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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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Contents

Editorial

Time is gone when education was more book based. Now we talk more about skill based education and the students of science are into it since day one. One has to be still more rigorous today that's why more projects are given and more innovative work is to be done.

When this innovation and quality production was not there, India's biggest pharma Industry Sun Pharma shares fell over 3 per cent. According to Nomura's estimates, supplies from Halol contribute around $350-$400 million in US sales for the Indian pharma company. The Halol facility was inspected by the US Food and Drug Administration last year and received a Form 483 detailing possible deviations from good manufacturing practices. Sun currently faces supply constraint for some products due to the compliance issues at the Halol facility.

Promotion of Indigenous System of medicines through R&D by young scientists is a must. Our Government also is trying to cut prices of key drugs to fifty per cent as our drugs are at present priced very high.

India by 2028 is going to be the most populous country overtaking China as per UN report and our sex ratio is also unfavorable. This is definitely a bad news. Therefore, the only solution to look after common men is Herbal Industry which will grow through cultivation and growth of Pharma Industry which in the last 9 months of 2012-2013 has grown 22%. The high potential of indigeneous herbs is before you, rest I leave to you to ponder upon.

My sincere thanks to all those who have contributed for bringing out this issue and to all the Board Members of UJPAH, contributors of research papers, participants, specially Dr. P.K, Garg, V.C. U.,T. University and Dr. Rajendra Dobhal, Director General of UCOST, all staff members, eminent scientists and last but not the least Dr. I.P. Saxena for his timeless efforts to bring out this journal in time and organise this seminar with contribution of Dr.I.P.Pandey, Dr. Himmat Singh, Dr. B.B. Raizada. May Almighty protect us all from the fury of winter season and also from the water borne diseases to arise in the coming months.

(Dr. S. Farooq)
Chief Editor

Chemical Constituents of *Urtica ardens Leaves*

S.C. Sati, Maneesha D. Sati anδ I.P. Panδey*
Department of Chemistry, H. N. B. Garhwal University (A Central University)
Srinagar, Uttarakhand, India. 246174
*Prof. Emeritus in Chemistry E-mail: sati_2009@rediffmail.com

Abstract- 3β-hydroxyurs-12-en-28-oic acid (1) and methyl palmitate(2) were isolated from alcoholic extract of leaves of *Urtica ardens*. The structures of these compounds were characterized by means of chemical and spectral methods including advanced 2D NMR studies. These compounds were first time isolated from this species.

Keyworδs: *Urtica ardens, Urticaceae,* 3β-hydroxyurs-12-en-28-oic acid and methyl palmitate

Introδuction

Urticaceae is a large family of about 45 genera and 550 species found in tropical and temperate regions, 21 genera and 120 species in India. The plants of this family are herbs, undershrubs or rarely trees, without latex; epidermal cells often cystoliths, stem fibrous, leaves alternate or opposite, simple, stipulate or not. Flowers are minute, unisexual, regular, usually cymose, sometimes crowded on enlarged receptacle. Perianth lobes 3-5, sepaloids, free or united and stamens are equal to tepals, inflexed in buds, pollens 3-5 porate, spheroidal, stenopalynous. Ovary 1-locular, 1-erect ovulate, style simple and fruit achene or drupe.

Urtica ardens, vern. Stinging nettle, belong to family Urticaceae is a perennial, erect, pubescent herbs or shrubs often attaining to 2.5m high; stem greenish-pale, bark fibrous, petioles, leaves, branches covered with stinging bristles. Flowers are small, pale green, clustered on spreading, axillary 4-8cm long, paniculate cymes. Male flowers with 4-perianth segments and 4 stamens. Female perianth segments 4, unequal, inner ones twice longer than outer ones. Achenes ovoid, pale-brown, hairy, enclosed by persistent perianth. Flowering and fruiting season is August to January .The plants of genus Urtica is distributed throughout the world including Paraguay, Uruguay, Brazil, southwest of Hubei province, China, Asia, America, Europe, Iran, Greece and Turkey.

Material anδ Methoδs

General

Melting points were recorded on Perfit melting point apparatus. UV spectra were measured on a Perkin-Elmer Lambda-25 spectrophotometer (methanol as solvent) and IR spectra were recorded on Perkin-Elmer spectrum RX I FT-IR spectrophotometer using KBr discs. NMR spectra were obtained on SFO1 300 MHz-Bruker NMR spectrophotometer (300 MHz for ^{1}H and ^{13}C NMR, TMS as internal standard). MS were recorded on HRMS microOTOF-Q II 10328 (Bruker Compass data Analysis). Column chromatography was carried out on silica gel (Merck 10-40 μ) precoated plates (0.5 mm thick layer) were visualized by spraying with 7% H_2SO_4 as universal spraying reagent along with other specific reagents for particular class of natural products.

Plant Material

Whole plants (10 kg) of *Urtica ardens* were collected from the Auli, Chamoli Uttrakhand India in October 2014. The plant was identified from Department of Botany, HNB Garhwal University Srinagar Uttrakhand. A voucher specimen (GUH-6890) was deposited in the same section.

Extraction anδ isolation

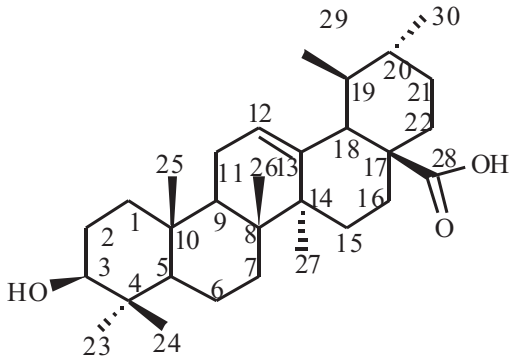
Shade dried coarsely powdered whole plant of *Urtica ardens* (10 kg) was extracted (three times)

exhaustively with 95% ethanol (5L) at 30-50 °C temperature for 16-18 hours on a heating mantle. The extraction mixture was filtered and solvent evaporated to dryness under reduced pressure to yield black residue (400 g). The crude extract was fractionated with petroleum ether and ethyl acetate (repeatedly three times) by soxhlet apparatus to yield petroleum ether (90 g), ethyl acetate (130 g), crude extracts. The ethyl acetate soluble (130 g) was pre-absorbed onto silica gel (Merck, 60-120 mesh, 200 g) and then allowed to run over the silica gel (500 g) packed column. The elution was started with $CHCl_3$:MeOH by increasing polarity of MeOH (5-17%). The fractions (100 ml of each) obtained from column were collected and deposited in same conical on the basis of TLC analysis. The elution afforded two compounds in pure form (purified by recrystallization).

Compound -1

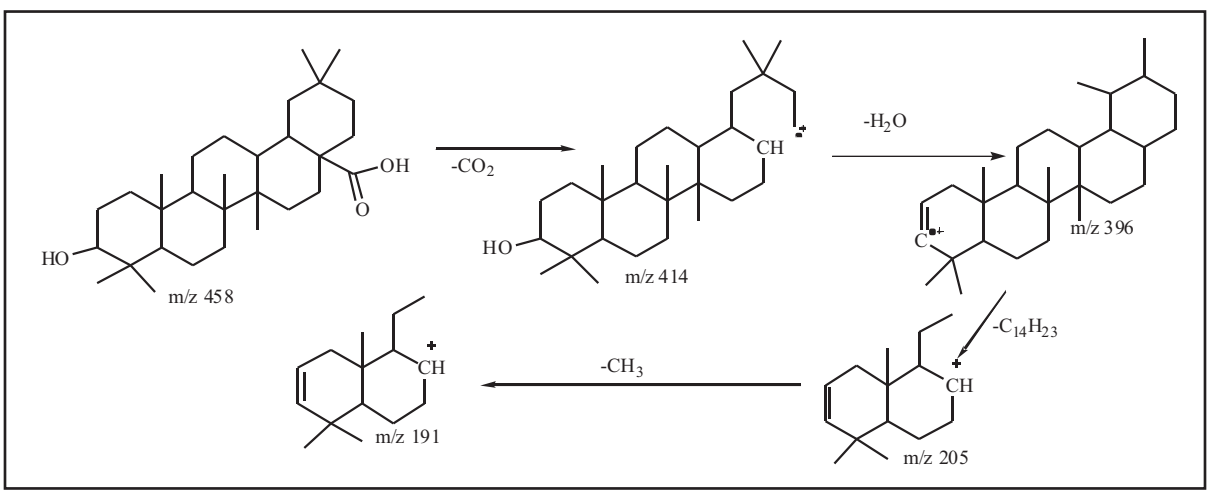
Pale yellow powder (161 mg); m. p. 270-272°C; IR $\upsilon_{max.}$ 3360 cm^{-1} (-OH), 2910 cm^{-1} (Aliphatic C-H), 1715 cm^{-1} (CO), 1656 cm^{-1}, 1465 cm^{-1}; HR-ESIMS (m/z): 558.73 $[M]^+$ (100%), 414.43 (22%), 396.93 (19%), 205.47 (20%), 191.07 (26%); NMR data see Table-1; Elemental analysis: (found C, 77.89; H, 10.76; O, 11.35%; calcd. For $C_{30}H_{48}O_3$: C, 78.81; H, 11.66; O, 11.23%).

It was obtained pale yellow powder (161 mg); m. p. 270-272°C and molecular formula $C_{30}H_{48}O_3$ proposed from its molecular ion at m/z 458 73 [M+H+H] in MS (positive mode) and elemental analysis. Compound SS-6 was given the positive test with Salkowski and Liebermann-Burchardt reagents which is characteristic for triterpenoids. The band at 1656, 1715 and 3360 cm^{-1} exhibited by IR spectrum were characteristic for olefinic, carboxylic and hydroxyl functional groups



Figure– Chemical structure of compound-1

respectively. The ^{13}C NMR spectra showed 30 carbon signals including a carboxylic (δ 18.5, C-28) and an oxygenated substituted carbon (δ 78.3, C-3). In mass spectrum, the fragments at m/z 205



Proposed mass fragmentation of compound-1

and 191 were characteristic for triterpenoids. All the spectral data were consistent with the reported data of 3β-hydroxyurs-12-en-28-oic acid. (Simone et al., 2008).

Table-1 ^{13}C anδ ^{1}H (300 MHz) NMR δata of compounδ in MeOD

Position	^{δ}C ppm	^{δ}H ppm (J in Hz)
1	38.6	0.91 (m)
2	27.6	1.33 (m)
3	78.3	3.14 (m)
4	38.8	
5	54.6	0.95 (m)
6	19.2	1.54 (m)
7	34.3	1.46 (m)
8	40.2	
9	47.6	1.36 (m)
10	37.9	
11	23.1	1.95 (m)
12	124.8	5.21 (br., s)
13	138.6	
14	41.9	
15	29.5	2.8 (m)
16	25.3	2.82 (m)
17	47.1	
18	54.3	2.20 (d, J=11.3)
19	39.3	1.82 (m)
20	39.8	1.78 (m)
21	31.2	1.33 (m)
22	38.0	1.15 (m)
23	28.0	110 (s)
24	18.3	0.93 (s)
25	16.5	0.76 (s)
26	17.4	0.95 (s)
27	24.0	0.96 (s)
28	180.5	
29	17.8	0.90 (s)
30	21.4	0.97 (s)

Compounδ -2

Shiny white crystals (60 mg); m.p. 66-68 °C; IR $υ_{max}$:2917, 1734, 1180, 961 cm^{-1}; EIMS (m/z): 270 (calcd.), 274 $[M+4H]^+$ (60%), 242 (45%), 185 (100%), 102 (15%); see NMR data: Table-2; Elemental analysis: (found C, 75.55; H, 12.59; O, 11.85%; calcd. For $C_{17}H_{34}O_2$: C, 75.81; H, 12.06; O, 11.63%).

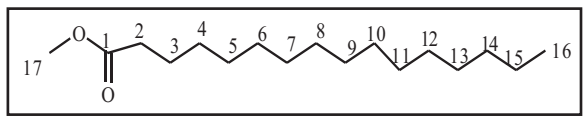
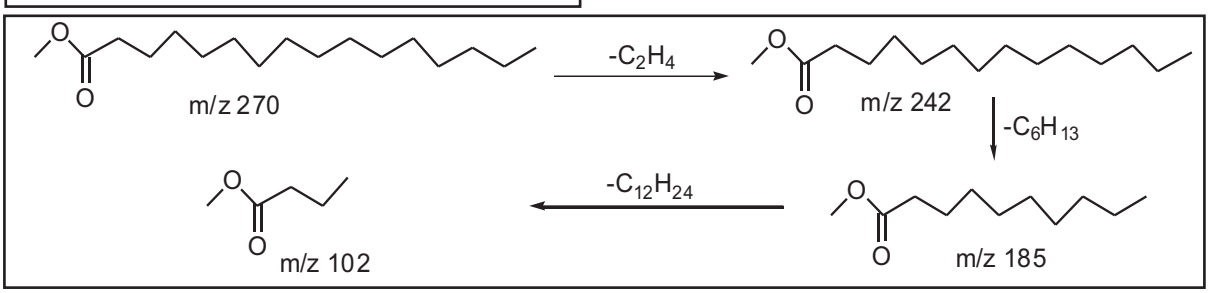


Figure – Chemical structure of compounδ -2

It was obtained as shiny white crystals (60 mg); m.p. 66-68 °C and molecular formula $C_{17}H_{34}O_2$ was proposed on the basis of its quasi-molecular ion at m/z 274.2720 $[M+4H]^+$ in HR-ESIMS (positive mode, calcd. 270) and elemental analysis. The IR spectrum exhibited bands at 2917 cm^{-1} characteristic of aliphatic C-H streching and a band at 1734 cm^{-1} was clearly expressive for the stretching in a carbonyl group. The ^{13}C and DEPT^{90} NMR spectra of compound showed 17 carbon signals including two methyl, fourteen methylene and one quaternary carbon. The ^{13}C NMR spectrum showed a highly downfield singlet at δ 174.04 indicating the presence of a carbonyl group whereas the signals appeared at δ 14.13 and 64.41 were due to the presence of one methyl and one methoxy groups. The sharp peak at δ 4.0 is due to the ester methyl located next to the carbonyl carbon and the triplets around δ 0.86 are from the terminal alkyl methyl in each of the methyl esters.



Proposeδ mass fragmentation of compounδ - 2

Table-2 ^{13}C, ^{1}H anδ DEPT (300 MHz) NMR δata of compounδ in $CDCl_3$

Position	$^{\delta}C$ ppm	$^{\delta}H$ ppm	DEPT
1	174.04		
2	34.44	2.2 (t)	CH2
3	25.05	1.6 (m)	CH2
4	25.96	1.28 (m)	CH2
5	28.67		CH2
6	29.18		CH2
7	19.27		CH2
8	29.29		CH2
9	29.50		CH2
10	29.55		CH2
11	29.60		CH2
12	29.63		CH2
13	29.67		CH2
14	31.94		CH2
15	22.71		CH2
16	14.13	0.86 (m)	CH3
17	64.41	4.0 (s)	OCH3

The methylene alpha to the ester group is at δ 2.2 and the beta group is at δ 1.6. The remaining CH_2 group protons have similar resonance frequencies and overlap in the range of δ 1.2–1.4. The total intensity in this region is the sum of the individual contributions from the remaining CH_2 groups in the molecule. This all spectral data (^{13}C, ^{1}H and DEPT[90]) are matched with reported data of methyl palmitate. Methyl palmitate previously reported from this genus by GCMS analysis of *U. dioca* (Iordache et al., 2009). Moreover, except carbonyl, there were no other multiple bonds present in compound (confirmed by IR and NMR spectra). Thus, it is clear that this multiple bond present in the form of ester. The mass spectrum of compound was found very informative in support of the structure confirmation. It showed a molecular ion peak at m/z 274.2720 (60%), which loses C_2H_4 to furnish an ion at 242.2815 (45%) whereas the important ions at 185.1139 (100%) and 102.1259 were due to the loss of C_6H_{13} and $C_{12}H_{24}$ moiety. Hence, the structure of compound was elucidated as methyl palmitate.

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Influence Of Foliar Extract of *Brassica juncea* On Pathogenesis Of Alternaria Blight (*Alternaria brassicae*)

Pramila[1,2]

1.Molecular Biology and Genetic Engineering, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar 263145, Dist. US Nagar (Uttarakhand), India.

2.Department of MLT & MM Uttaranchal College of Biomedical sciences & Hospital, Sewla Khurd, Transport Nagar, Dehradun (Uttarakhand), India 248001.

E-mail: pramila.unsiyal@gmail.com

Abstract - Rapeseed mustard production is widely effected by rapeseed-mustard disease Alternaria blight. Ten isolates of *Alternaria brassicae* were selected for the study of foliar extract on growth sporulation and pathogenecity. *Alternaria brassicae* isolates show higher growth and sporulation on mustard extract. Higher growth and sporulation on Varuna extract in comparison to Divya extract. Isolates grown on the Divya extract showed higher virulence in comparison to the isolates grown on Varuna extract and PDA alone. Mustard extract functions like a growth and sporulation inducer. Divya extract enhances virulence of the isolates while Varuna extract reduces the virulence in *in vitro* culture.

Keyworδs - Sporulation, Virulence, *Alternaria brassicae*, Alternaria blight, PDA, foliar extract.

Introδuction

The family Brassicaceae comprises one most important oil seed crop, rape seed mustard. Rapeseed mustard production is widely affected by rapeseed-mustard disease i.e. Alternaria blight [*Alternaria brassicae* **(Berk.) Sacc.**], White rust [*Albugo candida* **(Pers.) Kuntze**] and white rot [*Sclerotinia sclerotiorum* **(Lib.) δe Berry**] downy mildew complex and Powdery mildew. *Alternaria brassicae* having asexual life cycle and reproduces by asexual spores. The asexual spore is known as conidia.

Different individual show variability in morphological and physiological traits indicating existence of different pathotypes. Similar reports have been made in case of *Alternaria brassicae* which show morphological cultural pathogenic and molecular variability **(Awasthi anδ Kotle *1989,* Vishwanath *et al 1997,* Khan *et al* 2007, Pramila *et al 2014*).** Severity of disease Alternaria blight changes according to season, region and crop **(Chattopaδhyay *et al* 2005)**. Variability among the isolates of *Alternaria brassicae* grown on the different media also have been reported recently **(Sharma *et al* 2013).**

This situation requires the simultaneous study of pathogen variability with effect of different host plant media in relation to control which have no plant part. Difference in severity of disease in different host plant is due to their resistance and susceptibility toward pathogen while pathogens shows variability in different host **(Pramila *et al* 2014)**. Effect of different plant media on isolates of *A.brassicae* has been reported from different other state of India but not from Uttarakhand. Since the effect of different host media in relation to severity of disease or virulence of pathogens has not been studied. Present study showing such effect on the isolates of Uttarakhand.

