoroughly charred. The residue was cooled and moistened with 1 ml of sulphuric acid, gently heated until the white fumes was no longer evolved and ignited at 800 ± 25˚ C until all black particles disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool and few drops of sulphuric acid was added and heated. Ignited again as before, allowed to cool and weighed. The operation was repeated until two successive weighings did not differ by more than 0.5 mg (Indian Pharmacopoeia, 2010).

$$\% \text{ Sulphate ash value} = \frac{\text{Weight of sulphate ash}}{\text{Weight of crude drug taken}} \times 100$$

Results

Epiphytes with creeping or annulate rhizomes, branches ending in pseudobulbs. Stems of *D. macraei* are greenish turn yellow. Branches are golden yellow, fruits are golden yellow and roots are light brown in color. It is odorless and its taste is mucilaginous. Stem is pendulous and profusely branched in shape, branches are polygonal. Fruits are capsule in shape and roots are fibrous (Table-1, 2).

Transverse section of stem is circular or oval in outline. Epidermis is covered with thick layered yellow shining cuticle. Sclerenchyma cells are present next to epidermal layer. Vascular bundles are scattered in ground issue. Interstitial spaces are present in ground tissue (Plate 2-3).

Powder is shining or yellowish brown in color. When powder was treated with various reagents, lignified fibres, sclerenchyma cells, xylem parenchymatous cells, epidermal cells, unicellular covering trichomes, calcium oxalate crystals, vittae, raphides and tracheids are shown (Plate 4-11).

Table-1 Taxonomical Details.

Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Viridiplantae
Phylum	Streptophyta
Subphylum	Streptophytina
Infra phylum	Embryophyta
Class	Liliopsida
Order	Asparagales
Family	Orchidaceae
Subfamily	Epidendroideae
Genus	*Dendrobium*
Botanical name	*Dendrobium macraei* Lindl.

Table-2 Macroscopic/ Organoleptic Characteristics of *D. macraei*.

S. No.	Macroscopic/ Organoleptic characteristics	Inference
1.	Color	Stem- greenish turns yellow, Branches- golden yellow, Fruit- golden yellow, Roots- light brown
2.	Odor	Odorless
3.	Taste	Mucilaginous taste
4.	Shape	Stem- pendulous and profusely branched, Branches- polygonal, Fruit- capsule shape, Roots- fibrous
5.	Size	Stem- 12-13 cm in length, Branches- 4-5 cm in length, Fruits- 6-8 cm in length, Roots- 0.2-0.4 cm in diameter
6.	Texture	Stem-smooth, Branches- smooth and shining, Fruit- smooth and shining, Roots- slightly smooth and soft velamen coating

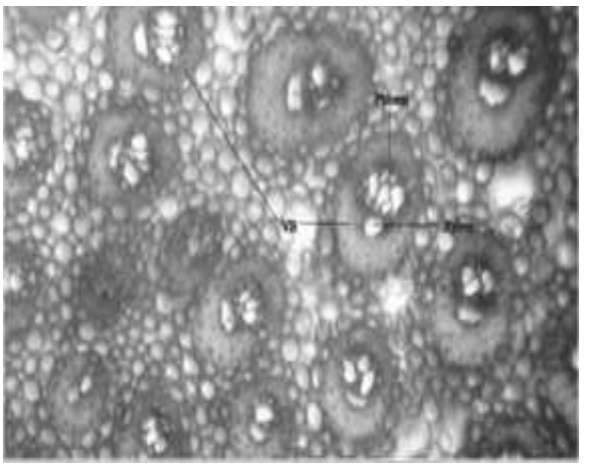

Plate- 2 Section representing vascular bundles of monocot stem of *D. macraei.*

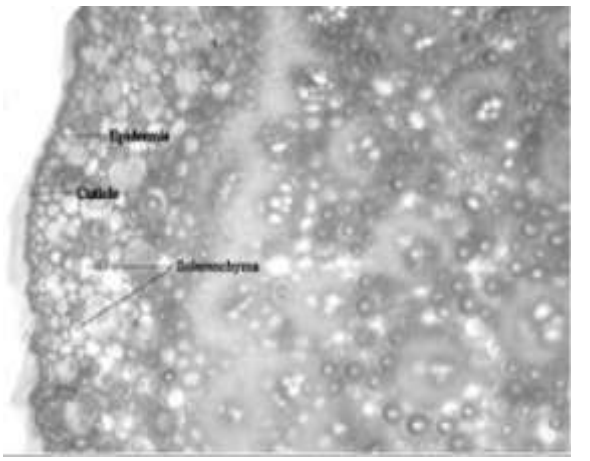

Plate-3 Section representing Epidermis and cuticle of monocot stem of *D. macraei.*

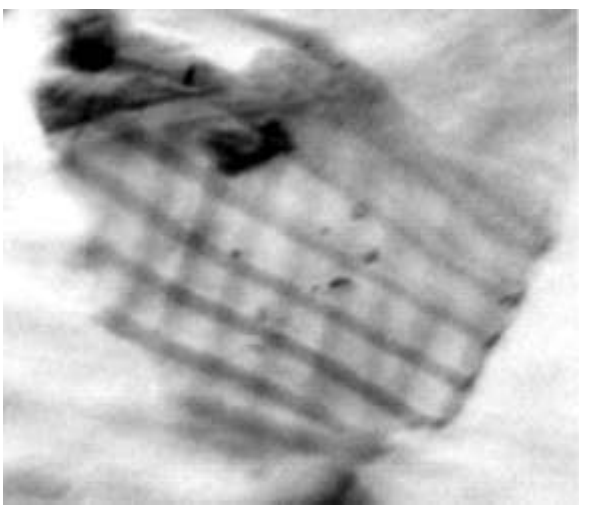

Plate-4 Epidermal cells (10X)

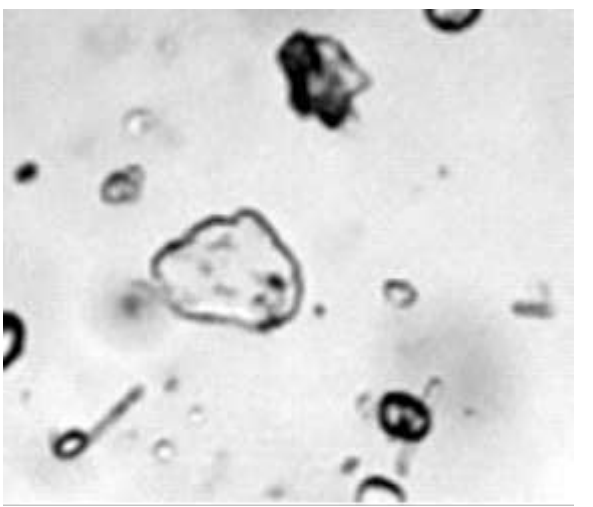

Plate-5 Calcium oxalate crystals (10X)

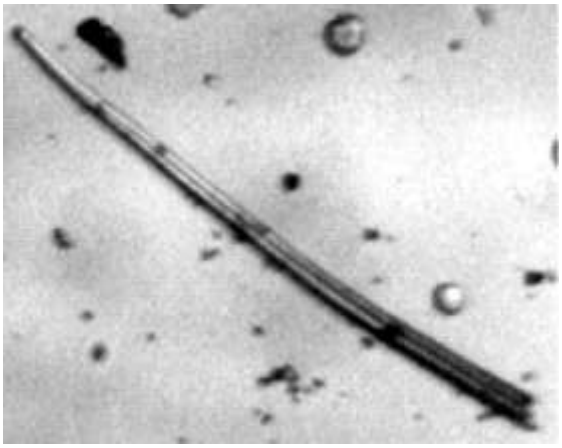

Plate-6 Lignified fibres (10X)

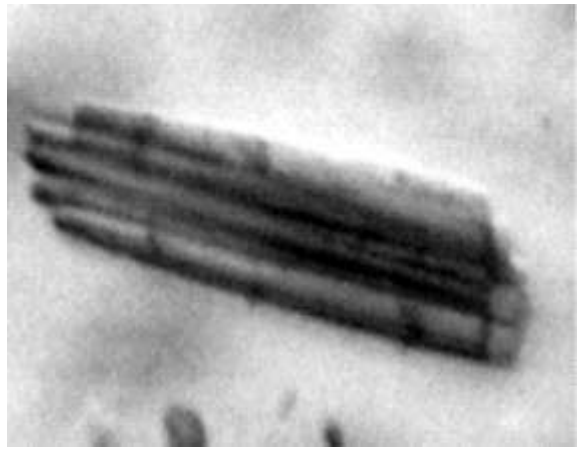

Plate-7 Raphides (10X)

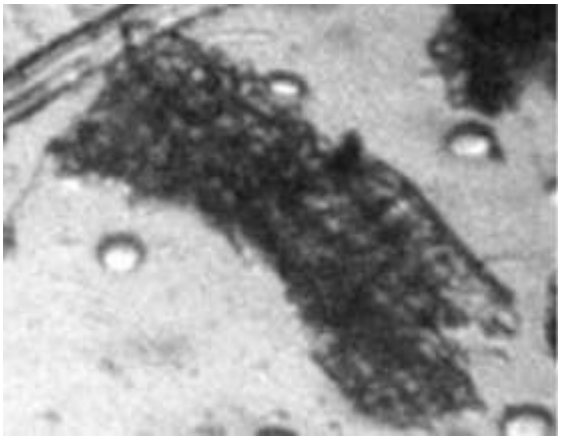

Plate-8 Vessels with spiral thickenings (10X)

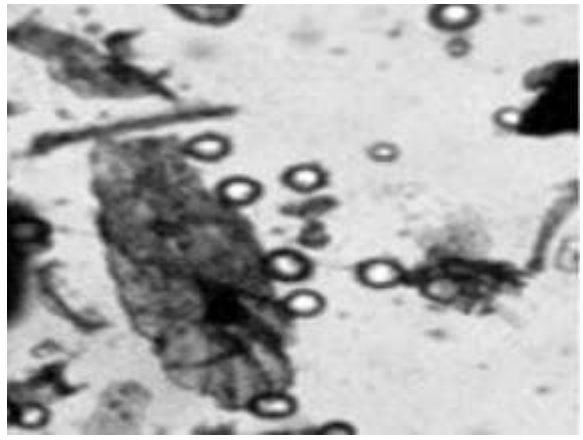

Plate-9 Lignified parenchyma cells (10X)

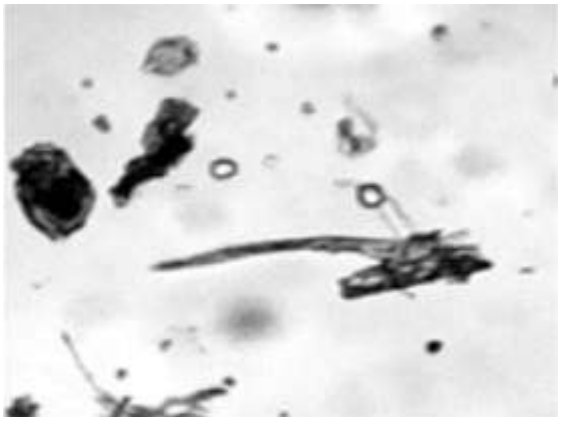

Plate-10 Unicellular covering trichome (10X)

Plate-11 Sclerenchyma cells (10X)

Physico-chemical parameters of *D. macraei* were evaluated to identify the authentication of plant material and to check the status of adulteration in plant which are presented in Table-3, 5.

Table-3 Moisture Content of *D. macraei*.

S.No.	Quality parameter	Results (% w/w)
1.	Moisture content	9.6%

Table-4 Extractive Values of *D. macraei.*

S.No.	Extractive values	Results (% w/w)	
		Hot extraction	Cold maceration
1.	Water soluble	12%	11%
2.	Ethanol soluble	11%	9%
3.	Acetone soluble	7%	5%
4.	Chloroform soluble	7%	5%
5.	Petroleum ether soluble	3%	1%

Table-5 Ash values of *D. macraei*.

S.No.	Ash values	Results (% w/w)
1.	Total ash	5.3%
2.	Acid- insoluble ash	1.3%
3.	Water soluble ash	3%
4.	Sulphated ash	7%

Discussion

The current study is emphasized on taxonomic studies, macroscopic and microscopic studies of D. macraei. The macroscopic characteristics of plant material were observed for differentiation of various parts of the plant from other same species of Dendrobium on the basis of color, odor, taste, shape, size, fracture and texture. If the sample was found to be significantly different, in terms of color, consistency, odor or taste, from the specifications, it was considered as not fulfilling the requirements *clearly shown in Table-1.* Section microscopic techniques shows the structural and cellular features of herbs which were examined in order to determine their botanical origins and assess their qualities. Transverse section cutting of stem of D. macraei was also observed which shows the features of monocot stem such as vascular bundles (contains xylem and phloem) in scattered form, cuticle sheath, epidermis just below the cuticle sheath, sclerenchyma cells and the difference between ground tissue and interstitial spaces is clearly shown in Plate 1-2. The powder microscopic method was useful for identifying species from fragments or powders and for distinguishing species with similar morphological characters; it may also be useful for evaluating the pharmaceutical quality of herbs. The powdered drug under the microscope showed fragments of parenchyma cells, xylem parenchyma, fibres, tracheids, calcium oxalate crystals, unicellular covering trichomes, raphides, sclerenchymatous cells, epidermal cells, vittae and vessels with spiral thickenings is shown in plates- 4 to 11.

Physico-chemical parameters help to identify the authenticated plant material and to check the status of adulteration present in crude drug material. In the present study moisture content in the coarse powdered drug was estimated as 9.6% shown in Table- 3.

Conclusion

The present study establishes the various pharmacognostic standards like macroscopic, microscopic characters, physico-chemical parameters and phytochemical analysis of D. macraei. Future prospects of the current investigations will be served as a standardization parameter.