Material anδ Methoδs

Collection of *A. brassicae* isolates

Plant material infected with *A. brassicae* was sampled randomly from different cultivars of *B. juncea* grown in the field of Crop Research Centre of G. B. Pant University of Agriculture and Technology, Pantnagar Uttarakhand, India. The isolates of *A. brassicae* were collected and designated as BJABI stands for *Brassica juncea*

Alternaria brassicae isolates (Table-1). These selected infected spots were washed 3 to 4 times in sterilized distilled water and then surface sterilized by dipping in 4% NaOCl solution for 1 min, followed by washing with sterilized water 3 to 4 times. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm petri dishes containing potato dextrose agar (PDA) and incubated at 25±2°C for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another Petri plate containing PDA medium, where it was grown for 15 days at 23±2°C in the BOD incubator. On the basis of their conidiophore and conidial morphology as described by Simmons (2007), the pathogen was identified as *A. brassicae* (Berk.) Sacc. and purified by single spore isolation method. The isolated fungal pathogen cultures were maintained on PDA slants at 4°C.

Table-1 *Alternaria brassicae* isolates infecting *Brassica* cultivars.

***A. brassicae* isolate**	**Host**	**Date of collection**	**Location**	**Latituδe anδ longituδe**	**Plant part**
BJABI-1 (VR)	*B. juncea* (Varuna)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-2 (DV)	*B. juncea* (Divya)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-3 (LM)	*B. juncea* (local mustard)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-4 (P7)	*B. juncea* (Pre 2007)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-5 (P10)	*B. juncea* (Pre 2010)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-6 (KR)	*B. juncea* (Kranti)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-7 (ND)	*B. juncea* (NDRE4)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-8 (JD)	*B. juncea* (JD-6)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-9 (KA)	*B. juncea* (Kanti)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf
BJABI-10 (AS)	*B. juncea* (Ashirwad)	5th January, 2011	Pantnagar, Uttarakhand	29°N, 79.3°E	Leaf

BJABI, *Brassica juncea Alternaria brassicae* isolates.

Preparation of supplementeδ meδia of leaf extract of *Brassica juncea* genotype Divya (most tolerant)/ Varuna (most susceptible)

The leaves were first washed under running tap water for 5 mts, then surface sterilized by washing with autoclaved distilled water for 5 mts. leaves were then washed with 70 percent ethanol for 1 mts again with autoclaved sterilized distilled water for 3 mts. Again washing was done with 0.1 % HgCl2 for 30 sec followed by 3 min washing with distilled water then dried by filter paper. Leaves were crushed into an autoclaved pestle and mortar and filtered by double muslin cloth. Thus 4 ml leaf extract was prepared from one gram leaf. The final concentration of PDA was maintained according 39gm/1 lit with extract. Media was prepared into 3 different concentration of leaf extract. 1 ml extract per

petriplate, 2 ml extract per petriplate and 3 ml extract per petriplate.

Inoculation of plate having meδia

Plates having media were inoculated with7 mm mycelia disk of fungal culture and incubated at 22^0C. These plates were observed after 10 and 15 days of incubation. Variability in growth was recorded by diameter measurement of colony in all the three concentration.

Different plates with different media were designed as follows :

Name of media	Name of leaf extract	Extract volume per plate
PDA without extract	-	- - -
PDA with leaf extract	Divya	1 ml 2 ml 3 ml
PDA with leaf extract	Varuna	1 ml 2 ml 3 ml

Ability/intensity of sporulation

Sporulation of different isolate was determined after 4 sub culturing and 10 days of incubation. 5 mycelia disc of 7 mm diameter size were cut from the equally distant and darkest part of the colony. Agar from the disc was removed. One such disc was placed per 1.5 ml eppendorf tube containing 200 µl suspension of autoclaved distilled water and lacto phenol in ratio of 9:1, with the help of sterilized needle. Thus we have five replicates of each isolate. The tube was vigorously shaken and disc was scraped by sterilized brush to get release of spores in the suspension. The suspension was used to determine the number of spore/ intensity of sporulation with the help of heamocytometer by following formula-

Number of spore/ml= average spore count per square X 104 X δilution factor

Infection

Infection was given to the healthy and 30 days old plant of *Brassica juncea* genotype Divya. Out of 10 isolates 7 were chosen to give infection. Infection was given in three parts for each isolate.

1. 10 plants (2 pots) were infected with suspension of those spore which grown on only PDA medium.
2. 10 plants (2 pots) were infected with suspension of those spores of the same isolate which were grown in PDA medium with Divya extract.
3. 10 plants (2 pots) were infected with suspension of those spores of the same isolate which were grown in PDA medium with Varuna extract.

Thus total 30 plants were infected for the study of virulence with control plants. Infection was given by spraying the spore suspension. Plants were placed in a moist surrounding in green house condition at 22-25^{0}C.

Recorδing anδ observation of Pathogenic changes

To confirm the changes in pathogenecity of isolates pathogenecity test was conducted in polyhouse condition. Observation for the disease development was taken at 5 days interval for 15 days. 10 leaves were randomly selected in each treatment. Average number and size of spot was counted per 10 cm^2 leaf area. Diameter of the spot was measured with the help of scale.

Average δisease inδex

Average disease index on leaf was taken at 5 days interval by use of the 0-5 rating scale as follow:

Table-2 Description of δisease scale (0-5)

Graδe Scale Description

0. No symptoms on the leaf.
1. 10-10 per cent leaf area infected and covered by spot no spot
2. 11-25 per cent leaf area infected and covered by spot
3. 26-50 per cent leaf area infected and covered by spot

4. `51-75 per cent leaf area infected and covered by spot

5- >75 per cent leaf area infected and covered by spot.

Disease index was calculated by use of following formula

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all individual ratings}}{\text{No. of leaves examined} \times \text{Maximum grade}} \times 100$$

Percent Disease Index (PDI) was worked out by using formula given by Wheeler (1969).

Results anδ Discussion

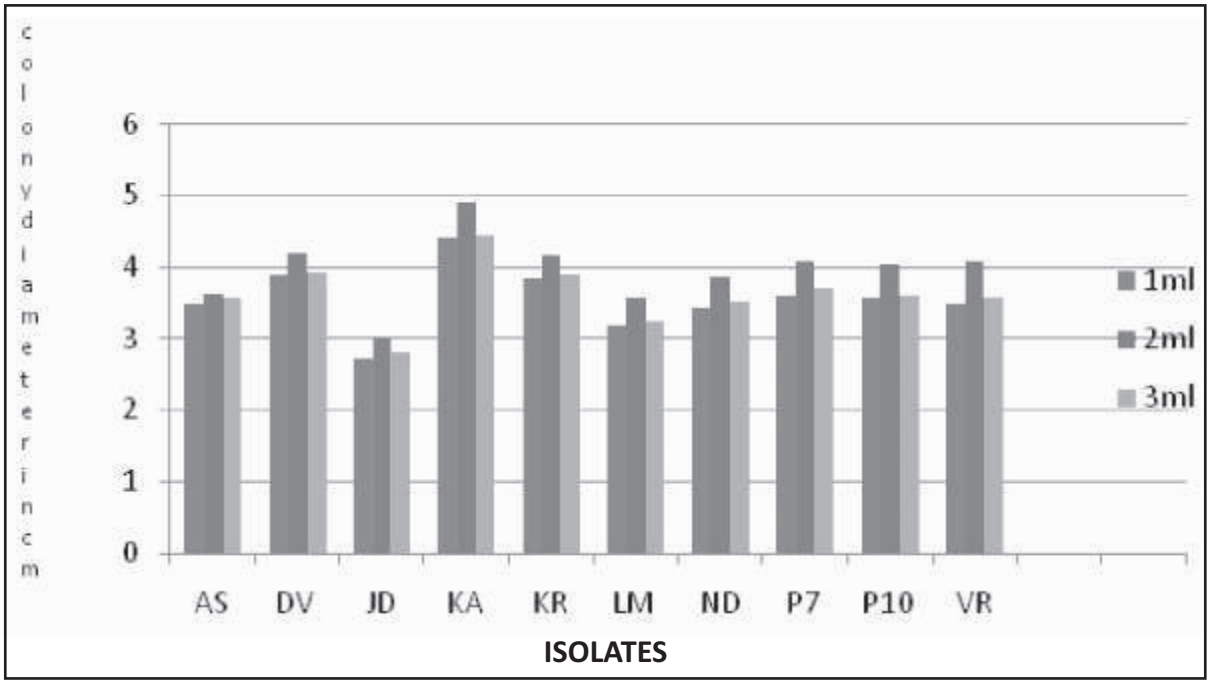
Colony size on δifferent meδium anδ δifferent concentration of plant extract

A. brassicae colony size was measured by the measurement of colony diameter. On the PDA medium KA (3.55 cm) showed highest colony size followed by DV, ND, VR, P7, AS, LM, KR and while JD (2.40 cm) showed lowest colony size. Presence of Divya extract as supplement increased the colony size in comparison to PDA alone. At the 1 ml concentration of Divya extract KA(4.42 cm) showed highest colony size followed by DV, KR, P7, P10, AS, VR,ND, LM, while JD (2.73 cm) showed lowest colony size. At the 2ml concentration of Divya extract again KA (4.90 cm) showed highest colony size followed by DV, KR, P7, P10, AS, VR,ND, LM, while JD (3.62 cm) showed lowest colony size. At 3 ml plant extract there was slight decrease in colony size. Here KA (4.45 cm) showed highest colony size followed by DV, KR, P7, P10, AS, VR, ND, LM, while JD (2.80 cm) showed lowest colony size.

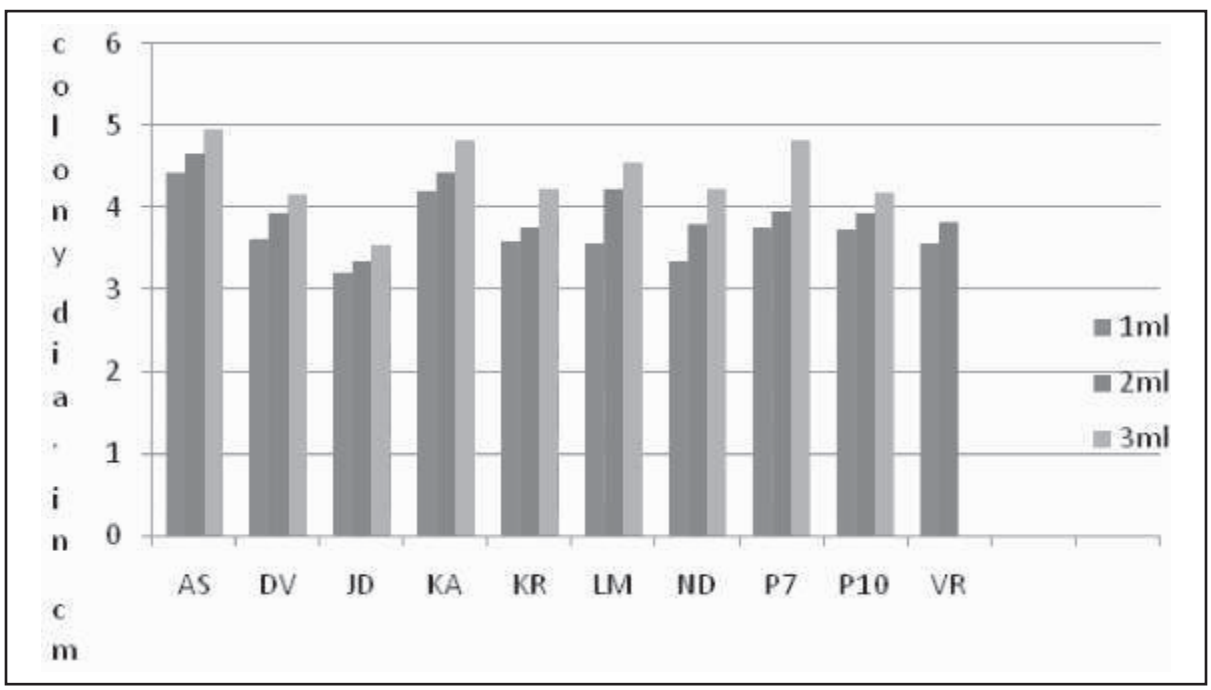
At the PDA medium supplemented with Varuna extract all the isolate showed higher growth than PDA and PDA medium supplemented with Divya extract. At the 1ml concentration AS (4.43 cm) showed highest colony size followed by KA (4.21 cm), P7, P10, DV, KR, LM, VR, while JD (3.20 cm) showed lowest colony size. At the 2ml concentration AS (4.67 cm) showed highest colony size followed by KA (4.43 cm), LM, P7, P10, DV, VR, ND, KR, while JD (3.33 cm) showed lowest colony size. At the 3ml concentration AS (4.95 cm) showed highest

Table-3 Comparative colony size of *Alternaria brassicae* isolates on δifferent PDA meδium anδ δifferent concentration of plant extract after 10 δays of incubation (colony δiameter in cm)

Isolate	PDA	PDA meδium supplementeδ with Divya extract				PDA meδium supplementeδ with Varuna extract		
		1ml	2ml	3ml		1ml	2ml	3ml
AS	3.07	3.49	3.63	3.57		4.43	4.67	4.95
DV	3.27	3.91	4.21	3.93		3.62	3.93	4.16
JD	2.40	2.73	3.02	2.80		3.20	3.33	3.55
KA	3.55	4.42	4.90	4.45		4.21	4.43	4.82
KR	2.82	3.85	4.18	3.90		3.58	3.74	4.23
LM	2.90	3.19	3.56	3.25		3.57	4.23	4.54
ND	3.27	3.45	3.87	3.51		3.34	3.79	4.22
P7	3.20	3.60	4.10	3.70		3.76	3.95	4.81
P10	2.80	3.57	4.05	3.60		3.72	3.94	4.19
VR	3.27	3.48	4.08	3.57		3.57	3.81	4.12



Colony δiameter of δifferent isolates on PDA supplimenteδ with Divya extract



Colony δiameter of δifferent isolates on PDA supplimenteδ with Varuna extract

colony size followed by KA, P7, LM,KR, ND, P10, VR, while JD (3.55cm) showed lowest colony size. From above observations this is concluded as plant extract increases colony growth.

Sporulation

At the PDA medium in the initial steps there was sporulation but after 4 subcultures there was no sporulation. This phenomenon was also observed by von Ramm C., Lucas G.B. (1963) that nonsporulating sectors develop often in culture even under optimum condition of sporulation. **Miller 1995, Shahin anδ Sheparδ 1979, Dalla** followed by KA, LM, DV, KR, AS, VR, P10, ND and JD showed the lowest number of conidia per ml When 2 ml of Divya extract was supplemented, with same incubation period isolate P7 (20) showed highest average number of conidia per ml followed by KA (18), DV, LM, P10, AS, KR, VR, while ND and JD showed the lowest number of conidia per ml. At the 3 ml concentration none of the isolates showed sporulation except P7. Reason for the decrease in sporulation with increase in extract concentration might be increase in the tolerant (T) factor of Divya plant which was less effective at lower concentration.

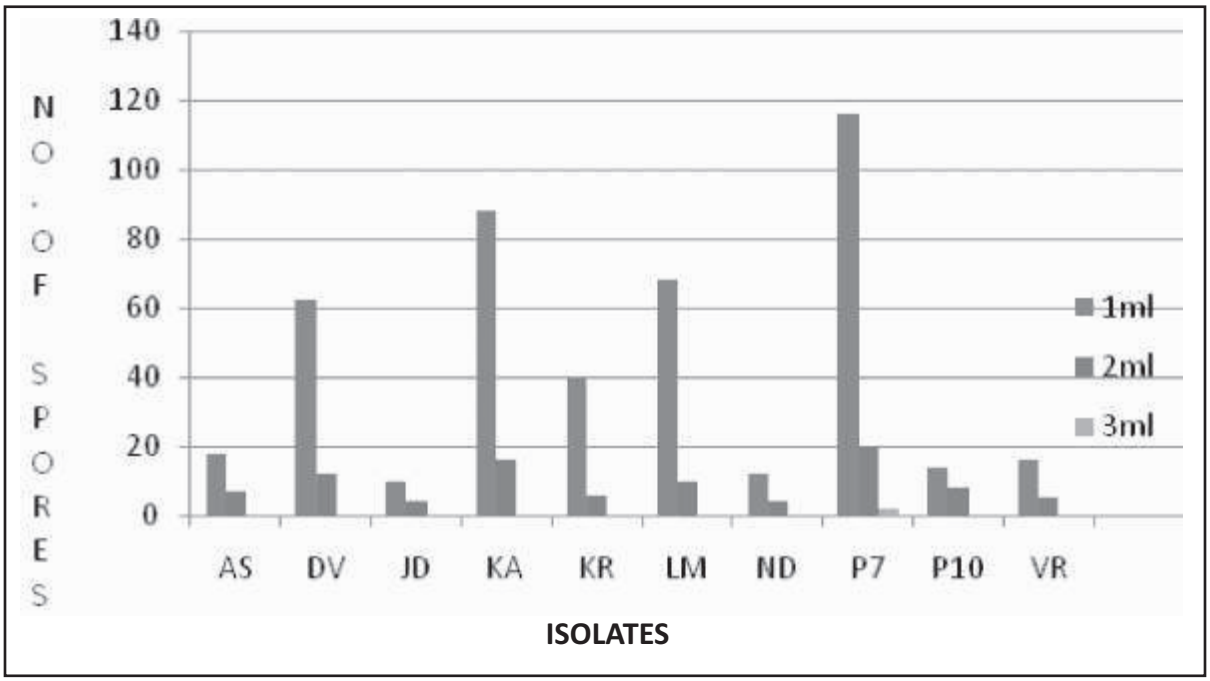
Table-4 Average number of coniδia formeδ after 10 δays of incubation at 22^{0}C in respect of *Alternaria brassicae* isolates on PDA meδium supplementeδ with δifferent concentration of plant extract (average number of coniδia per ml).