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Assessment of Diuretic Activity and Toxicity of *Withania Somnifera* (Asgandh) in Experimental Animals

Mohd Nadeem*, Mohd Urooj, Habibur Rahman and Shariq Ali Khan
Pharmacology Research Unit, Regional Research Institute of Unani Medicine, Aligarh-202002 U.P., India
*E-mail: nadeemmohd81@yahoo.com

Abstract- Aqueous extract of *Withania somnifera* (Asgandh) was evaluated for its diuretic and toxicity study in albino rats and albino mice. The diuretic activity was evaluated at the dose level of 100mg/kg and 300mg/kg body weight in the four group of rats. The result is compared with positive control furosemide at 4mg/kg, body weight. It is found that aqueous extract of Asgandh significantly increased the urine output at the dose levels of100mg/kg and 300mg/kg body weight along with furosemide as compared to control group. The Asgandh extract significantly increased the urinary sodium at dose of 300mg/kg body weight. The extract did not bring about the significant change in urinary potassium, chloride and urinary urea and uric acid. The toxicity study included acute and sub acute toxicity study. The acute toxicity study conducted in four groups of albino mice at dose levels of 1g/kg,3g/kg and 10gm/kg body weight of Asgandh extract for 24 hours revealed no abnormal behavior, no untoward symptoms and no mortality during the observation period of 24 hours. The subacute toxicity study conducted in three groups of albino rats at doses of 1g/kg and 3gm/kg body weight of Asgandh extract for 28 days revealed no significant change in biochemical hematological parameters. The organ to body weight ratio of liver, heart, kidney and spleen also remained unchanged with the effect of the drug extract.

Keywords: *Withania Somnifera*, Diuretic activity, Acute toxicity and Subacute toxicity

Introduction

Known as Asgandh in Unani, Ashwagandha in Ayurveda and winter cherry in English *Withania Somnifera*, Dunal, belonging to the family solanaceae, is well known for its healing properties in traditional system of medicine[1]. It is found to be beneficial in arthritis, diabetes, fluid overload and hypercholesterolemia[2-3] and has also been reported to possess antioxidant, aphrodisiac, anti-inflammatory, antitumour, hypotensive, antistress, immunomodulatary and CNS depressant properties[4-11]. The pharmacological effect of *Withania Somnifera* is attributed to *Withanolides,* a group of steroidal lactones[12].Since the diuretics play an important role in the cases of fluid overload like acute and chronic renal failure, hypercalciurea, cirrhosis of liver, the necessity of a potent and less toxic diuretic from herbal source is required to overcome the problems of doubtful efficacy and safety of synthetic medicines. Clinical investigation of the root of Asgandh reveals that it has potent diuretic activity, but the plant was not screened for its diuretic action in experimental animals. The toxicity study is a part of drug research and development. Hence, the diuretic study and toxicity of Asgandh was investigated in experimental animals.

Material and Methods

Procurement, Identification and Preparation of Drug

Asgandh root was procured from Dawakhana, Ajmal Khan Tibbiya College AMU, Aligarh and identified in the pharmacognosy section of Regional Research Institute of Unani Medicine, Aligarh. The roots were powdered and a known quantity was soaked in warm acidulated distilled water for 24 hours with occasional shaking and stirring. The soaked mass is filtered through a coarse filter paper at the end of 24 hours. The filtrate was dried over water bath and the aqueous extract thus obtained was used for pharmacology and toxicity study.

Diuretic Study

Diuretic study was carried out in four groups of albino rats of either sex of six each weighing between 100-150 gm. The study was conducted for a period of 10 days. Group I served as control receiving distilled water, while group II animals were given a single dose of frusemide (4mg/kg body weight. p.o) on the ninth day. Group III and IV animals were administered orally the aqueous extract of Asgandh at the doses of 100mg/kg and 300mg/kg body weight daily for nine days. On the ninth day, the animals of all the groups were hydrated with distilled water (25ml/kg body weight p.o) and kept in metabolic cages for collection of 24 hour urine samples. After centrifugation of the urine samples, urine volume was measured and the following estimations were carried out in the urine samples.

(i) Na^+ and K^+ estimation using flame photometer

(ii) Chloride estimation using Schales and Schales[13] method

(iii) Urea estimation using Fawett and Scott[14] method

(iv) Uric acid estimation using Henry Caraway's[15] method

Toxicity Study

Acute Toxicity Study

Acute toxicity study was carried out on four groups of albino mice of either sex of six each weighing around 20-25gms. Group I served as control and given distilled water orally. Group II, III and IV were given orally 1g/kg, 3g/kg and 10g/kg body weight, of the aqueous extract of Asgandh. All the animals were watched closely for 24 hours for adverse effect and mortality.

Sub Acute Toxicity Study

Albino rats of either sex weighing between 100-150 gms were randomly selected and divided into three groups of six animals each. Rats were kept fasted overnight (12hrs) with free access to water prior to administration of dose 1g/kg and 3g/kg body weight of Asgandh extract for 28 days. Group I was kept as normal control which was treated with distilled water for 28 days, while in the IInd group and IIIrd group aqueous extract of the Asgandh was administered orally at a dose of 1g/kg and 3g/kgbody weight for 28 days. The animals were observed for Gross behaviour (salivation, lacrymation, lethargy, sleep and coma) and mortality at 1, 2, 3 , 4 , 5 , 6 , 12 , 24 hours and thereafter once every day up to 28 days after drug administration. The animals were observed for body weight before commencement of the study and at termination of study.

On 29th day, blood was collected from the retro-orbital plexus of all the three groups of rats for estimation of SGOT, SGPT,Serum Alkaline phosphatase,Serum Cholesterol and Percentage haemoglobin, ESR, Total leukocyte count and Differential leukocyte count (% polymorphocyte count and % lymphocyte count). After collection of blood the animals in all the three groups were sacrificed and liver, heart, kidney and spleen were excised out for determination of organ to body weight ratio as compared to control group.SGOT, SGPT were estimated by Reitman's and Frankel method[16]. Alkaline Phosphatase was estimated by Bessey and Brock method[17]. Serum urea was estimated by GLDH, Ureas method given byTiffany *et al.,* [18], while Serum Creatinine was estimated by Jaffe's method given by Bower *et al.,*[19]. Serum HDL was estimated by Phosphotungstic Acid method given by Burstein *et al.,.*[20], while Serum cholesterol and Triglyceride were estimated by CHOD-PAP method given by Roeschlau*et al.,*[21] and GPO-Trindev method given by Mcgowan*et al.,*[22]. ESR and DLC were estimated by Westergreen and Leishman stain methods given in Medical Laboratory Technology by Mukherjee.[23]. TLC was estimated by Hemoaltometry method given by Plum[24]. Percent hemoglobin was estimated by Sahli's Acid Haematin method given by Newcomer[25].

Statistical Analysis

Statistical analysis was performed by using unpaired t test calculating p value at 5 % level. All values are expressed as Mean±SEM (standard error of mean). p value less than 0.05 found to be considered statistically significant.

Results and Discussion

Diuretic Study

The aqueous extract of Asgandh at the dose levels of 100mg/kg and 300mg/kg body weight significantly increased the urinary output in the rats as compared to control group. The increase in the urinary output caused by the Asgandh was comparable to that of the frusemide, a standard diuretic. The Asgandh extract significantly increased the urinary sodium level in the group treated with 300mg/kg body weight of the extract. The extract did not bring about any significant change in urinary potassium level. The urea and uric acid concentrations in the urine also remained unaffected by the extract of Asgandh (Table-1).

Pharmacological Study

Acute Toxicity Study

Careful observation of the mice subjected to this study revealed no abnormal behavior, no untoward symptoms and mortality during the entire observation period of 24 hours.

Sub Acute Toxicity Study

The biochemical and pathological investigation carried out on the blood samples of the rats at the end of four weeks of drug administration (1g/kg and 3g/kg body weight of the aqueous extract of Asgandh) showed no significant change in the values of SGOT, SGPT, serum alkaline phospatase and serum cholesterol of the drug treated rats as compared to normal ones. Haemoglobin, TLC, DLC and ESR estimations revealed no significant effect of the Asgandh extract on these parameters (Tables-2 and 3). The organ to body weight ratio of liver, kidney, heart and spleen also remained unchanged with the effect of the drug extract (Table-4).

Table-1 Effect of Aqueous Extract of *Withania Somnifera*(Asgandh) on Urinary Output, Urine Sodium, Potassium, Chloride and Urine Urea and Uric Acid in Albino Rats

PARAMETERS→ GROUPS↓	Volume of urine ml/24 hours	Sodium Meq/24 hours	Potassium Meq/24 hours	Chloride Meq/24 hours	Urea g/L	Uric Acid Mg/100ml
Normal Control (n=6)	2.91±0.24	0.0246±0.00	0.584±0.030	0.246±0.01	57.85±8.42	312.50±53.13
Frusemide 4mg/kg (n=6)	4.633***±0.16	0.0336±0.00	0.618±0.089	0.145*±0.041	66.185±9.27	250.00±43.06
Asgandh 100mg/kg (n=5)	4.02**±0.80	0.027±0.00	0.647±0.156	0.292±0.04	51.422±10.50	290.00±124.64
Asgandh 300mg/kg (n=6)	4.633***±0.18	0.156**±0.04	0.5905±0.02	0.195±0.04	46.183±7.24	345.83±64.68

Values are mean ± SEM
n= no. of rats in a group
p*<0.05, p**<0.01,p***<0.001

Toxicity Study

Table-2 Effect of Aqueous Extract of *Withania Somnifera* (Asgandh) on Biochemical Parameters in Albino Rats

PARAMETERS→ GROUPS↓	SGOT Units/ml	SGPT Units/ml	SALP KAU	Serum Cholesterol mg/100ml
GPI Normal control (n=6)	87.50±2.23	78.50±6.73	42.06±5.33	83.23±4.63
GPII Asgandh 1gm/kg (n=6)	79.50±3.30	74.83±7.44	47.08±1.83	78.08±9.15
GPIII Asgandh 3gm/kg (n=6)	82.16±3.76	77.83±3.93	44.26±4.26	94.25±10.34

Values are mean ± SEM
n = no. of rats in a group
p*<0.05, p**<0.01,p***<0.001

Table-3 Effect of Aqueous Extract of *Withania Somnifera* (Asgandh) on Haematological Parameters in Albino Rats

PARAMETERS ► GROUP ▼	Haemoglobin gm %	TLC / Cumm	ESR mm/hr	DLC	
				% Polymorph count	% Lymphocyte count
Group I Normal Control (n = 6)	14.50 ±0.46	5158.33±499.40	2.33 ±0.4216	41.83 ±3.31	57.66± 3.50
Group II Asgandh 1gm/kg (n = 6)	13.46 ±1.11	6525.00±846.00	2.38 ±0.5577	42.33 ± 5.32	56.83 ± 5.52
Group III Asgandh 3g/kg (n = 6)	15.16 ± 0.89	5775.00±470.41	1.83 ±0.3073	40.66 ± 2.04	58.83 ± 1.97

Values are mean ± SEM
n = number of rats in a group.
p* < 0.05, p < 0.01, p*** < 0.001**

Table-4 Effect of Aqueous Extract of *Withania Somnifera* (Asgandh) on Relative Organ Weight per 100 gm Body Weight in Albino Rats

	Organ Weight/100gm Body Weight			
PARAMETERS ► GROUP ▼	Liver	Heart	Kidney	Spleen
Group I Normal Control (n = 6)	4.114±0.2212	0.391±0.01102	1.105±0.0551	0.421±0.0252
Group II Asgandh 1gm/kg (n = 6)	4.708±0.6218	0.428±0.04360	1.236±0.16125	0.588±0.1246
Group III Asgandh 3g/kg (n = 6)	4.446±0.3462	0.398±0.03092	1.225±0.08734	0.511±0.0648

Values are mean ± SEM
n = number of rats in a group.
p* < 0.05, p < 0.01, p*** < 0.001**

Conclusion

It may be concluded from the above observation that Asgandh extract possesses diuretic effect. As the result of the acute and subacute toxicity study revealed the Asgandh extract is safe at all the dose levels.

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Phytochemical and *In Vitro* Antioxidant Potential of Aqueous Leaf Extracts of *Brassica Juncea* And *Coriandrum Sativum*

Kanchan, P. K. Chauhan and I.P. Pandey*
Department of Biotechnology, Shoolini University, Solan, Himachal Pradesh, India
*Prof. Emeritus Chemistry, Dehradun, India
*E-mail: ippande@gmail.com

Abstract- In the present study, phytochemicals and *in vitro* antioxidant potential of aqueous leaf extracts of *Brassica juncea* and *Coriandrum sativum* leaves were determined qualitatively. The phytochemical screening of plants studies showed the presence of terpenoids, flavonoids, tannin and reducing sugar in aqueous extract of *Brassica juncea.* Alkaloids and glycosides were absent in it. Aqueous extract of *Coriandrum sativum* showed the presence of tannins, terpenoids, reducing sugars, flavonoids, and glycosides and alkaloids were absent in aqueous extract of *Coriandrum sativum.* It had been found that among the aqueous extracts of *Brassica juncea* and *Coriandrum sativum*, the higher activity of Catalase was observed in aqueous extract of *Brassica juncea* than in aqueous extract of *Coriandrum sativum.* The peroxidase activity was observed to be low in aqueous extract of *Brassica juncea.* The value of ascorbate oxidase activity and Vitamin C content was high in aqueous extract of *Coriandrum sativum*.The main purpose of the study is to evaluate phytochemicals and antioxidant activity of the used plants.

Keywords: Antioxidant, Phytochemicals, *Coriandrum, Brassica and* Flavonoids

Introduction

The medicinal value of plants lies in some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites. The most important of these bioactive groups of plants are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds[1]. *Coriandrum sativum* is an annual herb, of the family Apiaceae. It has eleven components of essential oils, six types of acids, minerals and vitamins, each having a number of beneficial properties. It also contains antioxidants which can delay or prevent the spoilage of food seasoned with this spice. A study found both the leaves and seed to contain antioxidants, but the leaves were found to have a stronger effect[2]**.** *Coriandrum sativum* has been documented as a traditional treatment for diabetes. A study on mice found that *Coriandrum sativum* extract had both insulin-releasing and insulin-like activity[3]. The Indian mustard (*Brassica juncea*) is an herbaceous, annual, of the family, Brassicaceae. It is an herbal plant. There is the role of detoxification and swelling that can fight infection and cause prevention of diseases, inhibit the toxicity of bacterial toxins, promote wound healing and can be used to aid in treatment of infectious diseases. *Brassica juncea* contains a lot of ascorbic acid. It can increase the oxygen content in the brain to stimulate the brain for oxygen use. Its seeds are high in calories; 100 g of seeds contain 508 calories. However, they are good sources of dietary fiber; recommended in cholesterol controlling and weight reduction programs. The main purpose of our observation is to evaluate phytochemicals and antioxidant activity of *Coriandrum sativum*, *Brassica juncea* as these plants are commonly used in food.

Material and Methods

Collection of plant material: The plant materials used were the leaves of *Brassica juncea* and *Coriandrum sativum* which were collected from local area of Solan in November 2015.

Extraction of plant material: The plants (powdered form) taken for the study was stored under refrigerated condition till use. The samples were prepared by extraction of plant (powdered form) with distilled water by using sonicator. On evaporating the aqueous mixtures on water bath, a crude extract was obtained from both the plants.

Phytochemical Investigation of *Brassica juncea* and *Coriandrum sativum*

Alkaloid, flavonoids: The presence of alkaloids and flavonoids was investigated by using method of Harborne, 1973[4].

Tannins: - The presence of tannins was investigated by using method of Trease and Evans, 2002[5].

Glycoside and Terpenoids: The presence of glycoside and terpenoid was investigated by using method of Siddiqui and Ali, 1997[6].

Antioxidant Activity of *Brassica juncea* and *Coriandrum sativum L*eaves Extract

Assay of Catalase activity: Catalase activity was assayed by the method of Sinha A. K, 1972[7].

Assay of Peroxidase activity: The assay was carried out by the method of Addy and Goodman, 1972[8].

Assay Procedure for Ascorbate Oxidase: Assay of Ascorbate Oxidase activity was carried out according to the procedure of Vines and Oberbacher, 1965[9].

Quantification of vitamins: The determination of ascorbic acid was carried out by the procedure given by Sadasivam and Manickam, 1997[10].

Results

Herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Phytochemicals have been found to possess a wide range of activities which may help in protection against chronic diseases. Qualitative analysis of aqueous

Table-1 Phytochemical Investigation of *Brassica Juncea* And *Coriandrum Sativum*

Plant extracts	Alkaloids	Glycosides	Terpenoids	Flavonoids	Tannins	Reducing Sugars
Aqueous extract of *Brassica juncea*	–	–	+	+	+	+
Aqueous extract of *Coriandrum sativum*	–	+	+	+	+	+

Reducing sugar: To 0.5 ml of each extract solution add 1 ml of water and 5-8 drops of Fehling's solution was added hot and observed for brick red precipitate.

Table-2 Enzymatic Antioxidant Analysis in The Extracts Of *Brassica Juncea* And *Coriandrum Sativum*

Samples	Catalase (μ/moles of H2O2 decomposed/min/g protein)	Peroxidase (Unit/mg protein)	Ascorbate oxidase (μ mole/ml)
Aqueous extract of *Brassica juncea*	**3.75**	**1.584×103**	**95.23**
Aqueous extract of *Coriandrum sativum*	**3.135**	**2.508×103**	**100.262**
	1 unit = μ/moles of H2O2 ecomposed/min/g protein	1 unit = mg of GSH utilized/min	1 unit = 0.01 O.D change/min

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

Samples	Vitamin C (mg/g)
Aqueous extract of *Brassica juncea*	**0.100**
Aqueous extract of *Coriandrum sativum*	**0.295**

extract of *Brassica juncea* showed the presence of Terpenoids, Flavonoids, Tannins and Reducing Sugars, while aqueous extract of *Coriandrum sativum* showed the presence of Glycosides, Terpenoids, Flavonoids, Tannins and Reducing Sugars. Qualitative analysis carried out on each plant extract are summarized in (table-1).

From clinical studies, it is shown that terpenoids strengthen the skin, increase the concentration of antioxidant in wounds, and restore inflamed tissue by increasing blood supply. A wide Varity of phenolic substances derived from edible plants have been reported to retain marked antioxidant and anti-inflammatory activities. Hence, it is too important to investigate the phenolic contents, the antioxidant activity and antimutagenicity of some extract of some herbs, and to determine the relationship between antioxidant activity, phenolic contents and antimutagenicity activity.

Antioxidant Activity of aqueous extracts of *Brassica juncea* and *Coriandrum sativum*: Considering the important role of oxidative stress in the pathogenesis of several neurological diseases, and the growing evidence of the presence of compounds with antioxidant properties in the plant extracts, the herbal extracts exhibited good sources of water soluble antioxidants, phenolic compounds and antimutagens. Antioxidants properties of various extracts from many plants have been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones[11] (table-2).

The Peroxidase activity was observed to be low in aqueous extract of *Brassica juncea* (1.584X103 units/mg proteins), while the activity is high in aqueous extract of *Coriandrum sativum* (2.5 X 103 units/mg proteins). The value of ascorbate Oxidase is high in aqueous extract of *Coriandrum sativum* (100.262 µ mole/ml) and low in aqueous extract of *Brassica juncea* (95.23 µ mole/ml). The present study recommends more consumption of these vegetables that may have potential health effects.

Non-enzymatic antioxidants: The concentration of non-enzymatic antioxidant (Ascorbic acid) in extracts of *Brassica juncea* and *Coriandrum sativum* were also assessed and the results are represented in (table-3).

Vitamin C content was high in aqueous extract of *Coriandrum sativum* (0.295 mg/ g tissue), whereas in Aqueous extract of *Brassica juncea* it is (0.100mg/ g tissue). Ascorbate has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells and shown to function as a reductant for many free radicals[12].

Discussion

Among the aqueous extracts of *Brassica juncea* and *Coriandrum sativum*, the highest activity of Catalase was observed in aqueous extract of *Brassica juncea* (3.75 units/mg protein) and the lowest in aqueous extract of *Coriandrum sativum* (3.135 units/mg proteins). According to the study of Valavala *et al.,* 2011 13, *Brassica juncea* leaf extract (BJLE) delayed the cataract progression along with preventing oxidative and osmotic stress. In plants, antioxidant enzymes namely Catalase[14] and peroxidase[15] have been shown to increase when subjected to stress conditions.

Conclusion

The results from the present study of phytochemical and antioxidant systems revealed that the leaf extracts from both the plants have phytochemical and antioxidant enzymes. The wide use of these plant leaves in the India in food may have inflammatory and anti hepatotoxic activity due to their antioxidant potency. However, further studies are necessary to examine underlying mechanisms of antioxidant and anti-inflammatory effects and to isolate the active compound(s) responsible for these pharmacological activities.

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Zero Cost Water Purification by L.E.D. Light and Copper Complex and its Effect on Plant Growth

*[1]Anil Kumar Gupta, [2]Lokesh Saxena and [3]Shikha Saxena
[1,2]Department of Chemistry, D.A.V. College, Kanpur, India
[3]Department of Chemistry, DAV (PG) College, Dehradun, India
*E-mail: ak_gupta56@yahoo.co.in

Abstract- The white light reflects seven colors through prism. All of these colors of light are used in light emitting diode bulbs to produce light that appears white. However, out of these colored lights, the orange and blue light may be well utilized for purification of contaminated household water. Purification of household contaminated drinking water by reverse osmosis gives clean water but with low T.D.S. Since household water is already chlorinated, this does not necessarily need reverse osmosis which may cause osteoporosis and arthritis problem in the long run. Therefore, a zero cost new technique of household drinking water was designed wherein light of different wavelengths is subjected through LED bulb into a copper vessel having water. The orange component of the light is absorbed by cupric ions to form hexa aqua copper (II) ion which kills the bacteria and the blue component of light kills the viruses. The ceramic filter candles fitted in the upper part of the copper vessel is able to maintain the T.D.S. between the ranges of 100 to 250. The noble features of this Zero cost Copper purifier over other known alternatives are mainly ; pH of the water is found to be 7.03 which is neutral but pH of the R.O. water was 6.54(slightly acidic); no plastic; no motor; nominal consumption of electricity; nominal price; optimum T.D.S.; portable and bactericidal. The water thus purified may be used for better growth/yield of plants; this information may be good news for farmers and gardeners.

Keywords: Reverse osmosis, Osteoporosis, Zero cost technique, Copper complex, LED.

Introduction

To produce one liter of purified water, an ordinary RO purifier wastes up to four liters of water and our agriculture including medicinal plants accounts for 80% of all water consumption. Several agencies worldwide are promoting zero maintenance and cost effective method for the purification of contaminated household drinking water. The purification of water by U.V. rays of the sun is not so effective. Purification by reverse osmosis is ideal for river water but for house hold water, it proves to be expensive and low down the TDS of water, below 100 and causes mineral deficiency in the people[1].

Ancient civilization exploited the antimicrobial properties of copper. Copper metal ions have toxic effect on Algae, Molds, Spores, Fungi, Viruses, Prokaryotic and Eukaryotic micro organisms even in relatively low concentration. Antimicrobial copper surfaces kill greater than 99.9% various kinds of bacteria within two hours of exposure. Greeks, Romans and others also used copper for treatment of intestinal warms. During the process of creating rust molecules, copper pulls electrons from the membrane of the bacteria's cell wall, lipids, oxygen or proteins[2,3,4].

Copper can interact with lipids, opening holes in the cell membranes. Copper damages the respirator chain in *Escherichia coli* cells and is associated with impaired cellular metabolism. Cupric ion, Cu^{2+} is believed to be responsible for the antimicrobial action[5,6,7,8].

Inactivation of micro organisms may be due to increased availability of cupric ions Cu^{2+} is believed to be responsible for antimicrobial action. Copper cations dissolve on surface of bacteria and causes damages penetrating in the bacteria cells. Copper complexes form radicals that inactivate virus[9,10].

Copper may disrupt enzyme structures and functions by binding to sulfur containing amino groups of proteins. According to health benefits of copper water, mentioned in Ayurveda, copper has anti- inflammatory properties and helps in arthritis pain[11].

Copper, also an antioxidant prevents cell damage and aging. Apart from this, it is a vital element required in melanin production, works as brain stimulant, helps in healing wounds faster and maintains digestive system.

Copper like all metals form coordination complexes with ligands. In aqueous solution Copper (II) exists as $[Cu(H_2O)_6]^{2+}$. This complex exhibits the fastest water exchange rate (speed of water ligands attaching and detaching) for any transition metal aqua complex. Adding sodium hydroxide causes the precipitation of light blue solid Copper (II) hydroxide.

$Cu^{2+} + 2OH^- \rightarrow Cu(OH)_2$

Upon adding excess of ammonia the precipitate dissolves forming tetra ammine copper(II).

$CuH_2O)_4(OH)_2 + 4NH_3 \rightarrow [Cu(H_2O)_2 (NH_3)_4] + 2H_2O + 2OH^-$

$[Cu(H_2O)]^{2+}$ exhibit tetragonal distortion to give $[Cu(H_2O)_4]^{2+}$. The stability constant of $[Cu(H_2O)_6]^{2+}$ can be calculated by thermodynmic data using $\Delta G^0 = -RT \ln K$ and the value of $\log K_1, \log K_2, \log K_3$ and $\log K_4$ give the value 4.3,3.6,3.04 and 2.3. Molecules of H_2O are replaced by aq NH_3 giving $[Cu(H_2O)_5]^{2+}$ aq $[Cu(H_2O)_4 (NH_3)_3]^{2+}$ aq $[Cu(H_2O)_5(NH_3)_3]^{2+}$ and aq $[Cu(H_2O)_4 (NH_3)_4]^{2+}$. During this process, ΔG^0 is found to be large and negative with the increase in disorder or increase in entropy.

White light emitting materials have attracted significant attention in recent years as key components in display and lighting devices based on LEDs. Even pigments in fruits and vegitables can produce light.The chlorophyll,the green pigment of leaves when exerted by ultravoilet light on it through a torch, produces a blood red colour.

Some scientists have effected white light emission from easily available plants extarcts- turmeric and red pomegranate seed juice-paying way for further research. The extract of red pomegranate contains polyphenols and anthocynines as dyes. In the extraction of curicumin, the root of the turmeric plant was grinded with ethanol as solvent.

This extract was separated through an appratus to obtain a clear yellow liquid that contained curicumin as the dye. Under ultravoilet excitation the extracts produced white light.

Human and animal are a primary source of bacteria in water. Bacterial contamination cannot be detected by sight, smell and taste. Coliform bacteria may not cause diseases but can be indicators of pathogenic organisms that cause disease like intestential infections, dysentery, hepatitis, typhoid fever cholera and other illness. The term pathogen is used to describle an infectious agent such as virus, bacterium, prion, fungus, viroid or parasite that causes diseases in its hosts.The host may be an animal,a plant,a fungus or even another microorganism[12,13,14,15].

There are three important things that a plant need to grow, sunlight, nutrient and water and so we hypothesized that the composition of water may effect plant growth. Some research has been done in the past in testing radish growth. Work done by Wan and Yahou (2005) in North China tested various soil-water potential on radishes using a drip irigation system to determine what amount of soil water and nutrients would produce the best result[16]. A review by Johnson, W. on factors affecting plant growth has also been published[17]. Based on such studies, we may consider the use of The LED Light and Copper Complex purified water for better growth of the plants.

Material and Methods

The equipment of copper purifier required two Copper vessels, a ceramic filter candle, a mixture of colored light emitting unbreakable non glass bulb. Out of two copper vessels, the upper vessel was fitted with the bulb at its top and ceramic

candle filter at the base. The lower part of the vessel was adjusted below upper vessel so as to collect the water drops poured by filter candle. The $[Cu(H_2O)_6]^{2+}$ complex and blue component of the colored light produced from the bulb are the main anti microbial component of the purifier and kill more than 99.9% of the pathogens. The bulb is shielded and fitted inside the closed copper vessel to limit the exposure. The human skin exposure to the LED bulb has no side effect of sunburn and skin cancer.

- First of all 1 litre of water taken from storage tank was placed inside the Copper purifier vessel fitted with ceramic water filter candle and LED bulb. The light was passed for two hours.
- Now, we had two samples A an B. Sample A was named as treated and B was named as untreated.
- One drop of sample A was then placed in a nutrient (Agar Agar) culture plate in an incubator for 24 hours at 35^0C for culturing.

Discussion

The principle of white light which results seven distinct colors through prism and their recombination in light emitting diode to give light that appears white, was exploited for the purification of the contaminated household water. Since copper is a transition metal, first of all influenced by the dissolved oxygen and surface oxidation of copper produces Copper (I) oxide. The Copper (I) oxide is then subsequently oxidized to copper (II) ion. When the mixture of colored light is passed from the bulb, the Copper ions absorb the orange component of the combined seven colored light (appearing white) to produce the octahedral hexa aqua copper complex $[Cu(H_2O)_6]^{2+}$.

Results

The $[Cu(H_2O)_6]^{2+}$ and the blue component of the light passing from the bulb are mainly found to be the anti microbial components of the purifier and were capable to kill more than 99.9% of the pathogen of household contaminated water Besides copper complexes, the irradiation of light of different energies from the bulb facilitates the purification of contaminated household water because highly energetic blue light kills or inactivate all the pathogens by destroying nucleic acids and disrupting their DNA leaving them unable to form vital cellular function. Ceramic candle filter is a simple device made out of clay and is used to filter drinking water and to remove turbidity or sediments to give drinking water; TDS less than 300 ppm.