isolate	PDA	PDA+ Divya extract				PDA + Varuna extract		
		1ml	2ml	3ml		1ml	2ml	3ml
AS	-	18	7	-		26	82	108
DV	-	62	12	-		12	38	44
JD	-	10	4	-		26	36	44
KA	-	88	16	-		54	84	114
KR	-	40	6	-		16	18	20
LM	-	68	10	-		10	24	36
ND	-	12	4	-		26	30	80
P7	-	116	20	2		74	112	146
P10	-	14	8	-		2	2	_
VR	-	16	5	-		20	22	36

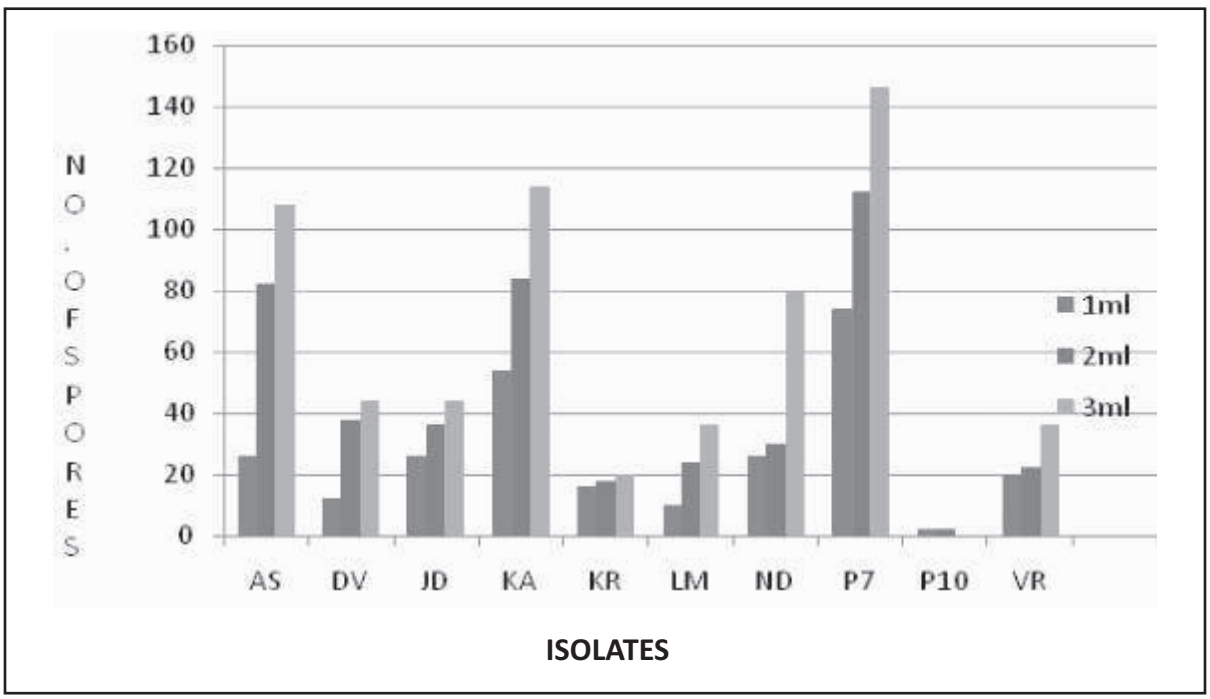
Pria et al, 1997; Avilla et al 2000, and **Vieira 2004** also reported that V8 juice medium, PDA, and media with plant parts or extracts of plants are used in protocol to induce sporulation of Alternaria species.

Isolates showed sporulation with Divya extract but the sporulation intensity of the isolates differ in this medium according to concentration. When 1 ml Divya extract was supplemented and after 10 days of incubation, number of conidia was counted. It was observed that P7 (116) showed highest average number of conidia per ml

When Varuna extract was supplemented with different concentration (1ml to 3ml) it was observed that as the concentration of extract increased, there is corresponding increase in number of conidia per ml, in all the isolates. This result confirmed that Varuna is considered as the susceptible variety of the Brassica juncea which means pathogen A.brassicae can easily survive and reproduces on this plant. When PDA media supplied by the Varuna extract isolates showed increment in sporulation with increase in the plant extract concentration.



Sporulation on PDA supplementeδ with Divya extract



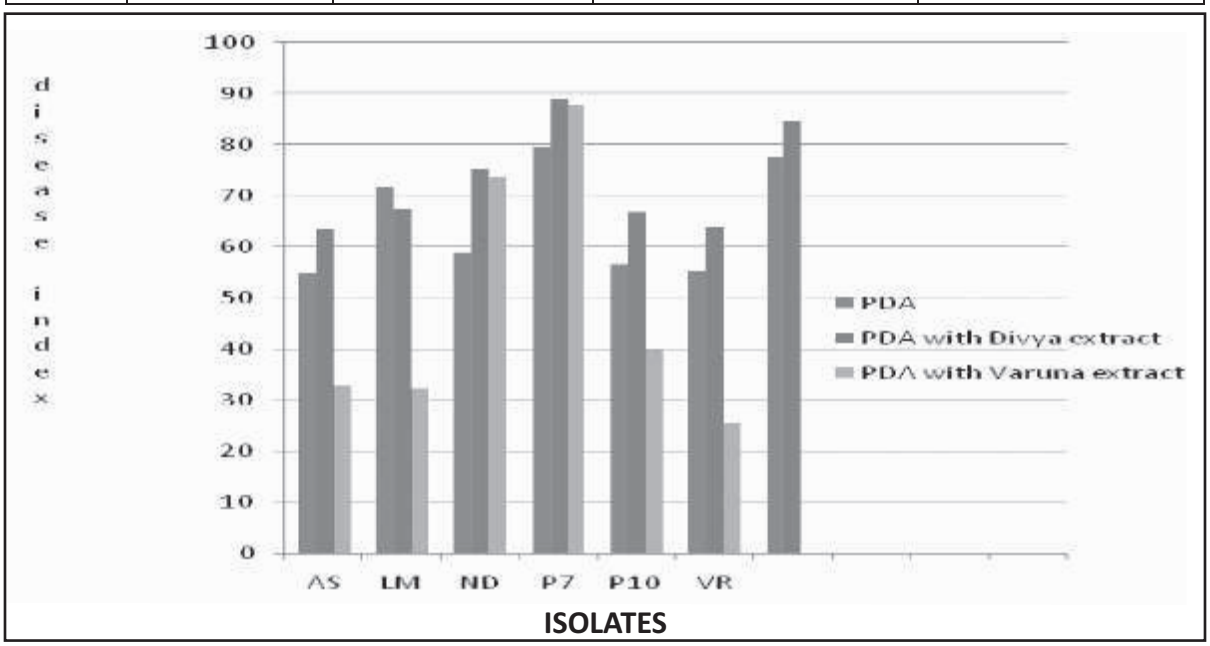
Sporulation on PDA supplementeδ with Varuna extract

Stuδy of virulence

Out of 10 isolates 7 isolates i.e. AS, KA, LM, ND, P7, P10, and VR were selected for the virulence test. Spore suspension of 2x103 spores/ml concentration was prepared. After infection Percentage disease index on leaf was recorded at 15 DAI (days after infection) in glass house. Spores of all isolate show highest disease index in PDA supplemented with Divya extract except KA which shows highest disease index in only PDA. While most of the isolates show higher disease index in PDA medium only in comparison to PDA supplemented with Varuna extract except LM and ND which shows higher disease index in PDA supplemented with Varuna extract.

Table-5 Determination of δisease inδex of plant infecteδ by *Alternaria brassicae* isolates Spore grown on δifferent meδia after 15 δays of infection

Isolate	Disease inδex after 15 δays			
	control	Spores grown on only PDA	Spores grown on PDA supplementeδ with Divya extract	Spores grown on supplementeδ with Varuna extract
AS	No infection	54.87	63.33	36.70
KA	No infection	71.56	67.54	32.23
LM	No infection	58.85	75.22	73.64
ND	No infection	79.59	88.94	87.77
P7	No infection	56.64	66.82	39.66
P10	No infection	55.27	63.76	25.45
VR	No infection	77.45	84.53	No infection



Isolate VR from Varuna extract did not show infection on Divya plant. Possible reason might be adaptability of isolate VR when it was grown on Varuna extract. So it is virulent for the susceptible variety only and could not overcome the defense system of Divya plant and hence could not develop disease. In the Divya extract isolates adopted itself to survive in a tolerant environment. Due to adaptability in resistant environment isolates show higher virulence on the same resiatant palnt Divya. In Varuna extract isolates adopted to the susceptible environment and could not develop disease with equal intensity in resistant environment. **Glavov anδ Mayama (2004**) studied variability in *A. alternate* tobacco pathotypes and conclude that some fungi belonging to genus *Alternaria* are plant parasite that easily can change when exposed under different condition.

Acknowleδgement

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Evaluation Of Phytochemical, Antioxiδant Anδ Antibacterial Activities Of *Coriandrum Sativum And Asparagus Racemosus* Against Uti Bacteria

Tanika Thakur, P.K.Chauhan anδ I.P Panδey*
School of Bioengineering & Food Technology, Shoolini University, Solan, HP, India
*Prof. Emeritus in Chemistry

Abstract - The present study was carried out to evaluate the phytochemical, antioxidant and antibacterial activities of methanolic extracts of *Coriandrum sativum* and *Asparagus racemosus* against the bacteria isolated from different UTI patients. From different biochemical tests three bacterial species i.e. *E. coli, P. mirabilis*, and *Staphylococcus auerus* have been isolated. Phytochemical screening of the *C. sativum* and *A. racemosus* showed the confirmation of saponins, alkaloids, tannins, reducing sugars, glycosides, flavonoids, and terpenoids. Antioxidant activity of *C. sativum* and *A. racemosus* extracts were assessed by using DPPH method; the antioxidant activity increased in a concentration dependent manner. *A. racemosus* and *C. sativum* showed maximum %age inhibition of 44% and 42% respectively. Antibacterial activity of plant extracts was carried out using discs diffusion method against recovered isolates. The methanolic extract of *C. sativum* showed maximum zone of inhibition against *Proteus mirabilis* (19mm), *E. coli* (17mm) and *Staphylococcus aureus* (16mm) whereas *A. racemosus* showed the maximum zone of inhibition against *Staphylococcus aureus* (20mm), *E. coli* (18mm) and *proteus mirabilis* (16mm). Both plant extracts have high medicinal value. These results suggest that traditional folk medicine could be used as a guide to our continuing search for new natural products with potential medicinal properties.

Keyworδs: *Coriandrum sativum, Asparagus racemosus, Escherichia coli, Proteus mirabilis*, Urinary tract infection (UTI).

Introδuction

UTI is an infection that affects the part of urinary tract. The main causal agent of UTI is *Escherichia coli* however other bacteria, viruses or fungi may rarely be the cause (Amdekar *et al*., 2011). Symptoms of UTI include the urge to urinate frequently which may reoccur immediately after the bladder is emptied, a painful burning sensation when urinating, discomfort or pressure in the lower abdomen. The urine often has a strong smell, looks cloudy or may be contain blood. Risk factors include female anatomy, sexual intercourse and family history. *E. coli* is the cause of 80-85% of urinary tract infection with *Staphylococcus saprophyticus* begins the cause in 5-10% (Nicolle, 2008). Other bacterial causes include: *Klebsiella, proteus, pseudomonas and enterobactor*. Women are more prone to UTIs than men because in female the urethra is much shorter and closer to the anus (Nicolle, 2008). Medicinal plants are known to have their therapeutic potential for treatment of several ailments of humans. In Ayureveda and Unani system of medicine, a large number of Indian medicinal plants are used to treat infectious diseases (Padmini *et al*., 2010). According to World Health Organization (WHO), about 80% of world population use medicinal plants to treat human diseases (Serrentino, 1991). The forests of Himachal Pradesh said to have been the birthplace of Ayurveda are known to supply a very large proportion of the medicinal plants requirements of India with one estimates quoting figures as high as 80% of all Ayurvedic drugs 46% of all unani drugs and 33% of all allopathic drugs developed in India. Medicinal plants are known to contain several compounds with antimicrobial properties

and the uses of these types of compounds are being increasingly reported from different parts of world (Saxena and Sharma, 2002). India is the biggest producer, consumer and exporter of coriander in the world with an annual production of around three lakh tonnes. It contains an essential oil (0.03 to 2.6%) (Nadeem *et al*., 2013). All parts of this herb are in use as flavoring agent and/or as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations. Coriander has been reported to posses many pharmacological activities like antioxidant, anti-diabetic, anti-mutagenic (Cortes *et a*l., 2004), antilipidemic (Sunil *et al*., 2012), anti-spasmodic (Alison *et al*., 1999). *Asparagus racemosus* is highly effective in problems related with female reproductive system. Charak Samhita written by Charak and Ashtang Hridyam written by Vagbhata, the two main texts on Ayurvedic medicines, lists *Asparagus racemosus* as part of the formulas to treat disorders affecting women's health (Sharma *et al*., 2003). Roots of *Asparagus racemosus* can be used against nervous disorders, dyspepsia, diarrhea, dysentery, inflammations, neuropathy, hyperdipsia, antioxidant, antitussive and certain infectous diseases (Goyal *et al*., 2003).

Material anδ Methoδs

Collection of plant material: The plant materials used were the dried leaves of *Asparagus racemouse* which were collected from forest region of Bajhol (Solan) Himachal Pradesh. The seeds of *Coriandrum sativum* used were purchased from market (Solan) Himachal Pradesh.

Extraction of plant materials

They were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were dried in sunlight then made into fine powder. 10g Powder of both plants were mixed in 100ml of methanol and incubated for 48-72 hours at 40◦C in shaker. Filter the mixture after the incubation. Methanolic mixture was dried on water bath for 4 hours to make fine paste for further phytochemical, antioxidant and antibacterial investigations.

Isolation anδ characterization of microorganisms

A total of 100 samples of urine were collected from different hospitals of different locations of Solan, HP. The samples were inoculated in nutrient broth for 24 hrs for enrichment. 1ml of each sample was added to 9 ml of nutrient broth for sufficient enrichment and incubated at 370 C. The enriched urine samples were streaked on selective media bismuth sulfite agar (BSA) with the help of calibrated loop and incubated at 37^0C for 24 hrs for recovery of isolates. Morphological characteristics and biochemical reactions of recovered isolates were studied. The biochemical tests carried out were urease, citrate utilization, catalase, indole, hydrogen sulphide production, methyl red voges-proskaeur (MRVP) sugar fermentation tests.

Phytochemicals analysis of *C. sativum* anδ *A. racemosus* plant extarcts

The plant extracts were screened for the presence of reducing sugars, alkaloids, saponnin, tannins, flavonoids, anthraquinones, phlobatannin, steroids, terpenoids and cardiac glycosides (Ayoola *et al*., 2012).

Antioxiδant activity assay of *C. sativum and A. racemosus* plant extracts

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging assay was done by the procedure of Sutharsingh *et al*., 2011

Antibacterial activity of *C. sativum and A. racemosus* plant extracts

Disc Diffusion Methoδ

In this method 0.1ml of inoculums of test organism was spread on Mueller Hinton Agar media. A single filter paper disc placed gentely with the help of forceps. Add desired amount of methanolic extracts (20µl) from each plant extracts 100mg/µl. Then incubated the plates for 24 hrs at 37°C & observed the plates for zone of inhibition in mm by zone measuring scale.

Results

In this study total 10 Isolates were recovered from urine sample of UTI patients. The isolates were characterized and identified on the basis of morphological characteristics such as gram staining reaction, colony characteristics and biochemical test. Different bacterial isolates were confirmed by staining and biochemical tests (Table-1) and by growing them on their selective media. Based on cultural, morphological and biochemical characteristics of the organisms isolated, total three bacteria were identified from different samples.

Phytochemical screening of *C. sativum* anδ *A. racemosus*

The phytochemical screening of the plants studied showed the presence of flavonoids, reducing sugar, sponinis, alkaloids, terpenoids, tannins and glycosides. *C. sativum* and *A. racemosus* showed the presence of flavonoids, reducing sugars, sponnins, alkaloids, terpenoids, gilcosides, and tannins. Phytochemical analysis of plant extacts showed in the table-2.

Antioxiδant activity of methanolic extracts of *C. sativum and A. racemosus*

DPPH radical scavenging assay

The radical scavenging activities of *plants* extracts/fractions showed that the polar fractions scavenged the DPPH radicals more significantly. The scavenging of radicals ***increased with increasing concentrations of the extract/fractions which showed similar amount***

Table - 1 Biochemical characterization

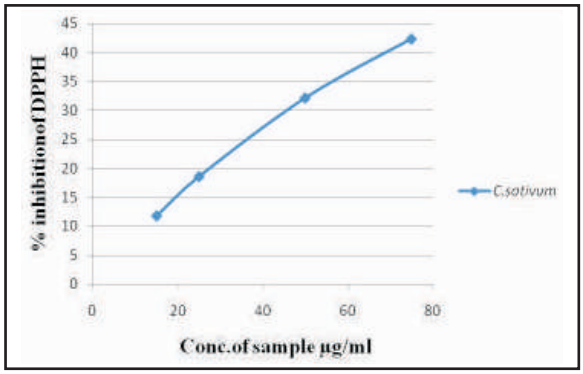
Sample No.	Inδole	MR	VP	Citrate	Catalase	Organism Iδentifieδ
S1	+ve	+ve	-ve	-ve	+ve	*E. coli*
S2	-ve	+ve	-ve	+ve	+ve	*Proteus mirabilis.*
S3	-ve	+ve	-ve	-ve	+ve	*Staphylococcus aureus*

Table – 2 Phytochemical analysis of plant extracts (+ve = present, -ve = absent)

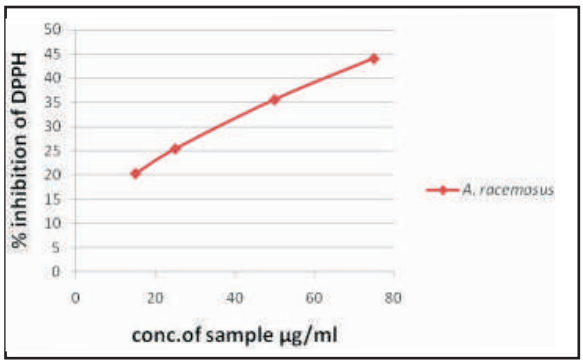
Sr. No.	Phytochemical	*C. sativum*	*A. racemosus*
1.	Alkaloids	+ve	+ve
2.	Tannins	+ve	+ve
3.	Reducing sugars	+ve	+ve
4.	Terpenoids	+ve	+ve
5.	Flavonoids	+ve	+ve
6.	Sponins	+ve	+ve
7.	Cardiac Glycosides	+ve	+ve

Table - 3 DPPH raδical scavenging activity of *C. sativum* anδ *A. racemosus*

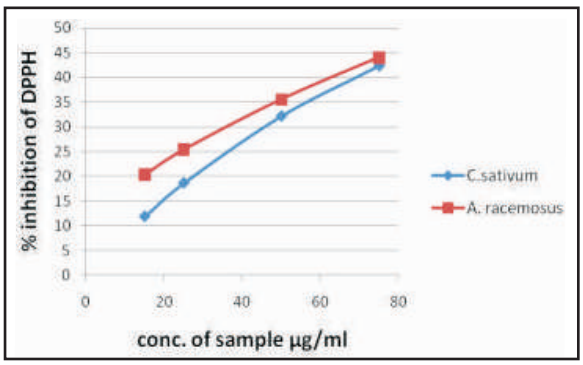
Conc. / Plants	[illegible]	25µg/ml	50µg/ml	75µg/ml	IC_{50}
Coriandrum sativum	[illegible]	18.64±0.001	32.20±0.0005	44.8±0.0011	88.16
Asparagus racemosus	20.33±0.0015	25.42±0.0015	35.59±0.0015	44.06±0.0015	88.90



Graph-1 DPPH raδical scavenging of *C. sativum*



Graph-2 DPPH raδical scavenging activity of *A. racemosus*



Graph-2 DPPH raδical scavenging activity of *A. racemosus*

of percent radical scavenging afterwards as showed in the table no.-3 and graph no. 1 and 2.