Conclusion

When the plates of sample A and B are removed from the incubator, not a single coliform bacteria colony was found in the treated sample A but in sample B colonies of coliform bacterias was significantly observed, even without staining. Hence, it was concluded that all of the coli form bacteria present in the sample of contaminated stored tank water was completely killed by copper complex and the blue light produced from this novel water purifier equipment. Absence of any other pathogens including virus was also confirmed due to absence of coli form bacteria in sample A. An application for financial assistance for patenting of this equipment was already sent by inventor to National Research Development Corporation of India on 02 /12/2015.

Advantages over other known alternative and substitutes

There is no use of plastic, ultraviolet rays, motor usage and water wastage. It kills pathogens completely and shows no side effects. It is portable, economical, ecofriendly and has low cost maintenance. Purified water had pH 7.03 hence not acidic. The equipment is also able to cut emissions according to conference of parties at Paris (2015) to tackle global warming issues.

On the basis of the research as carried out by Wan, S. and Yahou, K.[16] and reviewed by Johnson, W.[17], it may be suggested that the L.E.D. light and Copper Complex purified water may cause rapid

and robust growth of plants, medicinal or aromatic plants. This may be a useful information for farmers and gardeners. To gather further results, study on growth of different plants and their species in varied quality of water is in progress; the details of which will be published later.

Figure-1 Household Water Purifier

Figure-2 Treated and Untreated samples

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Antimicrobial Activity of Extract of *Berberis Lycium*

***Mansi Gupta[1] and *Ajay Singh[2]**

[1]Research Scholar, Department of Chemistry,Uttaranchal University, Dehradun, India.

[2]Associate Professor and Head Department of Chemistry, Uttaranchal University, Dehradun, India

[1, 2]*E-mail- *mansigupta87@yahoo.co.in* and ajay21singh@yahoo.com

Abstract- Herbal medicines are prepared from herbal plants by using their extracts or essential oils. Herbal medicines are used for their therapeutic and medicinal values. *Berberis Lycium* is a herbal plant which is found in uttarakhand hill areas. *Berberis* species are the most important and valuable among the plant kingdom. *Berberis Lycium* is an evergreen shrub belonging to the family of *Berberidaceae*. In this study, essentials were extracted from various parts of this plant. *Berberis Lycium* has not only the medicinal and therapeutic value but it is also used to prepare jams, jellies and pickles as well by the local inhabitants. The present experiments were carried out to study the antioxidant and antibacterial effect of the alcoholic and aqueous extracts of roots and fruits of the *Berberis Lycium* . Fruit and root extracts have shown good antimicrobial activity while fruit extract has shown antoxidant activity. In herbal drug or medicines, various plant parts such as leaves, fruits, flowers, stems, seeds and roots are used for preparing the medicines.

Keywords: Herbal medicine, Antibacterial, Antrioxidant, *Berberidaceae*.

Introduction

In the recent years, medicinal species that reside in natural areas have received scientific and commercial attention. In the United States, of the top 150 prescription drugs, at least 118 are based on natural sources. A child suffering from leukemia in 1960 faced a 10 percent chance of remission; by 1997, the likelihood of remission had been increased to 95 percent-, thanks to two drugs derived from a wild plant native to Madagascar. As medicinal plants receive increased scientific and commercial attention, there is increasing pressure on the wild plant populations from which most of the medicinal plants are harvested. Overharvesting of these medicinal plants have led some of the species to become endangered[1].

Berberis Lycium is, an evergreen shrub belonging to the family Berberidaceae. The genus Berberis comprises of as many as 500 species. Out of which 77 species are native to India. *Berberis Lycium* is an erect shrub which grows to a height of 3 meter with a thick woody shoot and covered with a thin brittle bark[2]. The flowers from this plant are hermaphrodite .It means both male and female organs are on the same plan and they are pollinated by Insects (i.e self-pollination). It is distributed in the temperate and sub tropical parts of Asia, Europe and America. Several species are grown in gardens for their ornamental leaves and bunches of the succulent, acidic and edible berries[3].

The flowering and fruiting season of *Berberis Lycium* is from the month of March to July. The flowers start appearing from the first fortnight of March and ends up to April. The fruits of *Berberis Lycium* are relished from the month of June- July[4] and are known as berries. These berries are mostly consumed in raw form, especially by the rural population. They are rich source of vitamin C and anthocyanin, although they have sharp taste. The fruits are also cooked and made in to preserve. Juice is also extracted from the fruits which are slightly acidic in nature. The leaves and shoots are also cooked in some parts of the world and are used as a tea substitute[5].

In order to maintain health status of human and animals, the control of microbial growth is necessary. Basically antimicrobial agents are those chemicals that kill or inhibit the growth of microorganisms. Such a substance can either be synthetic, chemical or a natural product[6]. In recent

years it was observed that multiple drugs resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs used for treatment of various infections[7]. Therefore, scientists throughout the world are now trying to search out the new antimicrobial substance from various sources including medicinal plants[8]. *Berberis lycium* was not only fully analyzed for its phytochemical property but its antibacterial, antifungal and antiviral activities were also not investigated in the past[9].

The aim of the present research was to identify the antimicrobial potential of *Berberis Lycium* against various microorganisms.

Material and Methods

In the present study, fruit extracts of *Berberis Lycium* were tested against bacteria and fungi. The study was conducted in microbiology Lab., Uttaranchal University, Dehradun.

Collection of Samples

The fruit samples of *Berberis Lycium* were collected from hilly area of Nanital. The plant was identified in FRI,Dehradun.

Preparation of Samples

Fruits of *Berberis Lycium* were subjected to shadow drying followed by oven drying at 60^0C for over night and then converted into powdered form in a grinder. Total 30g of samples was added to 200 ml of methanol and extracted in Soxhlet apparatus for 4 hours at a temperature less than the boiling point of the solvent. The extract was further concentrated by rotary evaporator and the residue was stored for further process. Where as in case of aqueous media, same amount of sample was dissolved in water and boiled, filtered and saved for further process just like the above process.

Antimicrobial Activity

Kirby–Bauer antibiotic testing is also known as Disc diffusion antibiotic sensitivity testing. It is a test which uses antibiotic impregnated wafers to test whether bacteria are affected by antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If the antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of the antibiotic is enough to stop growth.

The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest near to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion is used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotic to combat a particular infection.

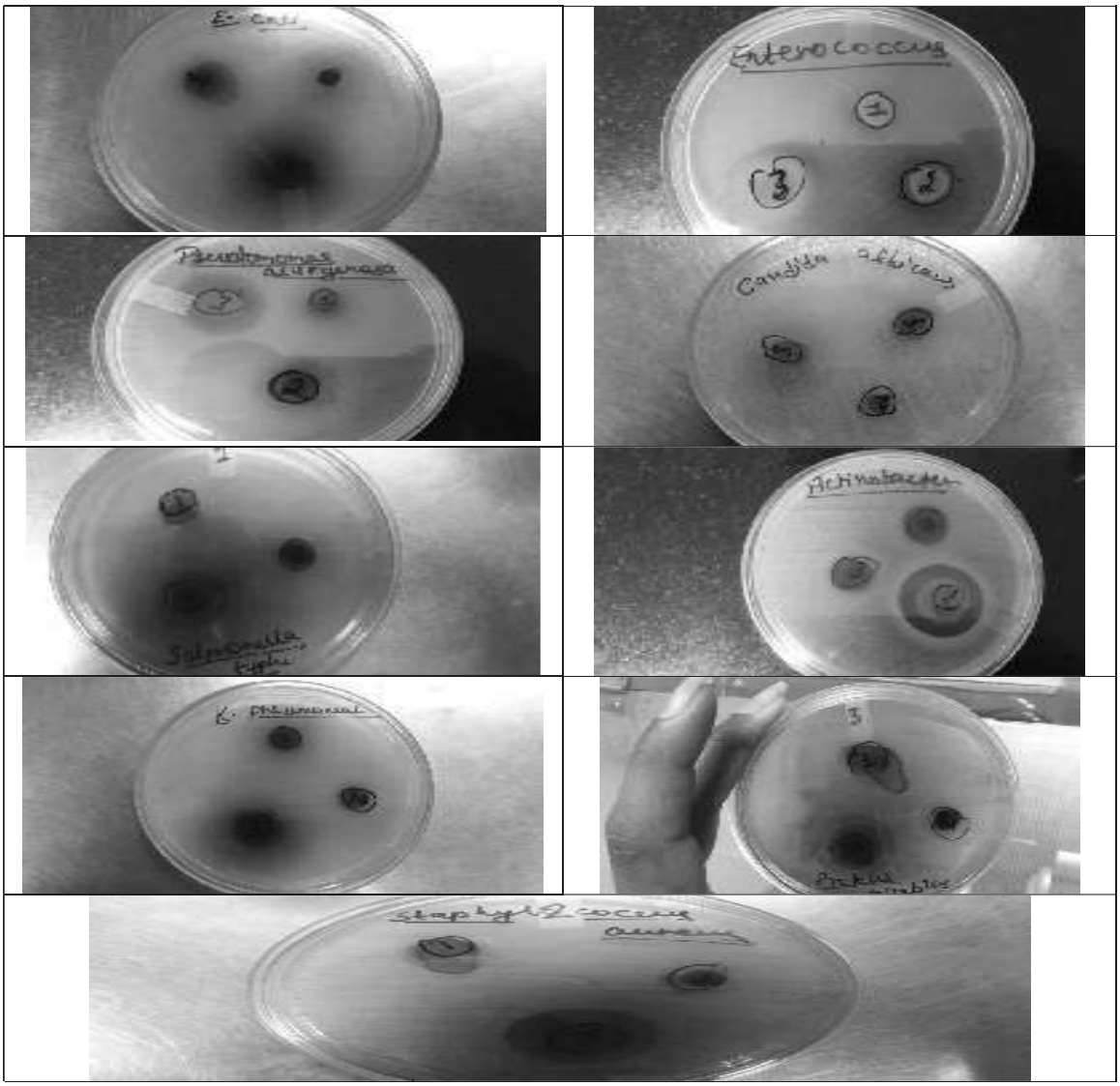
In similar way, fruit extracts of *Berberis Lycium* were tested against various microorganisms i.e. bacteria and fungus.

Results and Discussion

The fruit extracts of *Berberis Lycium* were prepared in two different solvents and tested against microorganisms. The fruit extract with water sample [1] while the fruit extract with methanol is sample [2].The results pertaining to antimicrobial activity of fruit extracts are summarized in the table and shown in the figures.

Table– Minimum Inhibitory Concentration (MIC) (in mm) of Fruit Extract of Berberis Lycium

S.No	Strains	Water Extract (Sample 1)	Methanolic Extract (Sample 2)
1.	*Pseudomonas aeruginosa*	09	10
2.	*Enterococcccus*	14	15
3.	*Acinetobacter baumannii*	13	20
4.	*Salmonella typhi*	resistant	resistant
5.	*Staphylococcus aureus*	14	14
6.	*Proteus mirabilis*	14	13
7.	*Escherichia coli*	resistant	07
8.	*Klebsiella pneumoniae*	07	08
9.	*Candida albicans*	resistant	resistant



Figures- Antimicrobial activity of fruit extract of *Berberis Lycium*

In the present study, the methanol and aqueous fruit extracts of *Berberis Lycium* were applied to control the growth of different microorganisms and it was found that methanol extracts have provided better results as compared to aqueous extracts. Therefore, methanol is considered as a useful solvent for assessment of antimicrobial activities[10]. In the present study, methanol and aqueous root extracts of *Berberis lycium* were applied against 8 bacterial strains as well as 1 fungi. It was observed that methanol extract has inhibited the growth of these organisms significantly as compared to aqueous extracts .The results obtained during this study revealed that fruits of *Berberis lycium* contains some active phytochemicals which have ability to control the growth of some microorganisms[11,12]. Therefore, this study will highlight the antimicrobial activity of *Berberis lycium*. The microorganisms which were affected by plant extracts could have some difference in their cell walls or inheritance antimicrobial resistance genes as plasmids can easily be transferred among bacterial strains or fungal strains. Therefore, on the basis of the results obtained in the present study, the fruit extracts of this plant can be helpful for development of new and useful drugs in pharmaceutical industry for the treatment of various infectious diseases.

Conclusion

This in vitro study shows that the fruits of *Berberis lycium* exhibit good amount of antimicrobial activity i.e it shows a great variation in antibacterial activity while the fruit extract in fungal solution showed resistant.This means that the fruits of *Berberis lycium* must be containing some bioactive components that can be considered useful for further research. Furthermore, it can be useful for treating various infectious diseases and pharmaceutical industries also.

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Morning and Night Blooming Flowers: A Comparative Phytochemical Evaluation

S.Farooq, *Zafar Mehmood and A.K.Dixit
*E-mail: zafarmehmood31@gmail.com

Abstract- Flower scent in the blooming flowers is nature's blessing to mankind to have pleasant fresh awakening air with fragrance of flowers in the morning and similarly with night blooming flowers to have a fragrance which may help them to sleep well. Blooming is also a mode of communication between flowering plants and their animal pollinators. Just to compare these plants, a study is being conducted on day and night blooming flowers to establish the difference in their phytochemistry as well as the chromatographic pattern of both the types of flowers, also to find out the constituents of the volatile oils giving energy and freshness and drowsiness from night blooming flowers. Four different flowers with two night's bloomings (*Cestrum nocturnum* and *Polianthes tuberosa*) and other two days blooming flowers (*Helianthus annus* and *Hypericum roseum*) were taken for this study. Phytochemical analysis as well as the chromatographic band pattern clearly revealed the difference between night and day flowering. The results showed the absence of alkaloids in night blooming flowers while both the day blooming flowers indicated the presence of alkaloids, on inhaling which may provide energy.

Keywords: Night blooming flowers, Phytochemistry, Chromatographic analysis

Introduction

Night-blooming jasmine is an evergreen woody shrub growing to 4 metres (13 ft) tall. A powerful, sweet perfume is released at night. “All flowers have evolved mechanisms to achieve successful pollination. *C.nocturnum* produces an intensively fragrant “volatile” scent during the night to attract its pollinator[1].

Flower fragrances probably originated to deter herbivores; however, visiting insects selectively chose those flowers which provided food. Another evolutionary scenario could be that moths would be more likely to find a mate also visiting the same flower. The circadian cycle also attracts species that are frequent during the night, for example the noctuid moth.

Scents are usually produced from the metabolism of the cell membrane, with the corolla, pollen or nectar often being involved. It may be that it reduces olfactory fatigue in the pollinating insect. A “flashing” scent may be more noticeable than a steady scent. Pulsed scents may travel longer distances.

Odours are especially prominent in “primitive” plants. There are four categories of odour described as heavy, aromatic, lemon and foxy. The fragrance of *Cestrum nocturnum* has been described as a mix of musk and heliotrope.

The first interesting point is that the scent release is an endogenously (internally) controlled circadian rhythm, i.e. it is found in a cyclic (24h circadian) manner both in constant darkness and constant light at constant temperature. The Earth's 24-hour light/dark cycle though in nature in constant conditions, this rhythm is a period of roughly hours, it is affected by temperature, with the cycle lengthened in colder temperatures and reduced at higher temperatures. As mentioned, in the previous answer, there may be a number of reactions which would lead to the release of scent, therefore, if one reaction was temperature-dependent the cycle would be extended[2].

It is needless to mention that Jasmine is one flower that is famous for its sweet fragrance. Greenish white in colour, night Jasmine is considered as the best moon garden flower. This tiny, tubular flower blooms exclusively at night spreading its strong perfume around[3].

These flowers are rather special for they bloom exclusively at night and can turn any regular garden in an enchanting 'moon garden'.

Polianthes tuberosa (Rajnigandha) means "The Fragrance of the Night". It is a flower that is both mythical and magical. Its nectar is said by some, to have special powers and its scent magical to all who experience it. Tuberoses are popular flowers in floral arrangements and their scent is used to produce perfumes the world over.

Polianthes come from Mexico and the above is probably more familiar as a florist's cut flower.

They are however, quite easy to grow in a pot and long-lasting as a cut flower in water (with lots of changes) Flowering tubers have to be discarded as they will not flower again[4].

The tuberose is a night- blooming plant thought to be native to Mexico along with every other species of Polianthes. It grows in elongated spikes up to 45 cm (18 in) that produce clusters of fragrant waxy white flowers that bloom from the bottom towards the top o the spike. It has long and bright leaves clustered at the base of the plant and smaller, clasping leaves along the stem. The oil extracted from the flowers is used to make perfume[5].

Material and Methods

Plant material

Cestrum nocturnum; *Polianthes tuberosa* ; *Helianthus annus* and *Hypericum roseum* flowers were collected from Mussoorie forest Dehradun, Uttarakhand (India) in the month of April, identified and authenticated by department of pharmacognosy, the Himalaya Drug Company, Dehradun. A voucher specimen has been deposited in medicinal plants herbarium in the Museum. The collected flowers were dried in shade and finally grinded to powdered form and stored in polythene bags for further use.

Extraction

150 gm air dry powdered flowers were treated with 1250 ml of methanol by soxhlet extraction technique for 18 hrs. It was concentrated to dryness under reduced pressure and controlled temperature using rotary evaporator. The methanol extract yielded final extracts. The collected flower extracts were stored in a refrigerator.

Phytochemical screening

The phytoconstituents present in methanol extract were analyzed by using standard qualitative method[6,7]. The flower extract was screened for the presence of biologically active compounds like alkaloids, flavonoids, tannins etc**.**

Alkaloids

Five milligrams of extract was dissolved in twenty milliliters of dilute HCl and then filtered.

Mayer's test: 5 milliliters of filtrate was treated with Mayer's reagent. Yellow colour precipitate indicates presence of alkaloid.

Wagner's test: 5 milliliters of filtrate was treated with Wagner's reagent. Brown reddish precipitate indicates presence of alkaloids.

Dragendroff's test: 5 milliliters of filtrate was treated with Dragendroff's reagent. Red precipitate indicates presence of alkaloids.

Hager's test: 5 milliliters of filtrate was treated with Hager's reagent. Yellow precipitate indicates presence of alkaloids.

Flavonoids

Alkaline Reagent test: Extract was treated with few drops of NaOH solution. Formation of intense Yellow color which becomes colourless on addition of dilute acid (HCl or H_2SO_4).

Molisch Test: 5 ml filtrate was treated with a drop of alcoholic naphthol solution in a test tube. Formation of Violet ring at the junction indicates the presence of carbohydrates.) indicates the presence of Flavanoids.

Lead Acetate test: Extract was treated with few drops of Lead Acetate solution. Formation of intense Yellow coloured precipitates indicates the presence of flavanoids.

Shinoda's test: Small quantity of extract was dissolved in alcohol. To that few piece of magnesium with concentrated hydrochloric acid was added dropwise and heated. Appearance of magenta colour indicates the presence of flavonoids.

Sulphuric acid test: Extract was treated with few drops of concentrated sulphuric acid . Yellow orange colour indicates the presence of flavonoids.

Tannins

Ferric Chloride Test: 100 mg of the extract was boiled with 20 ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered. Filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicates the presence of tannins.

Results and Discussion

The results for phytochemical screening of *Cestrum nocturnum*, *Polianthes tuberosa, Helianthus annus and Hypericum roseum* are summarized in Table-1. The Preliminary phytochemical screening revealed the presence of active phytoconstituent such as alkaloids, flavonoids and tannins etc. Out of which falvonoids are among the most widespread group in all types of flowers. It is noted that alkaloids are present only in day blooming flowers (*Helianthus annus* and *Hypericum roseum*) but absent in night blooming flowers *(Cestrum nocturnum* and *Polianthes tuberosa) w*hile tannins were detected only in flowers of *Cestrum nocturnum*.

Conclusion

The present study was aimed to perform phytochemical evaluation of Night and Day blooming flowers. From the study, it is concluded that alkaloids are absent in the night blooming flowers of *Cestrum nocturnum* and *Polianthes tuberosa* while showing presence in flowers of *Helianthus annus* and *Hypericum roseum*. Phytochemical studies in Night and Day blooming flowers laid down a platform for more research work in this direction.

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Table-1 Comparative Phytochemical Evaluation of Night Blooming and Day Blooming Flowers

S.No.	Flowering plant	Phytochemicals		
		Alkaloids	Flavonoids	Tannins
1.0.	***Cestrum nocturnum***/Night blooming jasmine	**Absent**	Present	Present
2.0.	***Polianthes tuberose***/Rajnigandha/Night blooming	**Absent**	Present	Absent
3.0.	***Helianthus annus***/sunflower/day blooming	**Present**	Present	Absent
4.0.	***Hypericum roseus/****Choli phulya*/day blooming	**Present**	Present	Absent

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Phyto-Pharmacological Review of *Calotropis procera* – A Nature's Drug House in Tropical Countries

Versha Parcha, Noor Fatma, Deepak Kumar and Alok Maithani*
Department of Applied Chemistry, Sardar Bhagwan Singh PG Institute of Biomedical Sciences and Research, Balawala, Dehradun. India.
*E-mail: alok_maithainii@rediffmail.com

Abstract- The use of traditional medicine and medicinal plants in the most developing countries, as a normative basis for the maintenance of good health, has been widely observed. In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from the traditional healers. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs. In our present study, we thoroughly investigated the phyto-pharmacological potential of *Calotropis procera* which will be useful to explore effective botanical medicines, if sincere research is carried out in this direction.

Keywords: *Calotropis procera,* Phyto-constituents, Pharmacological Activity, Botanical Medicines and Latex.

Introduction

Calotropis procera is a drought resistant and salt tolerant shrub of India and an important drug of Ayurveda[1]. It is native of Asia and widely found in China, Malaysia and Vietnam. It is a small, erect and compact shrub which is used in several traditional medicines to cure various diseases. There are two common species of Calotropis, viz. *Calotropis gigantea* (Linn.) R.Br. and *Calotropis procera* (Ait.) R.Br described by the Sanskrit writers[2]. Both the species are used as substitutes for one another and are said to have similar effects. It grows in varieties of soils and environmental conditions and doesn't require cultivation practices. Each part of the plant bears excellent medicinal properties and used for curing different human ailments. The decoction of the plant is used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism, asthma and as an expectorant and purgative.

Taxonomic Classification [3, 4]

Kingdom	- Plantae
Subkingdom	- Tracheobionta (Vascular Plants)
Superdivision	- Spermatophyta (Seed Plants)
Division	- Magnoliophyta (Flowering Plants)
Class	- Magnoliopsida (Dicotyledones)
Sub class	- Asteridae
Order	- Gentianales
Family	- Apocynaceae (Milkweed Family)
Genus	- Calotropis
Species	- procera

Vernacular Name [1, 5]

Table-1 Various Common Names of *Calotropis procera* in Different Places

Countries	Synonyms	India	Synonyms
English	Crown flower,Giant Indian milkweed	**Hindi**	Madar, Aak
Malaysia	Remiga,Rembega	**Sanskrit**	Ravi
Indonesia	Bidhuri,Sidaguri	**Assamies**	Akand
Philippines	Kapal-kapal (Tagalog)	**Bangoli**	Akon
Laos	Kok may,Dok kap,Dok hak	**Cannad**	Ekka
Thailand	Po thuean, Paan thuean, Rak	**Kashmiri**	Acka
Vietnam	Bootng	**Malyalm**	Erikku
French	Faux arbre de soie	**Marathi**	Rui
German	Wahre Mudarpflamzer	**Urea**	Arkha
Italian	Calotropo	**Panjabi**	Ak
Spanish	Algodon extranjero	**Urdu**	Madar
Turkish	Ipekag	**Telgu**	Gilledu
Arabic	Oshar	**Gujarati**	Aakando

Botanical Description

Calotropis procera (CP) is a perennial, evergreen and soft wooded shrub. It usually attains height of 3 meter and rarely reaches 5 meter. White coloured latex is obtained when leaves or stems are cut[6-9].

Leaves: The leaves are opposite and externally short petioles, belong to hearts shaped base .The blades are broadly elliptic or nearly orbicular, short pointed to blunt at the apex. The leaves are light to dark green and contain nearly white veins. These are 7-18 cm. long and 5-12 cm. broad slightly leathery and a fine coat of soft hairs that are easy to rub off (Figure-1a).

Flowers: The species having white flowers is a most common and superior variety and is referred to as Calotropis procera. The corolla is slightly succulent and consists of 5 small triangular dirty white sepals, 5 thick ovate petals (1cm^2) (Figure-1d).

Fruits: The fruits are green inflated ,and spongy ovoid follicles that split open to release plumed, papery light brown seeds with white filaments .These are 3-4.5 inches long and 2-2.5 inches wide. The main flowering period is from March to October (Figure-1b).

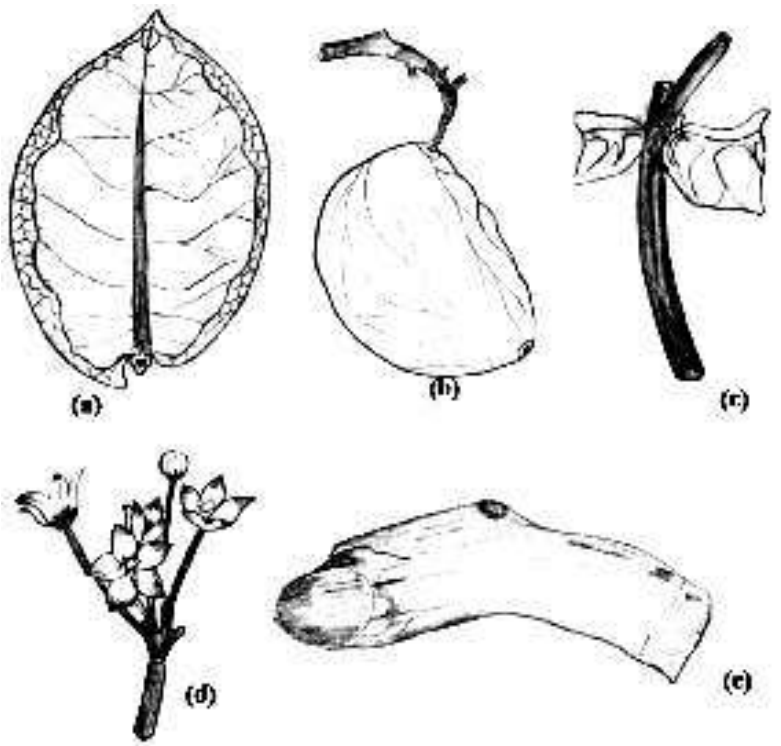


Figure-1 (a) Leaf; (b) Fruit; Stem (c); Flower (d) and root (c)

Root and Root Bark : Giant milkweed has a very deep, stout taproot with few or no near-surface lateral roots. Giant milkweed roots were found to have few branches and reach depths of 1.7 to 3.0 m in Indian sandy desert soils. The roots are covered with root bark with rounded head and rest of the portion spirally curved. The bark of the older root is cracked at places and is yellowish gray outside and yellowish white inside. The upper cork portion is spongy and rough while the inner portion of bark is smooth and mucilaginous (Figure-1e).

Phyto-pharmacological Discussion

Each part of *Calotropis procera* (CP) contains several phytoconstituents belonging to different classes of natural products, which make the genus Calotropis as the nature's drug house. About 70 chemicals have been isolated and characterized from different parts of this plant. The availability of various compounds and important pharmacological activity associated to each part of *Calotropis procera* is discussed in this paper.

Flower: Flower of *Calotropis procera* is rich in cardiac glycosides, carbohydrates, enzymes and steroidal contents. The major cardiac glycosides are calotropin **(1)**, calactin **(2),** calotoxin **(3),** calotropagenine **(4),** uscharin **(5),** voruscharin **(6),** uscharidine **(7),** uzarigenin **(8),** proceragenine **(9).** The carbohydrates present in it are D-arabinose **(10),** glucose **(11)**, glucosamine **(12)**, and rhamnose **(13)**. However, the most studied sterols in flower are lupeol **(14)**, stigma sterols **(15)**, cycloart-2,3-ene-3-B-25-diol **(16)**, multiflorenol **(17)**, clycosadol **(18)**, 3-epimoratenol, prosterol **(19)**. Two enzymes namely 3- protinase and calotropain **(20)**, have also been isolated from the flowers of *Calotropis procera*. The flower also contains one calotropenyl acetate **(21)**. The flowers of the plant

exhibit hepatoprotective activity, anti-inflammatory, antipyretic, analgesic and antimicrobial effect and larvicidal activity[10,11].