In the present study both the extracts had significant scavenging effect on the DPPH radical which was generally significantly increasing with the increase in the concentration from 15-75 µg/ml.

The following graph 3 shows the comparative dose-response curve of DPPH radical scavenging activity of the *C. sativum* and *A. racemosus*. At a concentration of 75µg/ml the scavenging activity of methanolic extract of *C. Sativum* was 42% while at the same concentration the scavenging activity of *A. racemosus* was 44%.

Antibacterial activity of *C. sativum* anδ *A. racemosus* plant extracts

Antibacterial activity of *C. sativum*

The antibacterial activity of *C. sativum* was evaluated by the discs diffusion method along with negative control. The antibacterial activity was assessed against bacterial strains at concentration 100mg/ml of plant extract. The methanolic extract of *C. sativum* showed antibacterial activity within the range of 16-19mm against *Proteus mirabilis* (19mm), *E. coli* (17mm) and *Staphylococcus aureus* (16mm).

Antibacterial activity of *A. racemosus*

The antibacterial activity of *A. racemosus* was evaluated by the filter paper discs diffusion method along with negative control. The antibacterial activity was assessed against bacterial strains at concentration 100mg/ml of plant extract. The methanolic extract of *A. racemosus* showed antibacterial activity within

Table - 4 Antibacterial activity of plant extracts

Sr. Noˑ	Recovereδ isolates	*Coriandrum sativum*	*Asparagus racemosus*
1.	*E. coli*	17mm	18mm
2.	*Stapphylococcus aureus*	16mm	20mm
3.	*Proteus mirabilis*	19mm	16mm

the range of 16-20mm against *Staphylococcus aureus* (20mm) *E. coli* (18mm) and *Proteus mirabilis* (16mm).

Discussion

In the present, study, the phytochemical screening of the *Coriandrum sativumn* and *Asparagus racemosus* showed the presence of flavonoids, reducing sugar, sponinis, alkaloids, terpenoids, tannins and glycosides. Chaudhary *et al*., (2014) observed the phytochemical screening of methanol and acetone extracts of *Coriandrum sativum* found the presence of some promising alkaloids, glycosides, flavonoids and amino acids. And Pathak *et al.,* (2011) also observed the presence of carbohydrate reducing sugar, terpenoids, protein and volatile oil in methanolic extracts of *Coriander sativum*. In the previous study the phytochemical screening of the *Asparagus racemosus* extracts revealed the presence of flavanoids, tannins, alkaloids, saponins and phenolic compounds. And according to Selvamohan *et al*., (2010) the phytochemical analysis showed the presence of tannins, glycosides, flavonoids, reducing sugars, anthraquinones in *A. racemosus* of the plant extracts. In the present study the scavenging activity of methanolic extract of *C. sativum* was 42% and *A. racemosus* was 44%. Wangensteen *et al*., (2004) observed that the antiradical activity of extracts of coriander for the DPPH radical inhibition was only 15% and Karmakar *et al*., (2012) also observed the antioxidant activity of ethanol extract of *Asparagus racemosus* on the basis of the scavenging activity of the stable DPPH free radical and 10% H_2SO_4.

In the present study the methanolic extract *C. sativum* showed antibacterial activity within the range of 16-19 mm against Proteus mirabilis (19mm), *E. coli* (17mm) and *Staphylococcus aureus* (16mm) and the methanolic extract of *A. racemosus* showed antibacterial activity within the range of 16-20mm against *Staphylococcus aureus* (20mm), *E. coli* (18mm), and *Proteus mirabilis* (16mm). Coriander is also reported to possess potent antimicrobial activity, thereby proving its efficacy as a medicinal herb, which is in line with other researches confirming medicinal effects of this plant. Patel & Patel (2013) observed that the methanolic extract of *A. racemosus* has shown average antibacterial activity against *E. coli* (16mm) and *Staphylococcus aureus* (13mm). And Rajendra *et al*., (2012) also studied the methanolic extract of *A. recemosus* for the antibacterial activity against isolated human pathogens and reported the zone of inhibition ranging from 3-15 mm. These results suggest that both the plant extracts have high value of medicinal effect including phytochemical, antioxidant and antibacterial activity.

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Comparative Stuδy On Antimicrobial Activities Of *Cordia Dichotoma* anδ *Sapium Sebiferum*

Suman Rawat[1]*, O.P.Sati[2], Ankita Sati[3] anδ Arti Tomar[4]

[1,3,4]Department of Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand (India)

[2]Department of Chemistry, H.N.B. Garhwal Central University, Uttarakhand (India).

*E-mail: sumanman.2008@rediffmail.com

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

Keyworδs: *Cordia dichotoma, Sapium sebiferum*, Antimicrobial activity, Phytochemical Studies

Introδuction

Cordia dichotoma forst. is a plant belonging to family *Boraginaceae*[1]**.** It is a tree of about 15 metres high, found spanning from north India and south China to Australia and Polynesia. **Leaves** are simple, alternate and entire to slightly lobed. The fruits of the plant are used as cooling, astringent, emollient, expectorant, anthelmintic, purgative and diuretic[2]. A number of pharmacological properties such as analgesic, anti-inflammatory and hepato-protective have been reported. *Cordia dichotoma* reduce the blood glucose level when compared to diabetic control group and exerts a significant hypoglycemic and antidiabetic activity[3]. Leaves used in Ulcers and in headache[4].

Sapium sebiferum roxb. *is* a plant belonging to family ***Euphorbiaceae.***[5] It is monoeciouces, deciduous small tree upto 13 m tall, stem often gnarled bark whitish grey with vertical cracks containing white latex. Leaves are alternate, broad rhombic to ovate in shape 3.8-8.5 cm long and have smooth edges, heart shaped. Fruits are three-lobed, three-valved capsules.[6] Its wood has been used to make various implements, toys, furnitures inferiors quality pencils, cricket bats and Chinese printing blocks.[7] In Chinese medicine, its oil is used as purgative and emetic. Resin from root bark is considered as purgative. The seed is antidote, emetic and purgative.[8]

Material anδ Methoδs

Collection of leaves of *Cordia dichotoma and Sapium sebiferum*

Leaves of *Cordia dichotoma* and *Sapium sebiferum* were collected from locality of Kachchi Garhi, Distt. Shamli (U.P.) and FRI, Dehradun respectively. Plant material was authenticated by **S. K. Srivastava** (Scientist D/HOD), Botanical Survey of India, Northern regional centre, Dehradun (BSI).

Extraction of leaves of *Cordia dichotoma and Sapium sebiferum* in δifferent solvents (Non-polar to Polar)

The collected plant material was washed with water to remove other undesirable material and then dried under shade. The air-dried leaves (200 gm) of *both* were crushed separately. The crushed leaves extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone, methanol by hot percolation

method using soxhlet apparatus. The extract was then evaporated till dryness to obtain residue. These using were concentrated under reduced pressure. The extract was used for antimicrobial activity.

Anti-microbial activity of δifferent extracts

The **anti-microbial activity** of the leaves of *Cordia dichotoma and Sapium sebiferum* was carried out. The leaves extracts were screened for anti bacterial and anti fungal activities.

Anti bacterial activity of leaves extract

In this study, the anti bacterial activity was studied against the micro organism and the bacterial cultures used in the study were: 1. *Escherichia coli,* 2. *Pseudomonas aeruginosa and 3. Bacillus cereus.*

These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37^0 c for about 18-24 hours and then stored at 4^0 c as stock for anti bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at 37^0c overnight. To test anti bacterial activity, the well diffusion method was used.

Culture meδia preparation

The microbiological media prepared as standard instruction provided by the HI-Media Laboratories, Mumbai. The media used for anti-bacterial activity Muller- Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121^0C at 15 psi for 15-30 minutes autoclave.

Plate preparations

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

Well δiffusion methoδ

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

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

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

Results

Table-1 Antibacterial activity of δifferent extracts of *Cordia dichotoma* anδ stanδarδ δrug Chloramphenicol, Streptomycin, Ampicillin.

S.No.	Test organism	Inhibition zone in mm					
		Pet.Ether	Chloroform	Methanol	Standard drug		
					Ampicilline	Streptomycin	Chloram-phenicol
1	E. coli	-	1mm	8.6mm	20mm	17mm	20mm
2	Bacillus cereus	-	2mm	24mm	15mm	16mm	18mm
3	Pseudomonas	-	11mm	5 mm	-	16mm	17mm

Table-2 Antibacterial activity of δifferent extracts of *Sapium Sebiferum Roxb* anδ stanδarδ δrug Chloramphenicol

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

Table-3 Antifungal activity of δifferent extract *Cordia dichotoma* anδ stanδarδ δrug Amphotericin-B anδ Clotrimazole.

S. No.	Test Organism	Inhibition zone in mm				
		Pet. Ether	Chloroform	Methanol	Standard drug	
					Amphotericin-B	Clotrimazole
1	*Aspergillus niger*	-	-	19mm	-	11mm
2	*Sclorotium*	-	4mm	6mm	-	_
3	*Candida- albicans*	-	-	24mm	-	12mm
4	*Rhizopus*	-	-	7mm	-	_

Table-4 Antifungal activity of δifferent extract *Sapium Sebiferum Roxb* anδ stanδarδ δrug Clotrimazole.

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

Discussion

The antimicrobial activity of leaves extracts of ***Cordia dichotoma*** was found active against E.coli, Bacillus cereus, Pseudomonas in chloroform and methanol extracts whereas leaves extracts of Pt. ether was found inactive against E.coli, B. cereus, Pseudomonas. The leaves extracts of methanol was found highly active against *Candida albicans and Aspergillus niger* while less active against *Rhizopus and Sclorotium.* The leaves extracts of Pt. ether did not find any activity. The results revealed that the **methanolic extract** has shown more degree of anti microbial activity than other extract when compared to the standard drug. It is due to presence of chemical constituents like carbohydrates, phenolic compounds, tannins, triterpenoids, saponins, terpenoids, proteins and aminoacids, this was confirmed by phytochemical studies. While in case of ***Sapium sebiferum*** the **acetonic extract** showed good antibacterial activity against B.cereus and B.pumilus and good anti-fungal activity against *T. flavurusclem* in comparison to the standard drug.

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A Stuδy on Extraction, Characterization anδ Moδification of Castor Seeδ Oil

Gaurav Rajput[2], I.P. Panδey[2], anδ Trivikram Rawat[2], anδ Gyanesh Joshi[3]

[1]Oil extraction, Environmental and Disaster Management Lab.,
Department of Chemistry, DAV (PG) College, Dehradun, Uttarakhand.
[2]4321 Foxfire Trl Portage, Ml 49024 USA.
[3]Cellulose and Paper Division, Forest Research Institute, Dehradun, Uttarakhand.
Corresponding author Email Id: ippande@gmail.

Abstract- The paper presents the complete study through extraction and characterization of both crude and refined castor oil with normal hexane. Degumming, neutralization and bleaching process using activated clay were used for the refinement of the oil produced. Iodine value for crude and refined castor oil produced, acid value, saponification value, specific gravity, refractive index was determined. The results were within the ASTM standard specifications. The iodine value obtained (83.70) for the refined oil indicates the utility of the oil as lubricant, hydraulic brake fluid and protecting coatings. The oil was modified via sulphation method and was tested on wooden material, paper and cloth. The test revealed that the modified oil is suitable to be used as a good dying agent and polish.

Keyworδs: Castor seed, Bleaching, Degumming, Adsorbent, Neutralization

Introδuction

Castor oil is a vegetable oil obtained by pressing the seeds of the Ricinus communis, a castor oil plant (Aliuddin, W., & Razdi, W., 2012). The common name "castor oil", from which the plant gets its name, probably comes from its use as a replacement for castoreum, a perfume base made from the dried perineal glands of the beaver (castor in Latin)[†]. Castor oil is a colorless to very pale yellow liquid with a distinct taste and odor once first ingested. Its boiling point is 313 °C (595 °F) and its density is 961 kg/m3 (Sigma-Aldrich, 2003). It is a triglyceride in which approximately 90 percent of fatty acid chains are ricinoleate. Oleate and linoleates are the other significant components (Castor IENICA, retrieved 2014-11-19). Castor oil is famous as a source of ricinoleic acid, a monounsaturated, 18-carbon fatty acid. Among fatty acids, ricinoleic acid is unusual in that it has a hydroxyl functional group on the 12th carbon. This functional group causes ricinoleic acid (and castor oil) to be more polar than most fats. The chemical reactivity of the alcohol group also allows chemical derivatization that is not possible with most other seed oils. Because of its ricinoleic acid content, castor oil is a valuable chemical in feedstocks, commanding a higher price than other seed oils.

Castor plant (Recinus Communis) from which castor beans and oil are subsequently derived grows naturally over a wide range of geographical regions and may be activating under a variety of physical and climatic regimes. The plant is however essentially a tropical species, although it may grow in temperate regions (Weise, 1983). Literature revealed that Castor beans contains about 30-35 percent oil (Marter, 1981; Weise, 1983) which can be extracted by variety of processes or combination of processes, such as hydrate presses, continuous screw presses and solvent extraction. However, the most satisfactory approach is hot pressing using a hydraulic press followed by solvent extraction (Marter, 1981; Salunke and Desai, 1941). The crude oil has distinct odour, but it can easily be deodorized in the refining process. Like any other vegetable oils and animal fats, it is a triglyceride which chemically is a glycerol molecule with each of its three hydroxyl group esterified with a long clown

fatty acid. Its major fatty acid is the unsaturated, hydroxylated 12-hydroxy, 9-octadecenoic acid, known familiarly as *Ricinoleic acid*. The fatty acid composition of a typical castor oil contains about 87% of ricinoleic acid. However, castor oil and its derivatives are used in the production of paints, varnishes, lacquers, and other protective coatings, lubricants and grease, hydraulic fluids, soaps, printing inks, linoleum, oil cloth and as a raw material in the manufacturing of various chemicals *sebacic acid* and *undecylenic acid*, used in the production of plasticizer and Nylon (Dole and Keskar, 1950). Prompted by above facts a study on extraction, characterization and modification of castor seed oil was undertaken and the results are reported herein.

Material anδ Methoδs

The raw material castor beans were purchased from the local market of Dehradun and the material was processed as follows.

Castor Beans Processing

The foreign materials and dirt present in castor beans were separated by hand picking, thereafter the cleaned beans were sun dried in the open until the casing splits and sheds the seeds. The beans were further dried in the oven at 65°C for 8hrs to a constant weight in order to reduce its moisture content which was initially at about 5 to 6%.The separation of the shell cotyledon was carried out using tray to blow away the cover in order to achieve a high yield. Finally mortar and pestle were used to crush the beans into a paste (cake) in order to weaken or rupture the cell walls to release castor fat for extraction.

Moisture Content of the Seeδs

5g of the cleaned seed sample was weighed and dried in an oven at 80°C for 8hrs. After drying, the sample was cooled in a desiccator and the weight was taken. The percentage moisture in the seed was calculated from the formula:

$$\text{Moisture} = \frac{(W1 - W2)}{W2} \times 100\%$$

Where, W_1 = Original weight of the sample before drying; W_2 = Weight of the sample after drying.

Extraction of Castor oil

500ml of n-Hexane was poured into round bottom flask. 20g of the sample was placed in the thimble and was inserted in the centre of the extractor. The apparatus was heated at 60°C and allowed for 4hrs continuous extraction using Soxhlet apparatus. The experiment was repeated for different weights of the sample, 30 g, 40 g and 50 g. After extraction was done, the crude oil samples were then placed to the rotary evaporator to remove the remaining solvent.

Determination of the Percentage Yielδ of Castor Oil Extracteδ

The percentage yield of oil extracted was determined by following formula:

$$Percentage\ of\ oil = \frac{W1}{Wo} \times 100\%$$

Where, W_o = Weight of seed sample subjected to extraction, and W_1 = Weight of oil extracted

Characterization of the Extracteδ Castor Oil

Aciδ Value

Equal volumes (25ml) of diethyl ether and ethanol were mixed in a 200ml beaker. The resulting mixture was added to 5g of oil in a 50ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with a standard solution of 0.1M NaOH and free fatty acid (FFA) was calculated asper published work (Aliuddin, W., & Razdi, W., 2012).

Determination of Saponification Value

Indicator method was use as specified by ISO 3657 (1988). 2g of the sample was mixed with 25ml of 0.1N alcoholic KOH was then added. The content was constantly stirred and refluxed for 60min. few drops of phenolphthalein indicator was added to the warm solution and then titrated with 0.5M HCl to the end point until the pink colour of the indicator disappeared. The same procedure was used for other samples and blank.