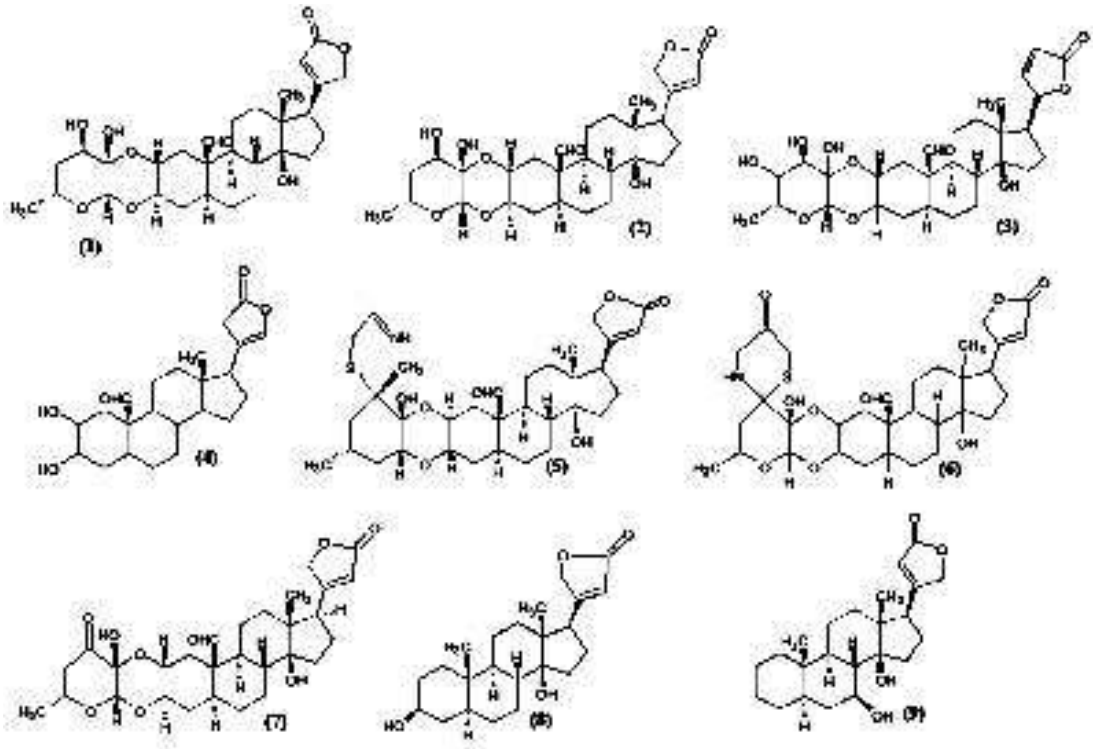
Zafar et al. in 2005 studied the effect of crude aqueous and methanolic extract of flowers of *Calotropis procera* on live *Haemonchus contortus* and observed the rate and extent of mortality and temporary paralysis. In their experiment to sheep infected with nematodes, they observed a significant reduction in egg count which indicates good antihelmentic activity of CP flower extracts against nematodes[12].

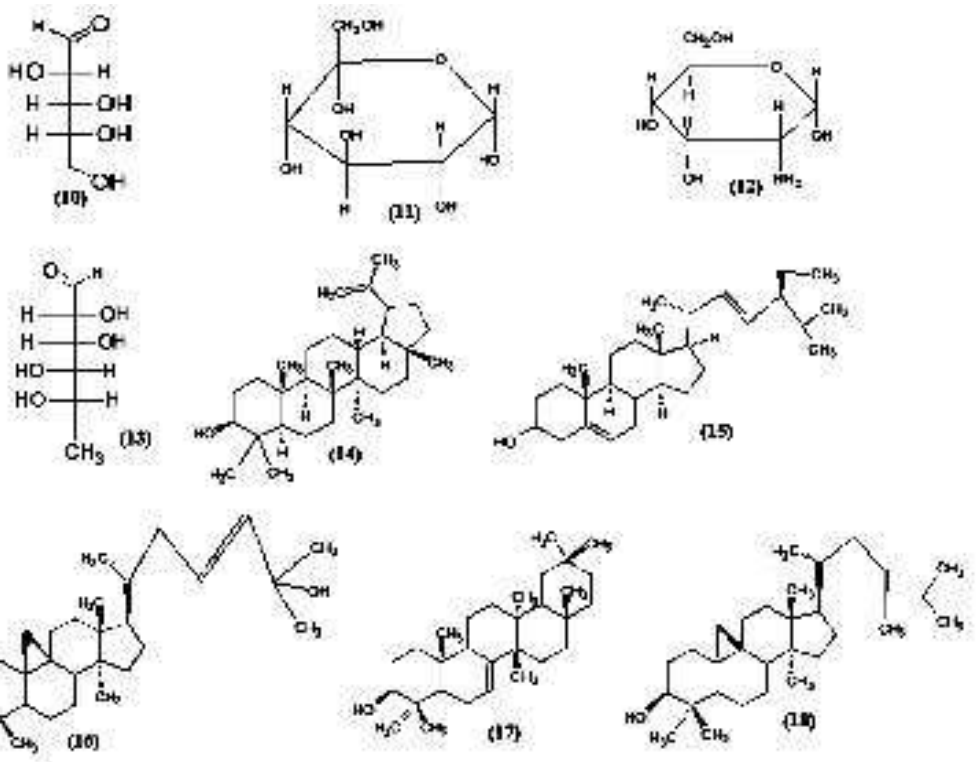
Setty et al. in 2007 reported hepatoprotective effect of ethanolic extract (70%) against paracetamol induced hepatitis in rats. The treatment with ethanolic extract (200mg & 400mg/kg) restored the altered levels of biochemical markers (SGPT, SGOT, ALP, Bilirubin and cholesterol, HDL, and tissue GSH) to almost levels in a dose dependent manner[13].

Mukharjee et al. in 2010 reported the CNS depressant activity of methanolic extract of fresh flowers of *calotropis procera* in an *in-vivo* model. Administration of standard drug Diazapam (1.5mg/kg body weight ip) and flower extracts (100mg/kg oral) produces significant CNS depression to the treated mice[14].

Sharma & Sharma in 2000, studied the antimalerial effects of ethanolic extracts of different oarts of *calotropis procera* .The IC 50 values were obtained from 0.11-0.47mg/ml against *P. falciperum.* It was observed that flower and bud extracts produce highest Ic values. Hence, these extracts are most active antimicrobial component in *Calotropis procera*[15].

Latex: Latex is a complex mixture of more than 09 cardiac glycosides which are calotoxin**(3)**, calactin**(2)**, uscharine**(5)**, voruscharine**(6)**, uzarigenine**(8)**, syriogenin**(22)**, proceroside**(23)**, cholin**(24)**. One enzyme named as trypsine was also isolated from latex of CP**(25)**. The presence of these compounds enables latex to be used as





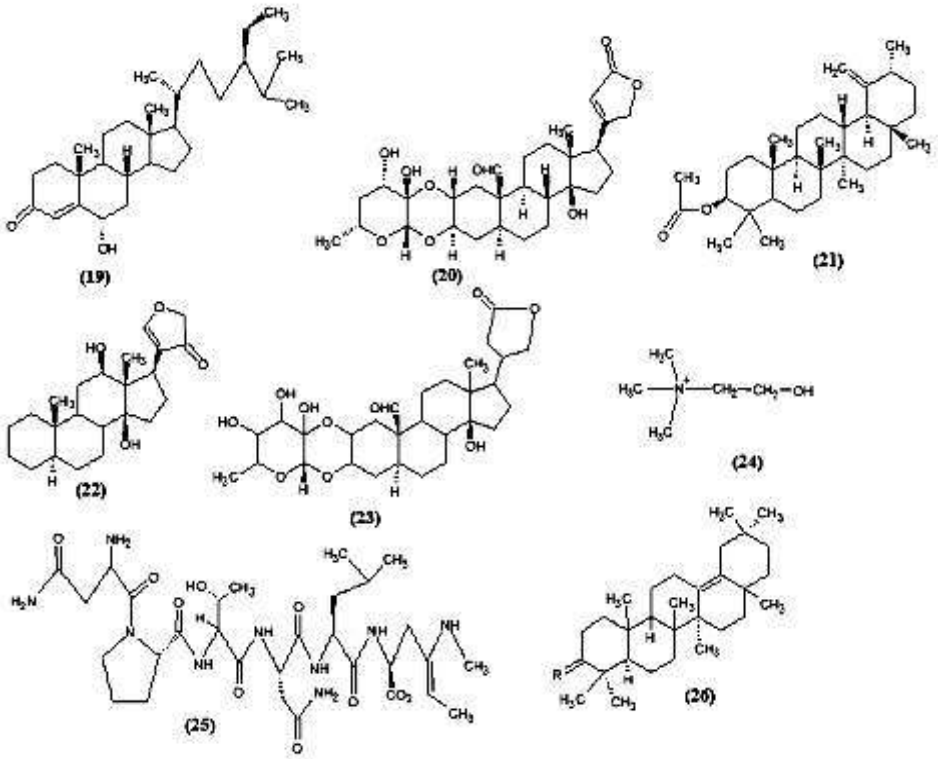
anti-inflammatory, analgesic, antinociceptive and antidiabetic remedy in folklore medicines.The Latex of the plant is reported to posses antiasthmatic, antileprotic, antirheumatism, anticold activity[16-18].

Kumar et al. in 2000 studied the analgesic effect against acetic acid induced writhing drug. Administration of dry latex at a dose of 830 mg/kg produces marginal analgesia in a tail- flick rodent model. The dose doesn't produce any toxic effects in mice and LD 50 was found to be 3000 mg/kg[19].

Vasconcelos and team in 2005 administered fraction of latex prote into male mice and observed a dose-dependent antinociceptive effect compare with the respective controls in all assays. Different doses of latex protein reduces the nociception produced by formaline and the effect was not reversed by pretreatment with naloxone. They observed that protein fraction of latex of *calotropis procera* possesses good antinociceptive activity[20].

Kumar et al. in 2005 observsd that daily oral administration of latex in diabetic rats at 100 and 400 mg/kg produced a dose dependent decrease in blood glucose and an increase in hepatic glycogen. It also restores the body weight and daily water consumption capacity of the treated animals. Dry latex also increases hepatic levels of endogenous antioxidants like, superoxide dismutase (SOD), calatase and glutathione and reduces the levels of thiobarbeturic acid reactive substance (TBARS) in alloxan- induced diabetic rats[21].

Al-Yahya et al. in 1985 studied the latex of *Calotropis procera* for its inflammatory activity in rats using pedal oedema and air pouch models of inflammation. Subcutaneous injection of aquous solution (0.1ml of 1) of dry latex into the plantar surface of paw produces inflammation. Maximum inflammation obtained one hour after the injection and was maintained for next one hour. The inflammatory activity (response) accompanied by an increase in vascular permeability that reaches



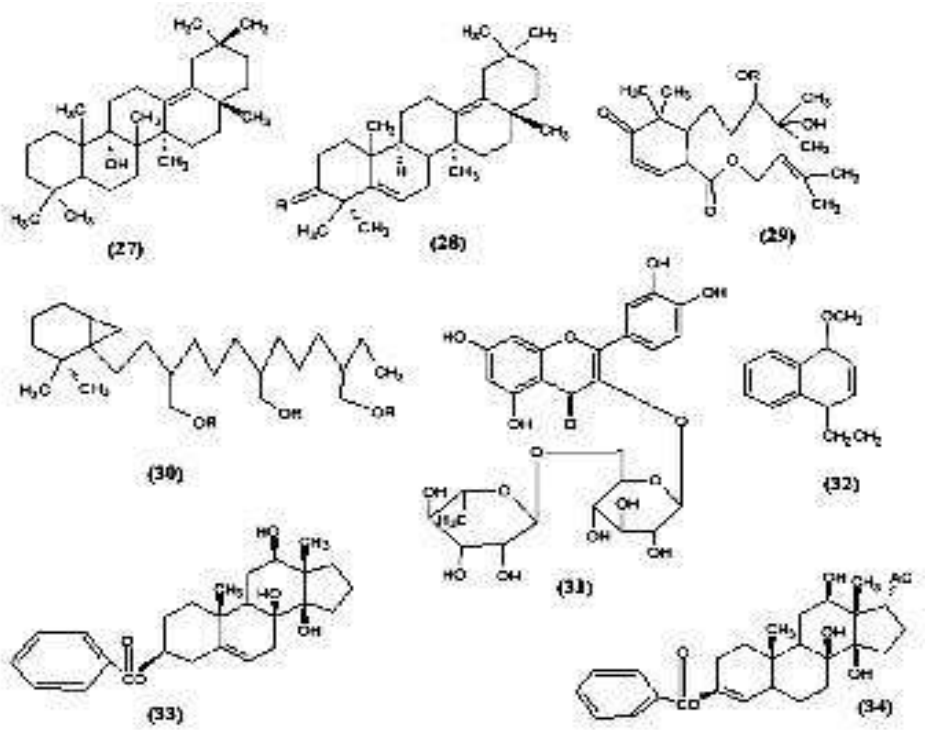
its maximum within 15 minutes. The model characterized for the exudates volume and its protein concentration and wet and dry weights of granuloma. Their study indicated that both the exudates volume and weight of granuloma was at maximum on day 5 after dry latex injection and the protein concentration peaked on the 3rd day. Further study of both the model for the anti inflammatory effect of various drugs, they showed that in padel oedema model phenyl butazone are more effective than prednisolone and almost complete inhibition was produced by mepiramine and cycloheptadine. In air pouch, model prednisolone is more effective than phenylbutazone in inhibiting the inflammation[22].

Kumar et al. in 2001 studied the anti-diarrhoeal activity of *Calotropis procera* dry latex. They evaluated its effect on intestinal transit, castor oil-induced intestinal fluid accumulation and electrolyte concentration in intestinal fluid. Dry latex produced a decrease in intestinal transit(27%-37%), compare with normal and castor oil treated animals. Dry larex significantly inhibited castor oil induced enteropooling, but it did not alter the electrolyte concentration in the intestinal fluid compared with castor oil- treated rats[23].

Uddin et al. in 2012 studied the antibacterial effect of various extracts of milky latex of *calotropis procera* using modified agar well diffusion method. Streptomycin was used as standard drug at the concentration of 2mg/ml. The crude methanolic extract exhibited good antibacterial activity with zone of inhibition ranging from 12-20mm diameter[24].

Roots and Bark Root

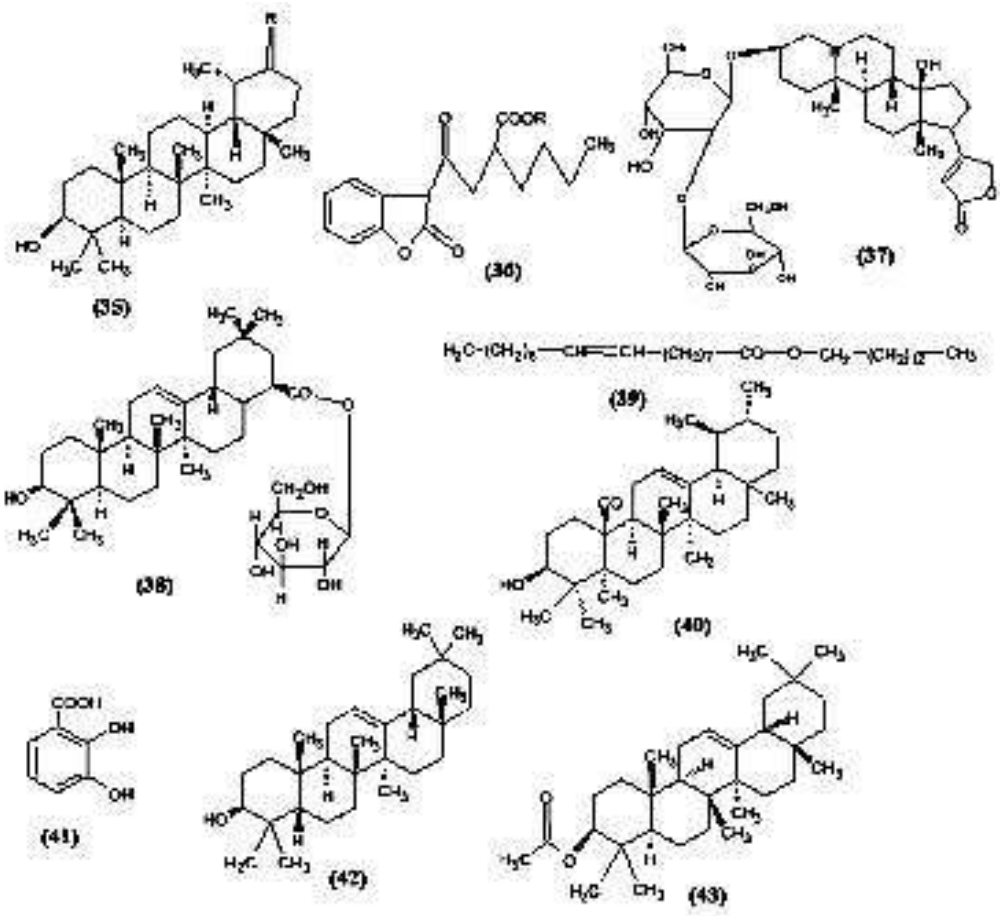
Root and root bark of *Calotropis procera* is rich in terpenoids, steroids, heterocyclics and napthalene derivatives. Terpenoids were mainly



Calotropaleanyl ester**(26)**, proceroleanol A and B**(27,28)**, Calotropsesquiterpenol**(29)**, Calotropsesterpenol**(30)**, Calotropursenyl acetate, quercetine -3- ratinoside**(31)**. One napthalene derivative namely Calotropnapthalene**(32)** was isolated from the root bark. Few sterols like benzoyllineolone**(33)**, benzoylisolineolane**(34)** and teraxasterol**(35)** have been isolated and characterized from the roots of CP. One heterocyclic compound named as chlorobenzofuranone**(36)** has also reported from the roots. Compounds like procerursenyl acetate**(37)**, proceranol-2**(38)**, n- tetradecanyl palmitoleate**(39)**, proceraursenolide**(40)**, pyrocatechuic acid**(41)** are also isolated from the root of CP.The root and root bark of plant are reported to have antidiarrheal and anticholeral activity[25-32].

Jalalpure et al. in 2009 studied anticonvulsant activity of root extract of *calotropis procera* in rats by using seizures induceed by maximal electroshock seizures (MES), pentylenetetrazol (PTZ), lithiumpilocarpine and kindling seizures. chloroform extract of *calotropis procera* roots showed the most significant ($P<0.01$), anticonvulsant effect by decreasing the duration of hind limb extension in the MES test. In the PTZ test, chloroform extract exhibited highly significant effect($P<0.001$) and aquous extract exhibited most significant ($p<0.01$)effect. By the study, they observed that the chloroform extracts and aqueous extracts of CP roots are useful in the absence of tonic clonic (grand mal), (petitmal) types of seizures[33].

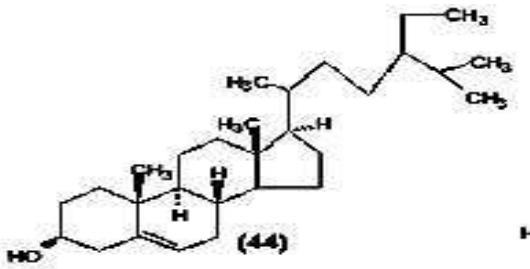
Mathura et al. in 2009 studied the anti-tumor activity of various root extracts of CP. Treatment of step 2 cancer cells with these extracts at different dosage of 1,5,10 and 25 mg/ml revealed that all the extracts except aqueous possess good cytotoxic effects. Among these, ethyl acetate extract showed strongest cytotoxic effect (96.3%) followed by methanolic (72.7%) and hexane extract (60.5%)[34].

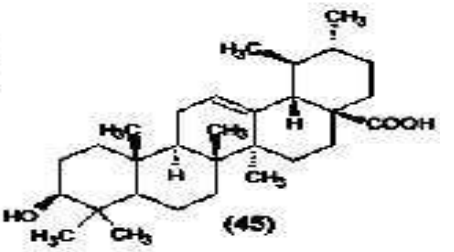


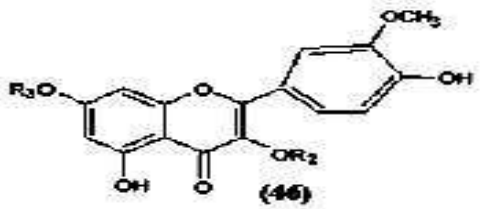
Danmalam et al. in 2007 studied the anticonvulsant of 70% ethanolic effect of root bark of *Calotropis procera* using maximum electroshock induced seizeures in chicks. The extracts proved to be effective in inhibiting electroshock induced seizures in chicks.The observed inhibition of hind limb tonic extention chicks was comparable to the effect of phenitoine(100% protection) in this model .Thus they suggest the usefulness of ethanolic extracts of *Calotropi*s proc*era* root bark in management of grandma epilepsy[35].

Leaves: Leaves of *Calotropis procera* have been reported to possess cardiac glycosides, terpenoides and sterols. Two major cardiac glycosides isolated from the leaves are calotropin**(1)** and calotropagenin**(4)**, where as terpenoids were mainly alpha-amyrin**(42)**, beta-amyrin and amyrin acetate**(43)**. Beta sitosterol**(44)** was the only steroid present in the leaves of *Calotropis procera.* Ursolic acid**(45),** ratinoside **(46)** and 3-thiozoline **(47)** was also isolated from the leaves of CP .The leaves of plant exhibit antirheumatism and antiasthmatic activity[36,37].

Yashmin et al. in 2008 evaluated antioxidant potential **of** methanolic and aqueous exract of leaves of CP using scavenging activity of the stable DPPH free radical, IC 50 of the methanolic extract was 110.25mg/ml indicating strong antioxidant effect, whatever the aqueous effect [38].







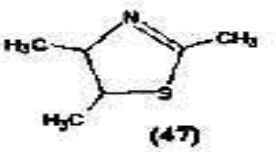


Table-2 Phyto-pharmacological Detail of Various Parts of *Calotropis procera*

Parts	Chemistry	Structure	Pharmacolical Activity	Reference
Flower	**Cardiac Glycoside**		Heptoprotective,	[13]
	Calotropagenin	**(4)**	Anti-inflammatory	[39-40]
	Calotropin	**(1)**		[39-40]
	Uscharin	**(5)**	Antimicrobial,	
	Calotoxins	**(3)**	Larvicidal	[41]
	Calactin	**(2)**	Antimalerial	[42]
	Voruscharin	**(6)**	Antiasthamatic	[43]
	Uscharidin	**(7)**	Antimoebic activity	
	Uzarigenin	**(8)**		
	Proceroside	**(23)**		
	Proceragenin	**(9)**		
	Syriogenin	**(22)**		
	Carbohydrates			
	D- Arabinose	**(10)**		
	Glucose	**(11)**		
	L- Rhamnose	**(13)**		
	Glucosamine	**(12)**		
	Sterols			
	Proesterol	**(19)**		
	Multiflorenol	**(17)**		
	Cyclosadol	**(18)**		
	Cycloart-23-Ene-3b-25 Diol	**(16)**		
	Lupeol	**(14)**		
	Stigmasterol	**(15)**		
	Calotropain	**(20)**		
	Calotropenyl acetate	**(21)**		

Latex	**Cardiacglycosides**		Antirabies	[44]
	Calotropin	**(1)**	Arthritis	[45]
	Calotoxin	**(3)**	Analgesic, Anti-inflammatory, antimicrobial, Antiliprotic	[41]
	Calactin	**(2)**		
	Uscharin	**(5)**		
	Voruscharin	**(6)**		
	Uzarigenin	**(8)**		
	Syriogenin	**(22)**		
	Proceroside	**(23)**		
	Trypsin	**(25)**		
	Choline	**(24)**		
Leaves	**Cardiacglycosides**		Antigout	[46]
	Calotropagenin	**(4)**	Antileprotic	[46]
	Calotropin	**(1)**	Antimalarial	[47]
	α-Amyrin	**(42)**	Antirhumatism	[46]
	β-Amyrin	**(42)**	Analgesic	[48]
	Amyrin Acetate	**(43)**		
	β-Sitosterol	**(44)**		
	Urosolic Acid	**(45)**		
	Ratinoside	**(46)**		
	3-thiozolin	**(47)**		
Root & Root bark	**Sterols**		Anticanceractivity	[49]
	Benzoyllineolone	**(33)**		[50]
	Benzoylisolineolane	**(34)**	Antimalarial	[51]
	Proceranol-2	**(38)**	Antirheumatism, Antiasthmatic, Antileprotic	
	Teraxasterol	**(35)**		
	Triterpenoids			
	Calotropsesquiterpenol	**(29)**		
	Calotropsesterpenol	**(30)**		
	Calotropleanyl Ester	**(26)**		
	Proceraleanol A	**(27)**		
	Proceraleanol B	**(28)**		
	Others			
	Calotropnaphthalene	**(32)**		
	Calotropbenzofuranone	**(36)**		
	Procerursenyl Acetate	**(37)**		
	N- Tetradecanyl Palmitoleate	**(39)**		
	Proceraursenolide	**(40)**		
	Pyrocatechuric Acid	**(41)**		
	Queretin–3-retinoside	**(31)**		

From the above vast literature search, it was observed that *Calotropis procera* itself contains enormous potential to cure various chronic ailments and is being recommended by various popular systems of medicines. Keeping this aim in mind, preliminary phytochemical screening and antimicrobial activity was performed with various extracts of CP latex. Significant effect was found

in Petroleum ether extract and the study further elaborated for investigation of antimicrobial principal (if any), which is under progress.

Conflict of Interest

There is no conflict of interest among all the authors.

Acknowledgment

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Antimicrobial Activity of *Ocimum* and *Capsicum annuum* Plant Extracts

***Ankita Kandwal, Shweta Kumari, Gunjan Bisht and Afshan Tarranum**
Department of Life Science, Beehive College of Advance Studies, Dehradun, India
*Email: ankitakandwal55@gmail.com

Abstract- Medicinal plants consist of component of therapeutic values and have been used as remedies for human diseases since long. The search for components with antimicrobial activity is gaining importance in recent times due to growing worldwide concern about the alarming increase in the rate of infection by antimicrobial resistant micro-organisms. Medicinal plants being as important natural resources and potentially safe drugs can play an important role in improving human health by contributing herbal medicines. Thus, present research work was designed to evaluate antimicrobial activity *Ocimum sanctum* and *Capsicum annuum* (green chilli) plant extracts.

Keywords: Antibacterial activity, Disc diffusion, Plant extracts.

Introduction

The Indian Himalayan Region supports approximately 1700 plant species of known medicinal value. A survey of World Health Organization (WHO) indicates that about 70 to 80% of the world population in the developing countries depends on herbal sources as their primary healthcare system. About two million traditional health practitioners use over 7500 medicinal plants all over the world (Hans S T, 1998).

Ocimum tenuiflorum is an aromatic plant in the family, *Lamiaceae* which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. It is an erect, many-branched subshrub (30–60 cm 12–24 in) tall with hairy stems and simple phyllotaxic green or purple leaves that are strongly scented. Tulsi is cultivated for religious and medicinal purposes, and for its essential oil. It is widely known across the Indian subcontinent as a medicinal plant and an herbal tea, commonly used in Ayurveda, and has an important role within the Vaishnava tradition of Hinduism in which devotees perform worship involving holy basil plants or leaves. This plant is revered as an elixir of life.

Capsicum annuum is a species of the plant genus *Capsicum* native to southern North America and northern South America. This species is the most common and extensively cultivated of the five domesticated capsicums. Although, the species name annuum means “annual” (from the Latin

annus "year"), the plant is not an annual and in the absence of winter frosts can survive several seasons and grow into a large perennial shrub. (Zhi Yun *et al*., 2007).

Methods

Collection of Samples

In systematic collection process, the samples of the respective species were colleted from adjoining areas of Selaqui, Dehradun.

Bacterial Culture and Culture Media

The bacterial cultures required for the experiment were obtained from Institute of Microbial Technology, Chandigarh, India in the form of slant. These cultures were maintained on Nutrient Agar media (NAM) at first being incubated at 37˚C for 3-5 days and then stored at 4˚C as stock culture assay.

Antibacterial Activity : The paper Disc diffusion method was employed. The Sterilized Whatman filter paper discs (6mm) were impregnated with 100 µL of plant extracts in different solvents. The Nutrient agar media (NAM) medium was prepared and transferred to the sterilized petridishes in such a way to keep a uniform depth of approximately 4mm. The100µl standardized inoculums prepared from solutions of *Clostridium* sp., *S. gordonii* and *P. vulgaris*, then spreaded over the media surface using a clean sterilized cotton swab. Discs were then placed on agar plates.

Bacterial Growth Inhibition Measurement : Bacterial growth inhibition was determined as the diameter around the discs. Zone of inhibition (ZOI) was measured in mm with the help of scale.