The expression for saponification value (S.V.) is given by:

$$S.V = \frac{56.1N(V0 - V1)}{M}$$

Where, V0 = the volume of the solution used for blank test; VI = the volume of the solution used for determination; N = Actual normality of the HCl used; M=Mass of the sample.

Determination of Iodine Value

The iodine value was determined as per ISO 3961 (1989) method.

0.4g of the sample was mixed with 20ml of CCl4. Thereafter 25ml of Dam's reagent was added to the flask using a safety pipette in fume chamber. The flask was then placed in the dark for 2 hours 30 minutes. At the end of the reaction, 20ml of 10% aqueous potassium iodide and 125ml of water were added. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow colour almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples. The iodine value (I.V) is determined as per the following formula:

$$\text{Iodine value (I.V)} = \frac{12.69C(V1 - V2)}{M}$$

Where, C = Concentration of sodium thiosulphate used; V1 = Volume of sodium thiosulphate used for blank; V2 = Volume of sodium thiosulphate used for determination, M = Mass of the sample.

Determination of Specific Gravity

Density bottle was used to determining the density of the oil. A clean and dry bottle of 25ml capacity was weighed (W0) and then filled with the oil, stopper inserted and reweighed to give (W1). The oil was substituted with water after washing and drying the bottle and weighed to give (W2). The expression for specific gravity (Sp.gr) is:

$$\text{Specific gravity (Sp. gr.)} = \frac{W1 - W0}{W2 - W0}$$

Determination of Viscosity

A clean, dried viscometer with a flow time above 200 seconds for the fluid to be tested was elected. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample. The viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to it's normal vertical position. The viscometer was inserted into a constant temperature bath at 28°C. The suction force was then applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flow freely from the upper timing mark to the lower timing mark was recorded.

Determination of Refractive Index

Determination of Refractive Index was done by refractometer. Few drops of the sample were transferred into the glass slide of the refractometer. Water at 30°C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value noted and recorded as the refractive index.

Determination of pH Value

2g of the sample was poured into a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. The sample was then cooled in a cold-water bath to 25°C. The electrode was calibrated with buffer solutions and the electrode was immersed into the sample and the pH value was read and recorded.

Refining of Extracted Castor Oil

Preparation of Clay

The clay sample was procured from the local market of Dehradun, Uttarakhand (India). The Clay preparation for oil refining was done

bymethod illustrated by Nkpa et al (1989) as follows:

The clay was ground and then mixed with water. Impurities such as sand and stone were removed. 5M HCl was added to the clay slurry and the mixture was then boiled for 2hrs at about 100°C. The resultant mixture was then washed with water to remove acid, dried and ground.

Degumming anδ Neutralization

Degumming and neutralization was done as per methods reported in literature (Nkpa et al., 1989; Carr, 1976).

The extracted oil was degummed by the addition of boiling water. The mixture was stirred for 3 minutes and allowed to stand in the separating funnel. Thereafter, the aqueous layer was removed. The procedure was repeated to ensure removal of most gums. For the neutralization, about 50g of the degummed oil was poured into a beaker and heated to 75°C, after which 40ml of 0.1M NaOH was added and stirred to a uniform solution. Sodium chloride (about 10% of the weight of the oil) was added to precipitate the soap formed. This was transferred into a separating funnel and allowed to stand for 1h; the soap formed was separated from the oil. Hot water was added again and again to the oil solution until the soap remaining in solution was removed. The neutralized oil was then drawn off into beaker.

Bleaching

60g of neutralized oil was poured into a beaker and heated to 90°C. 15% (w/w) activated clay was added to it. The mixture was stirred continuously for 30 minutes. The temperature was allowed to rise to 110°C for another 30 minutes. The content was filtered hot in an over at 70°C (Carr, 1976).

Moδification of the Refineδ Castor Oil:

20g of oil was warmed at 35°C. 15ml of concentrated sulphuric acid (98%) was then added and the reaction was allowed to completion with constant stirring. After, the product was washed with hot distilled water and left to stand for 2 hrs, after which water was then removed. And the sulphuric acid ester formed was finally neutralized with 10ml of 0.1m Sodium Hydroxide.

Results anδ Discussion

Results

The results obtained for various tests carried out for the sample are tabulated below:

Chemical analysis (Refineδ oil)

The physical properties of the crude and refined oil determined are presented in Table 1-3.

Table-1 Physical properties of the cruδe anδ refineδ castor oil

Description	Cruδe Castor Oil	Refineδ Castor Oil
Specific gravity	0.91	0.91
Viscosity at 28°C	9.87	6.02
Refractive Index at 28°C	1.42	1.45
pH	6.31	6.54
Colour	Amber	Amber

Table-2 Chemical properties of the cruδe anδ refineδ castor oil

Description	Cruδe Castor Oil	Refineδ Castor Oil
Acid Value [mg NaOH/g of Oil]	1.2	0.70
Saponification Value [mg KOH/g of Oil]	179.40	182.33
Iodine Value [g I_2 /100g of Oil]	86.42	83.70

Table-3 Determination of percentage oil extracteδ

S.N.	Description	Value
1.	The percentage of oil extracted	42.56 %;
2.	Percentage moisture content	4.15 %.

Table-4 ASTM specification for quality castor oil

Description	Value	Selecteδ
Specific gravity 20/25°C	0.957-0.968	0.962
Refractive Index, n_D	1.476-1.479	-
Saponification Value	175-187	181
Un-saponification Value	0.3-0.7	0.7 (Max %)
Iodine Value	82-88	85
Hydroxyl Value	160-168	160(Minimum)
Viscosity at 25°C	6.3-8.8 St	-
Acid Value	0.4-4.0	3
Colour (Gadner)	Not darker than 2-3	3.0 (Maximum)

Discussion

The results presented in the present work (Table 3) for the percentage moisture content, 4.15% shows a slight variation from the work reported earlier (Salunke and Desai, 1941). The percentage of the oil extracted, 42.56% is in good agreement with the range of the percentage oil content (30 – 55%) of castor beans as reported in literature (Marter, 1981; Weise, 1983).The variations in the quantity of percentage extraction of the oil further depends upon the variety of the material and mode of extraction (Janson, 1974).It is well documented that the best available method foe extraction of castor oil at present is by the use of hydraulic pressing (Marter, 1981; Salunke and Desai, 1941; Janson, 1974). The data presented in Table 1 presents a comparison between the physical properties of the crude and refined castor oil obtained in this work. The specific gravity values for both crude and refined oil are obtained to be the same, (0.9587). This is in accoardance with the reports published earlier (Lew Kowitseh J. I., 1909). Differences were observed between the value obtained for the viscosity of the crude and refined oil. The value of the viscosity of the crude castor oil (9.87st) was found to be out of the range of the recommended standard range of 6.3 – 8.8st (Lew Kowitseh J. I., 1909), whereas the refined oil's viscosity of 6.02st is fairly within the range. The decrease in theviscosity after refinement would be due to the removal of impurities present in the oil. The refractive index analysis shows that there is no significant difference between the value obtained for crude oil, 1.42and that of the refined oil, 1.45. Comparing this result with the ASTM values that ranges from 1.476 – 1.479 (ASTM, D960-52, 1952, Table 5), a little difference is noticed. However, this little difference can be considered being within an acceptable experimental error range which could be due to the presence of some impurities and other component of the crude oil mixture. In this way refractive index for both crude and refined castor oil is in accordance with ASTM specification.

Additionally, the pH value of the crude oil was recorded 6.31 which indicates that the oil is more acidic as compared to refined oil (pH 6.54). This may be as result of degumming and neutralization carried out during the refining process. The chemical properties analysis shown in Table 2 indicates that the acid value of crude and refined oil is 1.2mg NaOH/g of oil and 0.70 mg NaOH/g of oil respectively. Theresults show that the acid value is higher in crude oil as compared to refined oilwhich would be due to the presence of free fatty acids in the earlier one.Additionally, both values fall within the range as documented in the literature(Lew Kowitseh J. I., 1909). Table 2 shows the results for the saponification value of the crude and refined oil and it was179.40mg KOH/g of oil and 182.33mg KOH/g of oil respectively. The data shows that, for the crude oil, more alkaline would be required to enable it neutralize the available freely fatty acid librated by the oil, when compared with the refined oil. The saponification value of both crude and refined castor oil, are highly analogous with the result specified for standard quality castor oil (Lew Kowitseh J. I., 1909) as presented in Table 4.Furthermore, the resultsachieved for the iodine value of crude oil 86.42 revealsan increase in the average degree of un-saturation of the oil as compared toiodine value of refined castor oil, as such the amount of iodine which can be absorbed by unsaturated acids would be higher, compared to 83.70.These data reveals that both the crude and

refined oil could be classified as a non-drying oils (Lew Kowitseh J. I., 1909), since their iodine values are lower than 100. As a result of their agreement with standard, certainly, the oil could be used extensively as lubricants and hydraulic brake fluids.

In order to enhance the diverse applications of the castor oil refined oil was modified for a specific use by means of sulphation process to produce red oil which could be used in polishing, as a dyeing and wetting agent. The modified sulphated oil possess a high level of viscosity. The high viscosity of the modified oil would be due to the fact that the hydrogen liberated from the sulphuric acid added itself across the unsaturated bond of the oil thereby, yielding a saturated product. It was noticed that a little kind of pungent smell was observed from the sulphated castor oil. This may be as result of the excess un-reacted acid in the oil which has no effect on the intended end use. The modified castor oil was tested on wood, a piece of white cloth and a piece of paper.The modified castor oil stucked to the materials and a shining surface was obtained when the wood dried, while the colour is not easily removed from the cloth.

Conclusion

Castor oil has a well-known history of being used for many diverse applications. Due to the importance of the vegetable oils in the industrial, pharmaceutical, food industries, and also medical, there is an urgent need to produce more oil from the natural plant. In view of this, castor oil is a promising vegetable oil because it has several advantages; it is renewable, environmental friendly and produce easily in the rural areas, where there is an acute need for modern forms of energy. The study performed in the present work shows the percentage yield oil content of castor seed to be 42.56%. Although the yield of the extracted castor oil in the present work is little less than the earlier reports yet the quality of the oil cannot be overlooked.The castor oil produced in this research work was analyzed for specific gravity, viscosity, refractive index, acid value, saponification value and iodine value. The data shows that the refined castor oil is of good quality and could be recommended suitable for industrial usage. The refined castor oil was modified by means of sulphation and red oil was obtained which can be used as a wetting and dyeing agent and in the finishing of cotton and linen.

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Designing, Development anδ Permeation Stuδy of Diclofenac Ester Gel Using Eucalyptus Essential Oil as Permeability Enhancer.

Praδeep Chauhan[1], Versha Parcha[1], Ajay Kumar[1], Priyanka Dimri[1], Babita Gupta[2] anδ Alok Maithani[1]*

[1]Department of Applied Chemistry, Sardar Bhagwan Singh PG Institute, Balawala, Dehradun, Uttarakhand, India.

[2]Department of Chemistry, GGM Science College, Jammu, India

*E-mail: alok_maithanii@rediffmail.com

Abstract- Topical formulations are most commonly prescribed dosage forms for anti-inflammatory and dermatological disease. The effect of Eucalyptus essential oil on drug penetration rate across membrane is investigated in present study where the diffusibility of diclofenac and its esters in gels containing essential oils were analysed using Franz diffusion cell. It has been observed that highest membrane permeability was obtained in butyl ester derivative of diclofenac in gel formulation containing eucalyptus essential oil. This can be attributed due to higher alkyl chain in the compound as well as role of essential oil as a penetration enhancer.

Keyworδs: Eucalyptus, Essential oil, Franz diffusion cell, and Membrane permeability.

Introδuction

Diclofenac is an important drug of category NSAIDS belongs to chemical group phenyl acetic acid. Although, it is widely marketed as tablet doses forms but these days it is popularly formulated and marketed in semisolid formulations which include ointment and gels. Topical delivery applies drugs directly onto the skin that provides local effects and also penetrate deeper to give better absorption. Such formulation avoids GI irritation and prevent metabolism of drug in the liver but the permeability coefficient of drug is a major challenge for such formulations. However to increase membrane permeability various techniques may be adopt which includes increasing of drugs, incorporate of permeability enhancers, fluidization of skin etc.[1,2] In present study various esters of diclofenac were prepared to enhance their hydrophobicity or log P value, the ester were then formulated to gel form using essential oils as permeability enhancers[3,4,5]. The formulated gels from diclofenac esters with or without permeability enhancers were studied for *in-vitro* membrane permeability using Franz diffusion cell method.

Material anδ Methoδs

Preparation of Diclofenac Esters

Diclofenac was dissolved in different purified alcohol in the molar ratio 1:5 was added 4-5 drops of sulphuric acids and reflux for 6 hours under hygienic conditions. The reaction was monitored by TLC and after completion of reaction alcohol was distilled off. To the residue water was added, which was then extracted with diethyl ether and given washing with 5% sodium carbonate. The ether extract was dried over anhydrous sodium sulphate, concentrated and thus crude ester was obtained.

Extraction of Essential Oil

The samples of wild growing *Eucalyptus hybrid* plant were collected during the month of February from Balawala (Dehradun), which is located on the foot of lower Shiwalik mountain (Himalaya) at altitude about 650 m (a.s.l). The samples were identified by Dr Sandeep Dhyani, Faculty of Biotechnology, GRDPG IMT, Rajpur Dehradun, Uttarakhand. The samples were dried in shadow at room temperature for 10 days. Air-dried aerial parts of *Eucalyptus hybrid* deprived from wooden

parts (100 g) were subjected to hydro-distillation, using Clevenger-type apparatus for 3 h, according to the standard procedure. The obtained essential oils were dried over Na_2SO_4 and stored in a sealed dark vials, then kept at 4ºC prior to further analysis. The essential oil content was determined as percentage on fresh weight basis as average of three independent extractions of each site to minimize error (Percentage yield of essential oil was determined on fresh weight basis which was achieved by taking average of three extractions). Extracted oil were combined which was used for further analysis[6].

Formulation of Anti-inflammatory Gel

The present investigation include formulation of Diclofenac gel which contains 1 percent by weight of diclofenac and its derivatives as active ingredient, a medium comprising of water and glycerol in 3:7 in an amount sufficient to dissolve the drug and excipients. Carboxy methyl cellulose was used as gelling agent and an aliphatic amine in an amount sufficient to adjust pH of the preparation to about 6.7 – 7.7. Preservatives in prescribed concentrations were added and 1% essential oil was added as penetration enhancer into the formulation (Table-1).

In-vitro Membrane Permeability Stuδy

The in vitro penetration studies were performed for diclofenac and its esters using a franz diffusion cell. The dialysis membrane was mounted between the half cell keeping in contact with receptor fluid, 0.9% NaCl. The receiving chamber had a volume of 15 ml and the area available for diffusion was 2 cm^2. The top of donor cell was covered with an aluminium foil to prevent the evaporation of the drug. The concentration of penetrating drug was maintained uniform in all experiment by loading equal amount of drug and esters in methanol. In donor chamber temperature was maintained at 37^0C & the receptor fluid was stirred with a magnetic bar at 200 rpm. Fluid (1ml) was withdrawn from receptor cell at regular intervals and replaced by fresh normal saline to maintain the same volume (1ml) and withdrawn samples were analyzed after suitable dilution using the standard curve. Cumulative amount penetration was calculated for every half an hour upto 3 hours.[7-9]

Results anδ Discussion

The membrane permeability study of formulation is determined by Franz diffusion cell method. 1g of gel containing 20mg active drug (diclofenac and its esters) was applied to the dialysis membrane and the vessel and reservoir was filled with 0.9% NaCl solution. 1ml of solution was withdrawn at every 30 minutes upto 150min and concentration of the drug was determined using UV-spectrophotometer. Results are summarised in table-2. Results showed increase in concentration of drug with time. However, it was observed that the permeability improved when esters were prepared and highest drug diffusion was obtained in butyl ester of diclofenac. This directly is evidence greater diffusibility with increase in alkyl group. The results also recommend that the diffusibility of drug has improved when eucalyptus essential oil was aided

Table-1 List of active ingredient and excipients used in formulation of gel

Additives	Weight (in grams)	Role
Diclofenac Sod/ Diclofenac Esters	1.o	Active Ingredient
Sod CMC	5.0	Gelling Agent
Glycerol	5.0	Solvent
Propyl Peraben	0.2	Presetvative
Methyl Peraben	0.02	Preservative
Purified Water	q.s.	Solvent

Table-2 Percentage drug release in various gel formulations with and without addition of eucalyptus oil.

Time	DF	DF + Essential oil	Methyl Ester	Methyl Ester + Essential oil	Ethyl Ester	Ethyl Ester + Essential oil	Propyl Ester	Propyl Ester + Essential oil	Butyl Ester	Butyl Ester + Essential oil
30 min	13.9	14.0	14.7	13.6	16.7	24.8	20.5	24.9	20.2	33.2
60 min	18.3	24.6	20.5	24.4	33.1	33.6	29.6	36.9	33.2	38.1
90 min	26.3	28.6	28.4	36.2	42.2	39.6	33.8	45.3	41.1	48.1
120 min	33.9	41.5	38.6	45.1	58.7	48.8	45.6	66.3	51.3	62.6
150 min	45.2	48.9	46.8	49.9	55.7	52.5	51.9	72.5	64.6	81.9

to the formulation. Hence the eucalyptus oil can be exploited as a permeability enhancer in all topical delivery systems (Table-2).

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Antifungal Potential of *Murraya koenigii*

Shweta Tyagi anδ Aheesh Tyagi
Department of Biosciences, D.A.V. (PG) College, Muzaffarnagar (U.P), India
Affiliated to C.C.U. Merrut
E-mail: shewtatyagi@gmail.com

Abstract - The antifungal activity of alcoholic and aquoeus leaf extract of *Murraya koenigii* was tested against isolated fungal strains (*Penicillum crysogenum, Aspergillus niger, Aspergillus flavus and Fusarium)* by agar well diffusion method. All extracts showed antifungal activity against all isolated fungal strains. The acetone extracts of leaf (5%) exhibited maximum inhibitory activity against the *P.crysogenum, A. niger and A. flavus*. The ethanol extract of stem at 10% concentration showed minimum inhibition zone against *Fusarium* while acetone extract of stem (10%) gave maximum zone of inhibition against *Fusarium*. The acetone extract gave better antifungal activity in comparison to other extracts.

Keyworδs: *Murraya koenigii*, Penicillum crysogenum, Aspergillus niger, Fusarium

Introδuction

Murraya koenigii, a medicinally important herb of Indian origin, has been used for centuries in the Ayurvedic system of traditional Indian medicine and popularly used in Indian cuisine on a daily basis. (Maheswari and Cholarani, 2013). Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Ahmad *et al, 2001*) drug discovery and design (Mukherji *et al,* 2006). *Murraya koenigii*, a member of the family Rutaceae, is a deciduous to semi-evergreen aromatic tree found throughout India. *Murraya koenigii* commonly known as Meetha neem and Kari patta, in English known as Curry leaves, Girinimba and Suravi in Sanskrit, Karivempu, Karuveppilei and Karivepila in Tamil, Karepaku and Karuvepakuin Telugu. *Murrayakoenigii* contains acid, aldehyde, ester, ketone, alcohols **(Chakraborty *et al*, 1965).** The leaves are rich in mono-terpenoids and sesquiterpenoids which exhibit antifungal activities. It is used as an analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery and skin eruptions take care of indigestion, stomach ulcers (Manvi and Renu, 2011). So the aim of this study is to evaluate the antifungal efficacy of the aqueous and alcoholic extract of *Murraya koenigii* against *Penicillum crysogenum, Aspergillus niger, Aspergillus flavus and Fusarium.*

Material anδ Methoδs

The present work was carried out in the Department of Biosciences D.A.V. (P.G.) College Muzaffarnagar (U.P). The plant material (leaf and stem) of *Murraya koenigii*, were collected from the area of Muzaffarnagar. The collected plant material was washed with distilled water and then dried under in shade. The air dried plant material was crushed and extracts were prepared in distilled water and alcohol for antifungal testing. Plant dried material (5%, 10% & 15%) was dissolved in 50% solvent (Propanol, ethanol and acetone). Vortexing was done to mix them correctly and then kept in incubator at 30p C for 24 hr. After incubation, the mixture was centrifuged at 1000 rpm for 5 min at 4°C. The clear supernatant was referred to as crude plant extract and used further for antifungal testing.

Isolation, purification anδ iδentification of fungal strains from soil anδ fecally contaminateδ water

The fungal strains were isolated from soil and fecally contaminated water by serial dilution method (Cappuccino and Sherman, 2005; Aneja, 2009). The isolated colonies of *Penicillum crysogenum, Aspergillus niger, Aspergillus flavus*

and Fusarium were then streaked onto the fresh SDA plates.

Testing for antimicrobial activity

Antimicrobial activity of plant extract was tested by agar well diffusion method on SDA plate and measure the zone of inhibition in mm. (Khokra*et al*, 2008).

Results anδ Discussion

Table-1 shows the antifungal potential of *Murraya koenigii* leaves and stem at 5% concentration in different aqueous and alcoholic extract against isolated fungus strains. It is observed that the leaf extract prepared in acetone showed the maximum inhibition zone against *P. crysogenum, A. Niger* and *A. flavus.* While the extract of leaf in distilled water, propanol and ethanol showed same activity against *Fusarium* and *P. crysogenum*. The minimum inhibition zone is found in distilled water and ethanol extract against *A. niger*. All the alcoholic extract of stem showed same inhibitory zone against *P. crysogenum* while acetone extract showed maximum inhibition against *A.niger*. The minimum zone of inhibition is observed in propanol and distilled water stem extract aganist *A. flavus* and *Fusarium* respectively.

The antifungal potential of *Murraya koenigii* leaves and stem at 10% concentration in different aqueous and alcoholic extract against isolated fungus strainsis mentioned in Table-2. At 10% concentration of leaf extract prepare in distilled

Table-1 Antimicrobial activity of *Murraya koenigii* leaves anδ stem at 5% concentration in aqueous anδ δifferent alcoholic meδium for isolateδ microorganism (inhibition zone in mm± SD)

Microorganism	plant parts	D.W. (mm±S.D.)	Propanol (mm±S.D.)	Ethanol (mm±S.D.)	Acetone (mm±S.D.)
Penicillium crysogenum	leaf	19±1.82	19±1.88	19±1.82	20±1.99
	stem	19±1.85	20±1.83	19±1.91	19±1.82
Aspergillus niger	leaf	17±1.67	18±1.75	17±1.62	19±1.80
	stem	18±1.80	19±1.54	18±1.82	24±2.02
Aspergillus flavus	leaf	18±1.80	18±1.25	18±1.77	20±2.11
	stem	20±1.94	18±2.13	19±1.97	20±2.17
Fusarium	leaf	19±1.91	19±2.11	19±1.82	18±1.73
	stem	17±1.58	19±1.12	20±2.15	20±2.15

Table-2 Antimicrobial activity of *Murraya koenigii* leaves anδ stem at 10% concentration in aqueous anδ δifferent alcoholic meδium (inhibition zone in mm± SD)

Microorganism	plant parts	D.W. (mm±S.D.)	Propanol (mm±S.D.)	Ethanol (mm±S.D.)	Acetone (mm±S.D.)
Penicillium crysogenum	leaf	18±1.72	21±2.02	20±2.51	20±2.02
	Stem	18±2.10	19±1.82	20±3.41	19±1.92
Aspergillus niger	leaf	17±1.63	20±2.15	21±2.10	19±1.82
	Stem	19±2.51	21±2.01	21±2.12	20±2.17
Aspergillus flavus	leaf	17±1.52	19±1.82	20±1.43	18±1.78
	Stem	18±1.83	21±2.16	19±2.83	21±2.18
Fusarium	leaf	20±1.92	20±2.11	19±1.62	19±1.88
	Stem	20±2.41	18±1.83	15±1.85	22±2.20

Table-3 Antimicrobial activity of*Murraya koenigii* leaves anδ stem at 15% concentration in aqueous anδ δifferent alcoholic meδium (inhibition zone in mm± SD)

Microorganism	plant parts	D.W. (mm±S.D.)	Propanol (mm±S.D.)	Ethanol (mm±S.D.)	Acetone (mm±S.D.)
Penicillium crysogenum	leaf	21±1.97	20±2.11	19±1.82	21±2.12
	Stem	20±2.11	19±1.83	21±2.11	19±1.82
Aspergillus niger	leaf	21±2.16	21±2.31	18±1.80	20±1.82
	stem	21±2.32	18±1.62	20±1.83	20±2.36
Aspergillus flavus	leaf	19±1.75	20±2.01	19±1.92	21±2.01
	stem	19±1.85	19±2.19	19±1.90	22±2.24
Fusarium	leaf	20±2.32	19±1.95	20±2.15	19±1.82
	stem	18±1.80	21±2.15	19±1.19	21±2.11

water showed minimum zone of inhibition against all the isolated fungal strains. While the propanol and ethanol extract showed good inhibition. Ethanol extract and propanol extract show the maximum zone of inhibition *against A.niger* and *P.crysogenum* respectively. The extract (10%) of *Murraya koenigii*stem in acetone showed maximum inhibitory activity against *Fusarium* while minimum zone of inhibition is showed by *P. crysogenum*. The ethanol and distilled water extract of stem of *Murraya koenigii* gave minimum zone of inhibition against *Fusarium*.

Table-3 showing the antifungal potential of *Murraya koenigii* leaves and stem at 15% concentration in different aqueous and alcoholic extract against isolated fungus strains. The minimum zone of inhibition showed by *P.crysogenum* and *A.niger* in ethanol extract of leaf while the acetone extract of leaf gave maximum inhibition activity against *A. flavus* compared to the other extracts. The extract prepared in acetone of stem showed good inhibition against *A.flavus* and *Fusarium* while the ethanol extract and distilled water extract showed maximum inhibitory zone against *P. crysogenum* and *A.niger* respectively. The distilled water, propanol and ethanol extract gave same results against *A.flavus*.

Some reports also showed the similar findings. Jayaprakash and Ebenezer (2012) observed the antifungal efficacy of the ethanolic extract of *Murraya koenigii* against *Trichophyton mentagrophytes* and *Microsporum gypseum.* The methanolic extract of leaves of *Murraya koenigii* inhibited *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus uberis, Pseudomonas aeruginosa, Escherichia coli, Corynebacterium gravis* and *Bacillus cereus*. The hexane extract inhibited all microorganisms except *Staphylococcus epidermidis*, *Streptococcus uberis*and *Bacillus cereus*. The MIC values of the different methanolic extracts of leaves were found to vary greatly, and ranged from 8.25 mg/ml to 30mg/ml. Mathur *et al*, (2010) determined the antifungal activity against *Penicillium*, *Aspergillus niger, Aspergillus flavus* and *Fusarium*. Aqueous extract was found to be more active against *Penicillium* and *A. niger* in maintaining the protein and sugar content. Ethanol and chloroform extracts were found to be more active against *A. flavus* in maintaining the sugar content and *A. niger* in maintaining the protein content. The ethanol extract of *Murraya koenigii* leaves at 5% conc. show maximum activity against *Staphylococcus sps.*, followed by *Pseudomonas*, *E.coli* and *Bacillus sps.*. At the same time, there were also many reports that show antibacterial activity of plant leaves methanol extracts were measured by the zone of inhibition. It showed

maximum activity against *Pseudomonas aeroginosa* followed by *S.aureu*, *B.subtilis* and *E.coli*. (Zachariah et al, 2008)

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Phytochemical Investigation of Symplocos racemosa (leaves)

Shyam Vir Singh*, Sanδeep Negi, H.V. Pant, H.C. Joshi, R. Dhounδiyal anδ Deepali Singhal
Department of Chemistry, SGRR (PG) College, Pathribagh, Dehradun, Uttarakhand, India
**E-mail: shyamveer91084@rediffmail.com*

Abstract - Four compounds were isolated from the *methanol and* chloroform soluble fractions with ethanolic extract of the leaves of *Symplocos racemosa.* The structures of the isolated compounds were elucidated as luteolin (1), α-amyrin (2) and β-amyrin (3) by extensive spectroscopic studies like EI-MS, IR, UV, ^{1}H-NMR, ^{13}C-NMR (including editing technique like DEPT), COSY and HMBC.

Introδuction

Symplocos racemosa Roxb. belongs to the Symplocaceae family and in Sanskrit, was called Lodhra, Rodhra or Sarimata meaning "propitious", and "Tilaka" because it was used in Tilaka mark on the forehead. In India it was commonly known as 'Lodh'. A decoction of the bark was used for gargling and when the gums were spongy and bleeding. Roxburgh remarks that the bark was popular among the dyes of red in Calcutta and seemed to be used as mordant only. It grows abundantly in the plains and lower hills throughout North and East India, ascending in the Himalayas up to an elevation of 1,400 m. Lodh is a medicinal plant widely used by the traditional practitioners against various diseases as single or in compound drug. It has a wide range of usage in Ayurveda and Unani medicines. Its bark described as an emmenagogue tonic for the person of plethoric constitution and is useful in bowel complaints and ulcers. In the present study, there are four compounds were isolated from the *methanol and* chloroform soluble fractions with ethanolic extract of the leaves of *Symplocos racemosa.*

Material anδ Methoδs

Plant material

The plant material was collected from Gopeshwar, District Chamoli, Garhwal, Uttarakhand (India) in the month of March 2009. The authentification of plant material was made at the Department of Botany, HNB Garhwal University, Campus Pauri, Pauri Garhwal, Uttarakhand (India). A voucher specimen is available at the herbarium of Botany Department.

Extraction

Shade dried leaves (1 Kg) were exhaustively extracted with ethanol (60-80 ^{o}C) in a 5 Liter R.B. flask (500 g × 2.5 Ltr) followed by repeated extraction with ethanol. Every time, extraction was carried out for to 10 hrs. In each case the extract was filtered and concentrated under reduced pressure when brown syrupy mass (110 g from ethanolic extract) respectively was obtained.

Isolation

In this study, isolations of the compounds as fractions were carried out by column chromatography with the increasing polarity ratio of chloroform and methanol. Out of which, four fractions were responded in which three were crystallized with ethanol and one was found in powdered form. Elemental analysis were carried out with the various Spectroscopical techniques such as EI-MS, IR, UV, ^{1}H-NMR, ^{13}C-NMR (including editing technique like DEPT), COSY and HMBC etc.

Experimental

The UV spectrums were measured on a Hitachi 320. Perkin Elmer model 202 automatic recording spectrophotometer and Toshinwal manual spectrophotometer. The IR spectra were recorded KBr pellets on Perkin Elmer model 577 and KBr discs (JASCO – IR – 810 spectrometer). The ^{1}H NMR were recorded on Bruker spectrometer at 400 MHz, ^{13}C NMR at 100.533 MHz, DEPT, COSY-NMR and HMBC-NMR spectra were recorded on same instrument using TMS as internal standard. EI-MS spectra were recorded on

JMS – DX 300 (JEOL) with direct inlet at 70 eV. The melting points are uncorrected.

Spectral δata of the four isolateδ compounδs

Compounδ-1 UV (MeOH): 268 and 344 nm, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.56 (1H, dd, *J* = 9, 2Hz, H-6'), 7.36 (1H, d, *J* = 2Hz, H-2'), 6.85 (1H, d, *J* = 9 Hz, H-5'), 6.75 (1H, s, H- 3), 6.46 (1H, d, *J* = 2 Hz, H-8) , 6.28 (1H, d, *J* =2 Hz, H-6), ^{13}C NMR (100 MHz, DMSO-d6): δ 181.8 (C-4), 164.3 (C-7), 164.0 (C-2), 162.1 (C-9), 157.6 (C-5), 149.7 (C-4'), 146.0 (C-3'), 120.8 (C-6'), 119.0 (C-1'), 116.8 (C-5'), 113.2 (C-2'), 103.8 (C-10), 99.2 (C-6), 94.7 (C-8), EI-MS, *m/z*: [M+H] + 287.

Compounδ-2 Molecular formula: $C_{30}H_{50}O$, Molecular weight: 426.3825, M.P. : 197-198 °C, IR ν_{max} cm^{-1}(KBr): 3510, 3055, 1635, 820 cm^{-1} ^{1}H NMR ($CDCl_3$) (400 MHz) (δ ppm): 5.11 (1H, m, H-12), 3.19 (1H, dd, J= 10.0, 4.5 Hz, H-3), 1.02, 1.01, 1.08, 0.96, 0.93, 0.88, 0.85 and 0.80 (3H, each s, Me), ^{13}C NMR ($CDCl_3$) (100 MHz) (δ ppm): 144.3 (s, C-13), 124.1 (d, C-12), 78.8 (d, C-3), 54.4 (d, C-5), 47.7 (d, C-9), 47.3 (d, C-18), 46.9 (t, C-19), 42.3 (s, C-14), 41.6 (t, C-22), 40.9 (s, C-8), 39.1 (s, C-4), 39.0 (t, C-1), 37.0 (s, C-10), 34.0 (s, C-17), 33.3 (q, C-29), 33.2 (s, C-21), 31.7 (s, C-20), 28.2 (q, C-23), 28.0 (q, C-28), 27.4 (t, C-2), 26.5 (t, C-16), 26.3 (t, C-15), 26.0 (q, C-27),23.6 (t, C-11), 23.3 (q, C-30), 18.5 (t, C-6), 16.9 (q, C-26), 15.6 (q, C-25) and 15.5 (q, C-24), EI-MS m/z (rel. int. %): 426 $[M]^+$ (15), 411 (18), 408 (16), 393 (32), 257 (20), 218 (100), 207 (10), 203 (40) and 189 (55).

Compounδ-3 Molecular formula: $C_{30}H_{50}O$, Molecular weight: 427.40, M.P.: 270-272 °C, IR ν_{max} cm^{-1}(KBr): 3510, 3055, 1635, 820 cm^{-1}, ^{1}H NMR ($CDCl_3$) (400 MHz) (δ ppm): 5.11 (1H, m, H-12), 3.19 (1H, dd, J= 10.0, 4.5 Hz, H-3), 1.02, 1.01, 1.08, 0.96, 0.93, 0.88, 0.85 and 0.80 (3H, each s, Me), ^{13}C NMR ($CDCl_3$) (100 MHz) (δ ppm): 144.3 (C-13), 124.1 (C-12), 78.8 (C-3), 54.4 (C-5), 47.7 (C-9), 47.3 (C-18), 39.8 (C-19), 42.3 (C-14), 41.6 (C-22), 40.9 (C-8), 39.1 (C-4), 39.0 (C-1), 37.0 (C-10), 34.0 (C-17), 33.3 (C-29), 33.2 (C-21), 39.2 (C-20), 28.2 (C-23), 28.0 (C-28), 27.4 (C-2), 26.5 (C-16), 26.3 (C-15), 26.0 (C-27), 23.6 (C-11), 23.3 (C-30), 18.5 (C-6), 16.9 (C-26), 15.6 (C-25) and 15.5 (C-24), EI-MS m/z: 427 $[M+H]^+$, 419, 365, 325, 271, 163, 97.

Results anδ Discussion

Characterization of compounδ-1 as luteolin

Compound-1 was obtained as an yellow powder and its molecular formula was established as $C_{15}H_{10}O_6$ from its MS spectral data that showed [M+H]+ ion at *m/z* 287 which further supported by its ^{13}C NMR spectral data. The UV spectrum also showed absorption maxima at 268 and 344 nm suggested a flavonoid structure. The ^{1}H NMR spectrum showed the presence of three meta coupled aromatic doublets at δ 6.28, 6.46 and 7.36, one ortho coupled aromatic doublet at δ 6.85, one doublet of doublets at δ 7.56 corresponding to a ortho and meta coupled aromatic proton, and a singlet at 6.75; characteristic for a 5,7,3',4'-tetrasubstituted flavone. The ^{13}C NMR spectra showed the presence of twelve aromatic carbons; eight quaternary carbons, six methine carbons, and an unsaturated carbonyl carbon. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of COSY and HMBC correlations. A search in literature suggested the spectral data was consistent to luteolin (3′,4′, 5, 7-tetrahydroxyflavone). Therefore, on the basis of these data compound SR-1 characterized as luteolin (3′, 4′, 5, 7-tetrahydroxyflavone) as in Fig.1.