Phytochemical Screening : Tests were performed to ascertain the presence / absence of different constituents in each plant extract.

- **Test for flavonoids:** 2 ml filtrate was added to conc. HCl and magnesium ribbon. Pink-tomato red color indicated the presence of flavonoids**.**
- **Test for amino acids:** 1 ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple color shows the presence of amino acids.
- **Test for tannins:** 1 ml of the extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration.
- **Test for anthraquinones (Borntrager's test):** 1 ml of the extract solution was hydrolyzed with diluted Conc. H2SO4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.
- **Test for cardiac glycosides (Keller-Killani test):** 5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.
- **Test for Starch:** 0.01g of Iodine and 0.075g of potassium iodide were dissolved in 5ml of distilled water and 2-3ml of extract was added. Formation of blue color indicated the presence of starch.
- **Test for Proteins (Biuret's test):** To 1ml of hot aqueous extract, 5-8 drops of 10% w/v NaOH solution, followed by 1 or 2 drops of 3% w/v $CuSO_4$ solution were added. Formation of violet red colour indicated the presence of proteins.

Results

Current study targeted the extraction and assay of antimicrobial metabolites from the fruit and leaf extract. The extracts were dried and antimicrobial metabolites were extracted from them by Soxhlet's extraction procedure. This yielded different results in different solvents in each of the experiment conducted in the study. Sequential or successive solvent extraction is as good option for better solubility of many of the photochemicals.

Antimicrobial Activity Measurement

The antibacterial activity of the isolated extracts was assessed on the basis of ZOI observed which have been given in the table below.

As observed above *Ocimum tenuiflorum* and *Capsicum annuum* extract was most active against *P. vulgaris* while they showed moderate activity against other strains. The ZOI was compared with negative control DMSO.

Table-1 Phytochemical Tests for the Two Herbs In Different Solvents

	Ocimum tenuiflorum				*Capsicum annuun*			
Phytochemical tests	Ethyl Acetate	Ethanol	Methanol	Chloroform	Ethyl Acetate	Ethanol	Methanol	Chloroform
Flavonoids test	-	-	+	-	-	-	+	-
Amino acid test	-	-	-	-	-	-	-	-
Tannins test	-	-	+	+	-	-	+	+
Cardiac Glycoside	+	-	-	-	+	+	+	-
Anthraquinones test	-	-	-	+	-	-	-	+
Starch test	-	-	-	-				
Protein test	-	-	+	+				

but it is always necessary to know the photochemical extracted by each individual solvent so as to avoid the inclusion of unnecessary solvents for extraction process.

Figure-1–Protein Test of *Oscimum tenuiflorum* leaf extract

Table-2 Zone of Inhibition in Millimetre (mm) of Extracts Against Test Bacterial Strains.

Bacteria	Zone of inhibition(mm)			
	Ocimum tenuiflorum			
	Ethanol	Ethyl Acetate	Choloroform	Methanol
Clostridium sp.	7	-	10	-
S. gordonii	9	-	-	15
P.vulgaris	-	10	-	10
	Capsicum annuum			
Clostridium sp.	7	-	8	-
S. gordonii	12	9	-	10
P.vulgaris	9	10	-	15

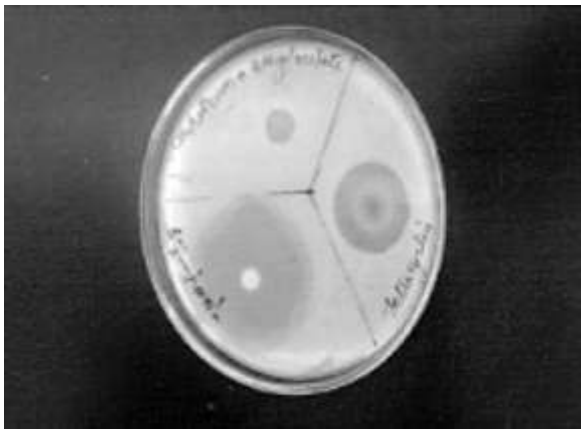

Plate-1 *Capsicum annuum* ethyl acetate, ethanol and chloroform extract against *Clostridium* sp.

Plate-2 *Ocimum tenuiflorum* methanol, ethanol and choloform extract against *Clostridium* sp.

Based on the above results, it can be concluded that the above extracts could be very good source for the extraction of antimicrobial components. These results were in agreement with studies conducted by Golshahi *et al.* in 2011 on *Ocimum sanctum* antibacterial activity The extracts could be used as a drug after proper pharmacological evaluation and clinical trials. The future prospects of the current research include further purification of extract and its evaluation for the presence of phytochemical components and their identification.

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About Flowers

Rose Flowers (*Rosa damascena*)

Mogra (*Jasminum officinale*)

Rajnigandha (*Polianthes tuberosa*)

Night blooming Jasmine (*Cestrum nocturnum*)

Rose Flowers (Rosa damascena)

Order : Rosales
Family : Rosaceae
Genus : Rosa
Species : R.damascena

Rosa damascena mill L,(Rose) commonly known as Damask rose. It is one of the most important species of Rosaceae family. Rosaceae are well- known ornamental plants and have been referred to as the king of flowers. At present time, over 200 rose species and more than 18000 cultivars form of the plant have been identified. Apart from the use of R. damascena as ornamental plants in parks, gardens, and houses, they are principally cultivated for using in perfume, medicine and food industry .However, R. damascena is mainly known for its perfuming effects. The rose water were scattered at weddings to ensure a happy marriage and are symbol of love and purity and are also used to aid meditation and prayer.

Beside perfuming effect, several pharmacological properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, antidiabetic, and relaxant effect on tracheal chains have been reported for this plant.

Rajnigandha (Polianthes tuberosa)

Order : Asparagales
Family : Asparagaceae
Genus : Polianthes
Species : P.tuberosa

Rajnigandha means “The Fragrance of the Night”. It is a flower that is both mythical and magical, its nectar said by some to have special powers and its scent magical to all who experience it. Tuberoses are a popular flower in floral arrangements and their scent is used to produce perfumes the world over.

The tuberose is a night- blooming plant thought to be native to Mexico along with every other species of Polianthes. It grows in elongated spikes up to 45 cm (18 in) long that produce clusters of fragrant waxy white flowers that bloom from the bottom towards the top o the spike. It has long, bright leaves clustered at the base of the plant and smaller,clasping leaves along the stem.

Jasmine Flower-Mogra(Jasminum officinale)

Order : Lamiales
Family : Oleaceae
Genus : Jasminum
Species : J.officinale

Flowers are presumed to be the divine grace of gods. The soothing fragrance of these flowers mesmerizes the senses of one and all. Endowed with the exotic perfumes, Jasmine flower is one of the most beautiful and fragrant amongst all the 15,000 flowering plant species in India. Majorly used as decorative flowers, these scented flowers are also used for medical motives and religious purposes.

Jasmine is also referred to as Mogra, Kundumalligai, Arabian Jasmine or Mallika. Its popularity in India can be easily understood as it is also praised as "Moonshine in the garden” by the Indians.

A prominent member of the family "Oleaceae" Jasmine is scientifically known as Jasminum officinale. An evergreen shrub, Jasmine is the pride of all beautiful gardens. It usually climbs up as a vine and reaches to a height of about 8 to 10 feet. Strangely the little flowers that this plant bears in huge clusters is simply about an inch each. The oval shaped green rich leaves, bear around five to nine leaflets which give the entire plant a very beautiful and artistic look. A symbol of purity and peace the beautiful jasmine flowers are white in color. Jasmine plant grows worldwide spreading its fragrance all over.

Night blooming Jasmine (Cestrum nocturnum)

Order : Solanales
Family : Solanaceae
Genus : Cestrum
Species : C.nocturnum

Night-blooming jasmine is an evergreen woody shrub growing to 4 metres (13 ft) tall. A powerful, sweet perfume is released at night. “All flowers have evolved mechanisms to achieve successful pollination. C.nocturnum produces an intensively fragrant “volatile” scent during the night to attract its pollinator.

No fragrant garden should be without this nocturnal beauty. While night blooming jasmine is a gorgeous plant with charming blooms, the scent also produces severe allergic reactions in some individuals.

Forth Coming Events

1. International Pharmacy Conference
   July 14-15, 2016
   Philadelphia, USA
   Web. http://pharmacy.pharmaceuticalconferences.com/
2. 2nd World Chemistry Conference
   August 08-10, 2016
   Toronto, Canada, web. http://chemistry.conferenceseries.com/
3. 3rd World Congress on Pharmacology
   August 08-10, 2016
   Birmingham, UK web. http://pharmacology.pharmaceuticalconferences.com/call-for-abstracts.php
4. 4th International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products
   August 29-31, 2016 Sao Paulo, Brazil.
   http://pharmacy.pharmaceuticalconferences.com/recommended-global-conferences.php
5. International Conference on Pharmaceutical Chemistry
   September 05-07, 2016
   Frankfurt, Germany web. http://pharmaceuticalchemistry.conferenceseries.com/
6. 3rd International Conference on Past and Present Research Systems of Green Chemistry
   September 19-21, 2016
   Las Vegas, USA, web. http://greenchemistry.conferenceseries.com/
7. World Congress on Chromatography
   September 21-23, 2016
   Amsterdam, Netherlands, web. http://chromatography.conferenceseries.com/
8. IUPAC 29th International Symposium on the chemistry of natural products and the 9th International conference on Biodiversity 24–27 September 2016
   Related Subjects- Biodiversity and Medicinal **chemistry, Toxicology, Biology**
   Izmir, Turkey, web. http://www.iscnp29-icob9.org/
9. 7th International Conference and Exhibition on Analytical & Bioanalytical Techniques
   September 28-30, 2016
   Orlando, USA
   web. http://analyticalbioanalytical.pharmaceuticalconferences.com/
10. International Conference on Applied Chemistry
    October 17-18, 2016
    Houston, USA, web. http://appliedchemistry.conferenceseries.com/
11. All Ayurvedic & **Herbal** Events in February **2017**, List of all Ayurveda **...**
    31 Jan-02 Feb **2017**. Moscow ... Popular speakers speaking at this **conference** are Lena GigerManuela ArcottaPascal Voggenhuber ... Ayurvedic & **Herbal**; Cosmetics and Beauty **Products** ... Organic & **Natural** Beauty Show ... New Delhi, **India**.
    10 times.com/ayurveda–**herbal.** Month, February.
12. Innopharm 2 is being organized by Paramita Health Care Society in association with Innovare Academics Sciences at M.P. Council of Science & Technology*, Vigyan Bhawan, Nehru Nagar, Bhopal MP, India from Feb 11-12, 2017. (About MPCST)
    Web. http://innopharm2.innovareacademics.in/
13. 10th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems March 13-15, 2017
    London, UK web. http://novel-drugdelivery-systems.pharmaceuticalconferences.com/registration.php
14. International Conference on Environmental Chemistry
    July 24-26, 2017
    Rome, Italy, web. http://environmentalchemistry.conferenceseries.com/call-for-abstracts.php

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The typed manuscript should not exceed 10 pages and tables and graphs should not exceed 10% of the typed manuscript.

Manuscript should be typed on one side of the paper with double spaces. The usual format of a manuscript comprises of Abstract (not exceeding 150 words), Introduction, Keywords, Material and Methods, Observations, Results, Discussion, Acknowledgements, References and Tables & Figures.

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

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The reprint of their articles will be supplied to the authors (First author) free of charge. Orders for additional reprints should be made along with the manuscript. They will be charged for postage and packing.

It is emphasized that the manuscripts should be neatly typed, double spaced throughout including tables on pages of uniform size with at least 2.5 cms margin on all sides. Author(s) should not break or hyphenate the words. Authors are required to include:

The chemical structure, formula and proprietary name of novel or ill defined compounds.

The w/w yield of prepared extracts in terms of starting crude material.

Complete formulation details of all crude drug mixtures.

The herbarium specimen number of plant(s) studied in case of less well known plants be cited using the collector and collection number (eg. Doe 123) as well as indicating the names of the herbarium institution where it has been deposited.

Keywords: Authors are requested to assign 3-6 keywords to the manuscript preferably taken from index Medicus of Excerpta Medica Index for abstracting and indexing purposes. They should be mentioned after abstract and before introduction.

References to literature are to be arranged alphabetically and to be placed at the end of the article and referred to by name and year chronologically. Standard abbreviations listed in the World list of Science Periodicals should be used. A sample citation is given here: Singh,G.; Dhar, U. and Singhal, A.K. Origin of Kashmir Saffron – a possible clue from weeds . *Sci.Cult*., 1976, 42:485-487.

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Vigyan Dham, Jhajra, Dehradun - 248 007
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Council Initiative for promotion of reverse pharmacology in Ayurvedic drug development

Uttarakhand State Council for Science & Technology (UCOST) was established in the last quarter of 2005 in Dehradun. Since its inception council has encouraged the research and development activities in the state and has funded projects in various disciplines of Science & Technology. Council provides financial assistance in R&D, International Travel supports, Entrepreneurship Development Program (EDP), Seminar/Symposium/ Conference/Workshop grants etc.In innovation promotion programthe grassroots level for application/ invention catering local needs and all individuals with demonstrable talent are being promoted. The council aims to forge partnership between Central and State Governments, NGOs, R & D institutions, academia and industry, Council will act as hub, maximizing collaboration between various organizations and promote science in multidisciplinary mode. As an initiative, Coordination Cell of the Council are being set up at various institutions.The council has established a state-of-art Regional Science Centre in Uttarakhand sponsored by NCSM, Kolkata, catering to needs of the people of state especially school going children. **The regional science Centre will also have an innovation lab sponsored by National Innovation Council.**

As far as medical science is concerned, UCOST **has initiated an ambitious "Drug Development" program to promote drug development in Ayurveda** within the ambit of reverse pharmacology and the guidelines laid down by WHO for the development of natural products. We have recently reviewed promising therapeutic effects of Herbo-mineral Formulations for prophylaxis of Chronic Pancreatitis and migraine, *Faltrikadi kwath* for prophylaxis of Hepatitis B and started to facilitate advanced R&D following reverse pharmacology. We are intended to work on drug development for some tropical diseases in near future under **Drug Development program provided that the aspiring Vaidya or Ayurvedic traditional healers have maintained meticulous record of their clinical work.**

I extended my best wishes to Universities Journal of Photochemistry and Ayurvedic Height for their endeavor in Herbal research.

Dr. Rajendra Dobhal
Director General

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