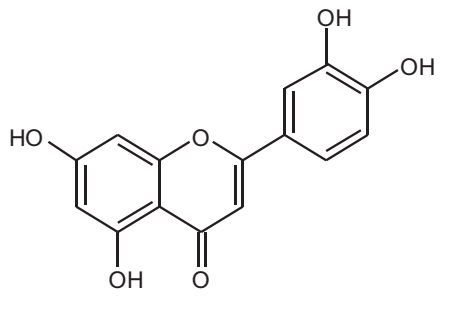

Fig. 1 Structure of Compounδ-1

Characterization of compounδ-2 as β-amyrin

Compound-2 was isolated as a white powder. The mass spectral data of compound SR-2 gave a molecular ion peak at m/z 427 corresponding to its $(M+H)^+$ ion suggesting the molecular formula as $C_{30}H_{50}O$, which was supported by the ^{13}C NMR spectral data. The ^{1}H NMR spectra of compound

SR-2 showed the presence of four methyl sinlgets at δ 0.70, 0.74, 0.86 and 0.92 as well as four methyl doublets that appeared at δ 0.71, 0.72, 0.86 and 0.87. Liebermann-Burchard reaction indicated compound 2 is having a terpenoid skeleton. The proton corresponding to a secondary hydroxyl group of a terpene moiety was appeared as a doublet of doublets at δ 3.24. Compound SR-2 also showed a proton at δ 5.13 as a triplet suggesting the presence of a trisubstituted olefinic bond. A search in literature suggested the spectral data was consistent to β-amyrin and on the basis of these data compound 2 characterized as β-amyrin as in Fig.2.

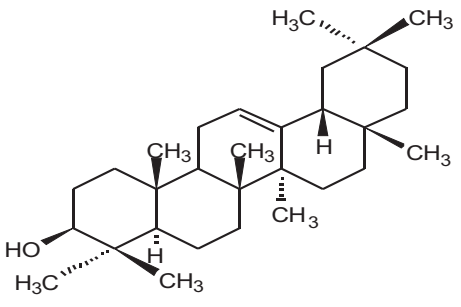

Fig. 2 Structure of Compounδ-2

Characterization of compounδ 3 as α-amyrin

Compound-3 was isolated as a white powder. The mass spectral data of compound SR-3 gave a molecular ion peak at m/z 427 corresponding to its $(M+H)^+$ ion suggesting the molecular formula as $C_{30}H_{50}O$, which was supported by the ^{13}C NMR spectral data. The ^{1}H NMR spectra of compound 3 showed the presence of six methyl sinlgets at δ 0.71, 0.74, 0.87, 0.91, 0.96, and 1.03, as well as two methyl doublets that appeared at δ 0.72 and 0.86. Liebermann-Burchard reaction indicated compound 3 is having a terpenoid skeleton. The proton corresponding to a secondary hydroxyl group of a terpene moiety was appeared as a doublet of doublets at δ 3.24. Compound SR-3 also showed a proton at δ 5.13 as a triplet suggesting the presence of a trisubstituted olefinic bond. The ^{1}H, ^{13}C NMR values for all the protons and carbons were assigned on the basis of COSY and HMBC correlations. A search in literature suggested the spectral data was consistent to α-amyrin. On the basis of these data compound 3 characterized as α-amyrin (Fig.3).

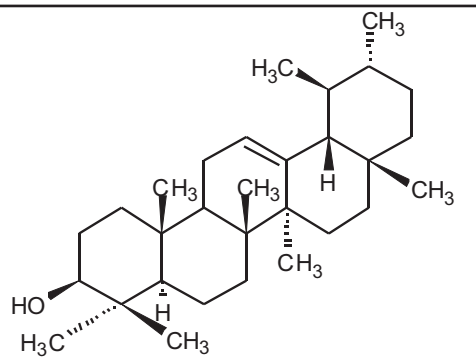

Fig. 3 Structure of Compounδ-3

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Inδian Traδitional Phytomeδicines: The Source of New Bioactive Compounδs Active *in vitro* Against Pathogens

S.Farooq, Z.Mehmooδ anδ A.K.Dixit

Abstract- The antibacterial activity of six medicinal plants was screened for different bacterial strains using methanol, Hexane and water as solvents. The leaves of six plants (*Pimenta dioica(leaves), Piper betel(leaves), Cannabis sativa(leaves), Lawsonia inermis (leaves),Saraca indica(leaves),* and *Cassia angustifolia (Leaves)* were dried and powdered before being subjected to soxhlation. All extracts were concentrated by using rotary evaporator. The phytochemical screening was carried out to know the compounds responsible for these activities. Methanol, Hexane and water extracts were tested against *Escherichia coli, Staphylococcus aureus, Salmonella enteritis* and *Pseudomonas aeruginosa.* The antibacterial assays in this study were performed by the agar-well diffusion methods so that they could be quantified by measuring the zones of growth inhibition diameters. The susceptibility of the bacteria to the crude extracts on the basis of zones of growth inhibition varied according to microorganism and extracting solvent. In most of the above mentioned plants, the methanol extract produced the highest activity when compared to Hexane and aqueous extracts. On the basis of the results obtained, it could be concluded that methanol could be used for extracting antimicrobial compounds from leaves of the plants used. The present study showed that the plant extracts possessed the antimicrobial activity against some pathogens associated with infections in the human beings.

Keyworδs: Phytomedicines, Antimicrobial activity, Antimicrobial Compounds, Bioactive Compounds

Introδuction

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983 and 1994 (Cragg et al., 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria.

According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India. Ayurvedic system of medicine has its long history of therapeutic potential. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995; Nascimento et al., 1990). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen et al., 1987) as well as tannin (Saxena et al., 1994).There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-

emerging infectious diseases (Rojas et al., 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Bylka et al., 2004; Shimpi and Bendre, 2005; Kilani, 2006). There is a continuous and serious need to discover new antibacterial and anti-fungal compounds for drug resistant pathogens and new infectious diseases (Majid et al., 2013). Thus it is a logical approach in drug discovery to screen traditional natural products. Here, we evaluate the potential of several plant extracts for antibacterial and antifungal activity against important human pathogenic bacteria and *Candida albicans*.

Material anδ Methoδs

Collection of plant material

Fresh and healthy leaves of different medicinal plants were collected from in and around Dehradun (Uttarakhand state, India).The plants were identified based on the taxonomical characteristics by Dr. Mayaram Uniyal, Department of Pharmacognosy, The Himalaya Drug Company Dehradun, India. The leaves were shade dried, powdered and used for extraction.

Test microorganisms

The antibacterial activity of the extracts were tested individually on G+ve and G-ve bacterial strains. All bacterial strains were obtained from IMTECH, Chandigarh India. The G+ve strain used was *Staphylococcus aureus MTCC 737 and* G-ve bacterial strains were *E.coli MTCC 1687; Psuedomonas aeruginosa MTCC 1688*, *Salmonella enterica MTCC 3858. and Candida albicans MTCC 3017.*

Preparation of aqueous extract

The finely powdered plant materials (100 grams) were boiled in 500 ml distilled water till one-fourth of the extract initially taken was left behind after evaporation. The solution was first filtered through double layered muslin cloth and centrifuged at 5000 g for 30 min and the supernatant was filtered through whatman No.1 filter paper under strict aseptic conditions and then the filtrate was collected in fresh sterilized bottles and stored at 4oC until further use.

Preparation of solvent extract

100 grams each of the powdered material was extracted with 500ml of methanol separately for 24hrs. The extract was filtered with sterile whatman filter paper No.1 into a clean conical flask. The solvent along with the sample was transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvent. The evaporated solvent so obtained was weighed and preserved at 4oC in airtight bottles until further use.

Phytochemical screening

Phytochemical screening was carried out to determine the presence of saponins, tannins, flavonoids, glycosides, triterpenoids,phytosterols and cardiac glycosides(Harbourne,1993 & Khandelwal,2000). The solvents used were methanol and distilled water.

Test for Saponins (Foam test)

About 200 mg of powdered sample was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent broth.Formation of foam indicated the presence of saponins.

Test for Tannins (Ferric chloride test)

About 200 mg of plant extract was treated with few drops of 0.1% ferric chloride and observed for blue or black colouration.Formation of blue black colour confirmed the presence of tannins.

Test for Alkaloids (Wagner's test)

About 0.5ml of extract solution was treated with 2-3 drops of Wagner's reagent (solution of Iodine in potassium iodide) and the formation of reddish brown precipitate indicated the presence of alkaloids.

Test for Flavonoids (Alkaline reagent test

To the extract solution, few drops of sodium hydroxide was added, formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute acetic acid indicated the presence of flavanoids.

Test for Sterols and Triterpenoids (Salkolwski's test)

The extract was treated with chloroform,few drops of concentrated H_2SO_4 was added, the test tube was shaken well and allowed to stand for some time. The appearance of red colour in upper layer indicated the presence of sterol and formation of yellow colour at the lower layer indicated the presence of triterpenoids.

Test for Cardiac Glycosides (KellerKillani test)

The extract was treated with chloroform and allowed to dryness. Then, 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride solution was added. The mixture was transferred to small test tube. 0.5 ml of concentrated H_2SO_4 was added along the sides of the test tube, the appearance of blue colour in acetic acid layer indicated the presence of cardiac glycosides.

Agar-well diffusion assay

Suspension of 24 h cultures of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes and Pseudomonas aeruginosa* was made in sterile normal saline. Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5mm diameter was used to make wells on the medium. 100 L of the various extract concentration were dropped into each, appropriate well (Atata et al;2003 & Bonjar,2004) Methanol solvent used for extraction apart from water was tested neat for each organism. The inoculated plates were kept in refrigerator for 2 hours to allow the extracts to diffuse into the agar15. The agar plates were incubated at 37oc for 24 h. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 30g of standard antibiotic streptomycin was used as positive control and respective solvents as negative controls.

Table- 1 ***List of medicinal plants and their part used for antibacterial studies.***

Meδicinal Plant	Family	Part useδ
Pimenta dioica	Myrtaceae	Leaves
Piper betel	Piperaceae	Leaves
Cannabis sativa	Cannabinaceae	Leaves
Lawsonia inermis	Lythraceae	Leaves
Saraca indica	Caesalpinaceae	Leaves
Cassia angustifolia	Leguminoseae	Leaves

Results anδ Discussion

Phytochemical screening on the crude methanol extracts of 6 medicinal plants were done using test tube. The results (Table- 2) revealed the presence of secondary metabolites such as saponins, tannins,alkaloids, triterpenoids and cardiac glycosides. Except *Pimenta dioica and Cannabis sativa* none of the other plants showed the presence of cardiac glycosides. Alkaloids and flavonoids were present in all extracts except *Lawsonia inermis* and *Saraca indica where alkaloids were absent*. The efficacy of Hexane, methanol and aqueous extracts of these medicinal plants against pathogenic bacteria showed varied level of inhibition (Table- 3). It was revealed from the result that each medicinal plant showed different degrees of inhibition against different microorganisms. The maximum zone of inhibition was observed in the case of *Candida albicans (22mm)* and *Staphylococcus aureus* (20mm) due to action of methanol extracts of *Pimenta dioica*,followed by *Lawsonia inermis* . The results revealed variability in the bactericidal concentration of each extract for given bacteria. It was clear from the present result that methanol extract exhibited pronounced activity against all the tested four bacteria. The highest antibacterial activity as seen with methanol extract might be

Table-2 Phytochemical Analysis of δifferent plant leaves extracts

S.No.	Name of the plants	Phytochemical constituents in leaf extracts					
		Alkaloiδs	Tannins	Flavonoiδs	Saponins	Terpenoiδs	Carδiac glycosiδes
1.0.	*Pimenta dioica(Linn.)Myrtaceae*	+	+	+	+	+	+
2.0.	*Piper betel (Linn.) Piperaceae*	+	+	+	+	+	-
3.0.	*Cannabis sativa L.Cannabinaceae*	+	-	+	+	+	+
4.0.	*Lawsonia inermis (Linn.)Lythraceae*	–	–	+	–	–	–
5.0.	*Saraca indica Caesalpinaceae*	–	–	+	+	–	–
6.0.	*Cassia angustifolia (Vahl.)Leguminoseae*	+	+	+	–	+	–

Table- 3 Antimicrobial activity of *different Plants* extracts against test organisms

S.No.	Test microorganisms	Diameter of zone of inhibition(mm)																	
		Pimenta dioica			*Piper betel*			*Cannabis sativa*			*Lawsonia inermis*			*Saraca indica*			*Cassia angustifolia*		
		HX	ME	AQ	HX	ME	AQ	HX	ME	AQ	HX	ME	AQ	HX	ME	AQ	HX	ME	AQ
1.0.	*Staphylococcus aureus MTCC 737*	15	20	10	12	18	14	10	17	15	12	20	15	10	19	14	14	18	15
2.0.	*E.coli MTCC 1687*	12	14	NAD	14	16	12	12	15	12	10	18	12	12	20	10	12	16	14
3.0.	*Pseudomonas aeruginosa MTCC 1688*	14	12	10	14	17	10	10	14	10	12	18	15	10	18	12	10	14	12
4.0.	*Salmonella enteric MTCC 3858*	10	15	NAD	12	16	12	10	16	12	10	20	15	12	16	12	10	12	10
5.0.	*Candida albicans MTCC 3017*	16	22	14	15	20	14	10	18	16	15	20	14	12	18	15	14	20	14

HX; HEXANE ME; METHANOL AQ; AQUOEUS NAD; NO ACTIVITY DETECTED

NAD; NO ACTIVITY DETECTED

due to the presence of alkaloids and tannins (Okemo, 1996). Broad spectrum activity of methanol extract tended to show that the active ingredients of the leaves were better extracted with methanol. Earlier studies had also shown the greater antibacterial activity of methanol extracts than other solvent extracts (Aqil et al.2003 & Kannan et al; 2009). With least or no antibacterial activity as seen with other solvent extracts, might be due to loss of some active compounds during extraction process of the sample and lack of solubility of active constituents in the solvent(Sampathkumar,2008). In spite of this permeability difference between Gram positive and Gram negative bacteria, the methanol extract had a broader spectrum of inhibitory activity. This showed the involvement of more than one active principle of biological significance. This study does not only show the scientific basis for some of the therapeutic uses of these plants in traditional medicine, but also confirms the fact that ethnobotanical approach should be considered when investigating

antimicrobial properties of plants(Iwu,1993 & Adesanya,2005).

The implication of the broad spectrum action of some of these extracts may help in selection of plants with antimicrobial activities for further phytochemical work on the isolation and the identification of the active compounds.

Conclusion

On the basis of the results obtained, it can be concluded that methanol can be used for extracting antimicrobial compounds from leaves. The present study shows that plant extracts possessed the antimicrobial activity against some organisms associated with infections and are highly resistant against antibiotics. Therefore, it suggests that the plant can be a source of oral drugs to be used in the treatment of opportunistic infections and may be a source for future drug formation.

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Stuδy of Fatty Aciδs Elementology anδ Total Protein of *Mentha Arvensis L*

Anita Rai[*a], N.P. Singh[b] anδ Sanjay Kumar Singh[b]
[*a] Department of Chemistry, PPN PG College, Kanpur, (U.P.), India
[b] Department of Chemistry, DBS College, Kanpur, (U.P.), India
*E-mail: raianita19@gmail.com

Abstract- India is well-known for its rich variety of medicinal plants. In the present investigation the plant, *Mentha arvensis L. (*family Lamiaceae) was studied. The highest saturated fatty acids were found present in it were Behenate and Palmilate 4.90% each. The unsaturated fatty acids were Heptadecadienoate 19.59%. Total 29 compounds were isolated from its extracts. Saturated and unsaturated fatty acids were isolated and identified by using GC-MS method. Six elements viz, copper, 11.17 (mg/kg) Iron, 5.8 (mg/kg) Sodium, 60.20 mg/kg) Magnesium, 16.20 (mg/kg) Potassium, 10.31 (mg/kg) and Zinc 12.47 (mg/kg) were isolated. Accumulation of Potassium was lowest and total protein was 42.44%, as established by kjeldhal method. Protocol of ISI-24-1-e was under of determination of total nitrogen, which was calculated using a nitrogen conversion factor of 6.25. The present study shows that *Mentha arvensis L.* is medicinally important because of fatty acids, elements and proteins as its content; this observation gives support to its use in traditional system of medicine to cure diseases.

Keyworδs: *Mentha arvensis L*, Fatty aciδs, Elements total, Protein, Plant.

Introδuction

Mint *(Mentha arvensis L)* belongs to the family Lamiaceae. It is locally known as pudina in Urdu and in English, as Mint which is cultivated abundantly throughout India. The aerial parts of the herb contains a great amount of fragrant chemicals resembling menthol, menthone, linalool, isomenthone, menthofuran, terpenes and piperitenone oxide, which are used in pharmaceuticals, food, flavor, cosmetics, beverages and industries. (Khanuja 2007; Patra *et al*, 2002 Verma, *et al* 2010). Lamiaceae herb family consists of more than 252 genus and 7000 species (Hedge, 1992). This family is known for the wealth of species with medicinal properties, which have been used since early period and numerous of these species are universal in Mediterranean region (Ali *et al.* 2000). According to WHO information that 70% of the people of developed countries depend on natural- products drugs for health care (Kumar *et al* 2004). *M. arvensis L* is distributed throughout the western Himalayas and is cultivated throughout world for use as a vegetable. It is used as a carminative, anti-spasmodic, anti peptic ulcer agent. It treats indigestion, skin diseases, coughs and colds in folk medicine. According to many researchers, the plant have 90% mint oil, monoterpenes, (menthone, menthofuran, menthyl acetate, cineole), sesquiterepenes, flavonoids, luteolin, menthoside, phenolic acids triterenes, tocopherols, betaine, rosmarinic acid, cyclenes; tannin; (Pino 1996, Liest, 1998). It serves as a good blood cleanser, antiseptic, antibacterial. Its leaves are used in care for insect bites.

Most of the chemical compounds used in the formulation of medicine are obtained from this plant. There are alkaloids, flavonoids, glycocides, saponins, triterpenes, carotenoids, fatty acid, eloeostearic acid; extract of which act as medicine. (Mors *et al.* 2000, Ferreira *et al.* 1992. Lindahl 1997. Singh 1997; Chisholm 1967). All types of treatment methods e.g. Hikmat, Ayurvedic, Allopathic and Homeopathic, are based on plants commercially (Mehmood *et al* 2003). Life is totally dependent on plants (Iqbal

2006, Wani *et al*. 2010). A number of physiologically important substances are synthesized by the plants (Kretovich, 2005). Plant's role in human nutrition is paramount, especially in malnourished communities of developing countries (Conway 1999; Mohamed 1992, Yanez *et al*. 1995). Fatty acids are organic compounds which are very important as human diet. They contain omega-3 fatty acids and poly unsaturated fatty acids which prevent diseases (Burgess *et al*. 1991; Wise et al. 1994).

Material anδ Methoδs

Collection of plant material

Mentha arvensis L were collected from Mentha & Allied Products Ltd, Rampur (March-April 2014).

The collected plant materials were washed with tap water followed by distilled water and dried in shade at room temperature for 24 days.

Extraction

The dried plant was chopped into small pieces and was dipped into 2 liter ethanol (EtOH) for about one month at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure at below 40°C using rotary evaporator, which yielded dark green gummy residue. The extracts were then partitioned with Ethyl acetate (EtOAc) and water, this procedure was repeated 3 times.

Column chromatography (CC)

The residue containing fatty acids fraction was separated on chromotagraph over silica gel. (70-230 mesh Merck) column. The column was first eluted with n-hexane and thereafter chloroform was added in order of increasing polarity. First fraction was eluted with pure hexane, fraction "A" was eluted from hexane: chloroform (85:15), fraction "B" from hexane: chloroform (80:20), fraction "C" from hexane :chloroform (75:25), and fraction "D" from hexane, chloroform (70:30).

Esterification

All fractions (A-D) were esterified with diazomethane, 0.5 mg of each fraction was dissolved in MeOH and 0.5 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated. The methylated fatty acid fractions were anyalized by GC-MS.

Gas chromatography-mass spectrometry (GC-MS)

The fatty acids (methyl ester) fraction were finally analysed and identified by GC-MS. The analysis was performed on JEOL JMS 600H Agilest 689 ON, equipped with 30m X 0.32 ZP-5 MS column, stationary phase coating 0.25µm. The column temperature was kept at 70°C for 2 min with increased at the rate of 4°C per min up to 250°C. Injector temperature 250°C, split ratio 1:45, the carrier gas (N_2/QHe flow rate 1.0ml/min. (Khanzada *et al.* , 2008 a.b. , 2013, 2014).

Elemental Assay

The samples were investigated for elemental anaylsis by using atomic absorption spectrophotometer (AAS), Hitachi Ltd. 180-50.S.N.5721. Appropriate working standard solution was prepared for each element (Khanzada et al. 2008, a.b, 2013, 2014).

Total protein analysis by kjelδhal methoδ

The sample was digested in H_2SO_4 (30ml) in the presence of catalyst $CuSO_4$ (1g) and K_2SO_4 (10g), after digestion sodium hydroxide (NaOH, 33%) was added followed by steam distillation; the distillate was collected in 20ml boric acid(4%). Then nitrogen content was determined by using titration with HCl (0.01N). A factor of 6.25 was used to evaluate total protein contents (Khanzada *et al*. 2008 a.b., 2013, 2014, 2015).

Total Protein Analysis

Protocol of ISI-24-1-e was found under determination of total nitrogen, which was calculated using a nitrogen conversion factor of

Table-1 Saturateδ fatty aciδs of Mentha Arvensis L analyizeδ as methyl ester

S.No.	Systematic Name	Common Name	Molecular Formula	Mol. Wt	R.R.T.	Rel. %ag
1	Methyl-n- heptenoate	Heptylete	$C_8H_{16}O_2$	144	17.37	2.18
2	Nonanoate	Laurate	$C_{13}H_{26}O_2$	214	19.63	3.40
3	n-Hexadecanoate	Palmilate	$C_{17}H_{34}O_2$	270	28.7	4.90
4	n-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	32.47	2.99
5	n-Docosanoate	Behenate	$C_{23}H_{46}O_2$	354	38.68	4.90
6	n-hexacoseonate	Cerotate	$C_{27}H_{54}O_2$	410	43.68	3.40
Total						**21.77**

6.25 (Khanzada *et al*. 2008, a.b, 2013, 2014, 2015).

Results anδ Discussion

The present investigation demonstrated the usefulness of *Mentha arvensis L* plant extract. This plant was analyzed for their fatty acid composition; the results obtained have been presented in (Table-1, -2). 29 total compounds were isolated, 6 SFAs and 23 UFAs and these tables also show their relative retention time (RRT) and relative percentage of their occurrence. The lowest and highest relative % of saturated fatty acids present *Mentha arvensis L* was that of Methyl-n-Heptenoate (2.18%) and n-Hexadecanoate (4.90%). The lowest percentage of unsaturated fatty acids Methyl (all cis)-eicosapentaenoate (0.91%)and the highest was of Heptadecadienoate, (19.59%). The percentage of SFAs and UFAs was 21.77% and 78.22 repectively.

In previous work of Pithecellobium dulce Benth. Fatty acid analysis, 9 saturated and 17 unsaturated fatty acids were identified and in *Calotropis procera* medicinal plant. There were 18 different fatty acids (FAs); saturated fatty acids were in greater quantity (52.92%) than unsaturated fatty acids (47.08%) (Khanzada et al 2008 a,b, 2013.). It was noted that fatty acids percentage (65.9%) in stem of *Calotropis procera* was higher than 58.7% of fatty acids present in whole plant extract of *calendula officinalis.* Flowers-extract contain total 37 fatty acids among which 13 were saturated 24 unsaturated; the unsaturated fatty acids were present in higher percentage than saturated ones (Khanzada *et al* 2014).

Total Protein Anaylsis

The total protein contents isolated from whole plant *Mentha arvensis L* was 42.44%. According to the previously reported work in *Tamarindus indica,* it was noted that 15.6% (Jamshoro), 10.8% (Nawabshah Ditt.) and 8.7% (Hyderabad Distt.) .(Khanzada et al. 2008a,b). The total protein content of T. indica was found to be lower (14%) when compared to an earlier report. (Arinathan *et al*, 2003). Its seed protein was 6.9% (M Pugalenthi *et al.* 2004). On comparison with the previously reported work in respect of different medicinal plants, protein from *Calotropis procera,* in leaf extracts 23.94, stem 8.94, bark 12.69 (Kalita *et al.* 2004). Watermelon seed protein, the high-quality dietary proteins, such as used in food formulations was recently reported by Ali et al. 2011. Total protein content in seeds of Pithecellobium dulce Benth was 67.11, while stems, roots and leaves contain, 15.72%, 10.58% and 13.75%

Table-2 Unsaturateδ fatty aciδs of *Mentha Arvensis L* analyizeδ as methyl ester

S.No	Systematic Name	Common Name	Molecular Formula	Mol. Wt	R.R.T.	Rel. %ag
1	Dodecenoate	Lauroleic	$C_{12}H_{22}O_2$	198	25.97	1.48
2	Tridecadienoate	Tridecadienooate	$C_{13}H_{22}O_2$	210	58.88	1.62
3	Tridecatrienoate	Tridecatrienoate	$C_{14}H_{22}O_2$	222	18.67	2.36
4	Methyl-2-tridecynote	Tridecynoate	$C_{14}H_{24}O_2$	224	28.12	1.33
5	Methyl tricosenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	29.32	1.63
6	2,4,5- tetradecatrienoate	Tetradecatrienoat e	$C_{15}H_{24}O_2$	236	25.28	2.72
7	Hexadecenoate	Palmileate	$C_{17}H_{32}O_2$	268	50.43	1.9
8	Heptadecadienoate	Heptadecadienoate	$C_{18}H_{34}O_2$	280	39.53	19.59
9	Octadecadienoate	Octadecadienoate	$C_{19}H_{34}O_2$	294	36.42	2.99
10	10-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	31.97	3.81
11	Nenodecenoate	Nenodecenoate	$C_{20}H_{38}O_2$	310	33.73	0.95
12	Methyl(all cis)eico-sapentaenoate	Eicosapentaenoate	$C_{21}H_{36}O_2$	316	61.7	0.91
13	Eicosadienoate	Eicosadienoate	$C_{21}H_{38}O_2$	322	47.45	1.63
14	9-Eicosenoate	Gadoleate	$C_{21}H_{40}O_2$	324	45.83	3.67
15	Heneicosenoate	Heneicosenoate	$C_{22}H_{42}O_2$	338	37.12	5.55
16	Methyl tricosenoate	Methyl tricosenoate	$C_{24}H_{46}O_2$	366	40.22	5.55
17	Tetracosadienoate	Tetracosadienoate	$C_{25}H_{46}O_2$	378	41.68	4.76
18	n-Pentacosenoate	Pentacosenoate	$C_{25}H_{48}O_2$	380	45.13	2.99
19	n-Hexacoseoate	Hexacosenoate	$C_{26}H_{50}O_2$	394	42.75	3.4
20	Methyl hexacoseonate	Hexacosenoate	$C_{27}H_{52}O_2$	408	51.08	1.63
21	Nonacosenoate	Nonacosenoate	$C_{30}H_{56}O_2$	450	43.25	3.67
22	Methyln-n-dotriacontanoate	Dotriacontanoate	$C_{33}H_{64}O_2$	492	54.55	1.77
23	n-Tritriacontanoate	Tritriacontanoate	$C_{34}H_{66}O_2$	508	57.28	2.31
Total						**78.22**

6 Saturated, 23 Unsaturated, Total compound = 29, Total %age of Saturated + Unsaturated fatty acid = 99.99, Mol. Wt = molecular weight, R.R.T. = Relative retention time, Rel.% age = relative percentage.\

respectively and flowers and fruits 14.76% and 10.50%. Recent work on Pithecellobium dulce Benth seeds show that they have rich sources of protein. (Khanzada *et al* 2013). In calotropis procera of Sindh, the highest percentage of protein was 50.80% of dry weight (Daulatpur), 32.11% (Nawabshah), 25% (Hyderabad) and (Jamshoro), and 29.45% from different sites (Khanzada *et al* 2008 b). Total protein in *Calendula officinalis L* flowers as reported is 30.20% (Khanzada *et al* 2014).

Elements Anaylsis

Six elements viz, copper, 11.17 (mg/kg); Iron, 5.8 (mg/kg); Sodium 60.20 (mg/kg); Magnesum 16.20(mg/kg); Potassium, 10.3 (mg/kg) and zinc 12.47(mg/kg) were isolated from *Mentha arvensis L*. Accumulation of Potassium 10.31 (mg/kg) was the lowest while sodium present with highest 60.20 (mg/kg) accumulation. On comparison with others, Magnesium 60.1 is present in T. Indica and minimum ratio in Copper, 0.76 (Khanzada *et al*. 2008a, Ishola et al 1990), Sodium 28.83, Calcium 315.28, Potassium 248.56, Magnesium 285.14, Phosphorus 369.47, Iron 7.14, copper 0.59, Zinc 6.94, Manganes 0.81 (Pugalenthi *et al*. 2004). Magnesium 71.10, Iron 2.0 Copper, 2.0, Sodium 8.0, Potassium (mg) 270.00 (Lewis *et al* 1964 , Anon, 1976, Duke 1981) detected mineral composition Calcium 36.6, Copper 2.10, Manganese 12.1, Sodium 8.90, Iron 45.5, Zinc 7.00, Potassium 1308, Magnesium 104 (Ajay *et al.* 2006). *T. Indica* seeds have Calcium, 10.00 Potassium 21.0 Sodium, 2.1. Magnesium, 15.0 Iron, 75.9. (Yusuf *et al* 2007) and Copper 9.09, Magnesium, 1.153, Sodium 62.1, Iron 31.7, zinc 13.2, Potassium 6.54 (Robert *et al* 2005).

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Effect of Season on *in vitro* Shoot Multiplication of Interspecific F1 Hybriδ (*E.tereticornis X E.grandis*) of Eucalyptus

Barkha Kamal anδ Vikash Singh Jaδon

Department of Biochemistry and Biotechnology, Sardar Bhagwan Singh (P.G.) Institute of Biomedical Sciences & Research, Balawala, Dehradun-248001, Uttarakhand, India

Corresponding author: E-mail: barkhabiotech2005@gmail.com

Abstract- The present investigation, explants collected during the month of July-Sept were most suitable for *in vitro* studies for micropropagation. 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 achieved in 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 axillary bud proliferation was influenced by the cytokinin and auxin concentration in the MS medium.

Keyworδs: Eucalyptus F1 hybrid, Mature nodal segments, MS medium, Micropropagation

Introδuction

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). Thus, the present study is aimed to describe an efficient in vitro tissue culture strategy and to assess the seasonal morphogenic response of the said commercially viable Eucalyptus F1hybrids under in vitro conditions.

Material anδ Methoδs

Plant Materials

Explants in the from of nodal segments(2-3 cm) were collected from mature, field grown, disease free and healthy plants of Eucalyptus for axillary bud proliferation and were washed with detergent (5-6 drops) and rinsed with tap water followed by sterilization of 0.1 % (w/v) $HgCl_2$ (Mercuric chloride) or $NaOCl_2$ (Sodium hypochlorite) and H_2O_2 (Hydrogen peroxide). Sterilization time varied with the explants used. They were finally washed thrice with autoclaved distilled water to remove the sterilants that can be harmful for explants.

Cultureδ Conδitions

All cultures were incubated at 25 ± 2°C temperature and relative humidity of 50-60 % maintained in culture room by air conditioners. All culture were kept under illumination of 16 hrs photoperiods with light intensity of 2500 lux obtained white cool fluorescent tubes of 40 watts (Philips, India).

Statistical Analysis

Data collected was analyzed using CRD design of experiments, as it is one of the most widely used designs in case of tissue culture experiments (controlled set of conditions and for homogenous materials) and gives best result. Minimum of 12 replicates were taken per treatment and each replicate consisted of at least 2 explants in each with repetition of experiments thrice. Degree of variation was shown by standard error and critical difference at 5%. Significance level was tested at 1%, 0.5% and 0.1% level. Arc sine transformed values were used in case of percentage value.

Results anδ Discussion

Explant Collection

Nodal segments containing axillary buds were found to be the best explants and were collected from 25-30 years old tree of Eucalyptus F1 hybrids growing in Forest Resaerch Institute (altitude 610 m, latitude 30 C N longitude 78E, annual rainfall 216 cm) for micropropagation. Other explant selected like shoot tip, leaf disc, met with complete failure. The suitability of the nodal segments has already been established in micropropagation of Eucalyptus hybrids (Bisht *et al*., 2000a reported in FRI-5 and Kapoor and Chauhan (1992) reported in FRI-14). The use of dormant axillary buds or nodes ensures allocation of reserved food and axillary substance for micropropagation. The technique offers the potential to raise thousands of plantlets from the nodal region of the existing clumps.

In the hybrid the bud break was influenced by the season of their collection and on the medium they were cultured. Cold and dry season adversely affected the bud break response the same has been reported by (Kapoor and Chauhan, 1990; Bisht *et al.,*1999; Bisht *et al.,*2000).

Effect of Season

In starting aseptic culture of the axillary buds for multiplication of the species, the main hazard faced was that of exudation of phenolic substance into the media. Probably because of this exudation of phenolic substances the explants could not survive and eventually died without regenerating buds. Media were changed 3-4 times at 2-3 days intervals from the beginning of the culture to overcome this exudation, but with little effect . Axillary bud explants were used from upper shoots of the adult trees or when new shoots arose. These shoots showed least phenolic exudation and responded to bud multiplication. In the present investigation, explant collected during the month of July-Sept were the best for *in vitro* studies for micropropagation (Table-1). Similary, Das and Mitra (1990) have reported exudation of phenolics was least in the coppiced shoots which responded to bud multiplication. They also observed explants collected during July-Sept were the best for *in vitro* studies for micropropagation. In Oct–Nov phenolic exudation was high even in the axillary bud explant from coppiced shoot. In May and June, this multiplication was almost negligible and associated with high amount of phenolic exudation.

It has been specified by Bonga (1982) that multiplication of shoot bud explant of both tropical and temperate trees is easier if the explant is taken from germinated seedlings and their phenolic exudation is also much less than the mature trees. But multiplication of trees through seed germination is not a satisfactory means of preserving the characteristics of a desired clone. However, there are very few reports in the literature, showing impact of season on *in vitro* axillary bud break. Das and Mitra (1990) have reported that the nodal explant of Eucalyptus collected during July –September responded favorably.

Surface Sterilization of Explant

Heavy bacterial and fungal contamination of nodal segments collected from mature tree was one of the major problems in the *in vitro* propagation of Eucalyptus species. Out of various sterilizing agents tried on FRI-6, it was observed that explants were very much susepltible to NaOCl or H_2 O_2 and majority of them were contaminated by fungus and bacteria and died after few days of inoculation and not even a single bud break was observed. When using 0.1%$HgCl_2$ for 15 min and followed by 1%Bavistin treatment for one min gave fruitful result and at this concentration up to 80 % nodal segments (explant) remained green and uncontaminated (Table-2). In earlier report on this hybrid 30% NaOCl treatment for 20 min gave fruitful results and at this concentration up to 50 % nodal segments remained green and uncontaminated (Joshi *et al*., 2003). On the basis of these findings

Table-1 Effect of season on axillary buδ break anδ phenolic exuδation using noδal segments in MS meδium supplementeδ with 1.5 mg/l BAP +1.5 mg/l NAA. Data recorδeδ after 5 weeks.

Months	Initial no. of replicates inoculateδ	Response %	Phenolics Exuδation
Jan	24	[illegible]	+++
Feb	24	29.16 ± 0.09	++
Mar	24	29.16 ± 0.09	++
Apr	24	20.87 ± 0.23	+++
May	24	8.30 ± 0.12	+++
Jun	24	12.50 ± 0.04	+++
Jul	24	33.30 ± 0.09	++
Aug	24	54.16 ± 0.09	+
Sept	24	62.30 ± 0.09	+
Oct	24	33.30 ± 0.02	++
Nov	24	8.30 ± 0.06	+++
Dec	24	4.16 ± 0.03	+++
Significance		***	
CD		0.388	

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

0.1% $HgCl_2$ for 15 min and followed by 1%Bavistin for one min treatment was used for surface sterilization of nodal segments.

Effect of Phytohormones

Shoot bud differentiation in cultured tissues is dependent on the auxin/cytokinin ratio in the medium (Bonga and Von Aderkas,1992). The requirement for exogenous auxin and cytokinin in the process of bud differentiation varies with the tissue system and apparently depends on the endogenus levels of the two hormones in the tissue viz, an auxin and a cytokinin. Presence of cytokinin in the medium leads to the promotion of bud differentiation and development. BAP is one of the cytokinin, which is usually used in the establishment and multiplication of a wide range of plant species.

In FRI-6 (*E.tereticornis X E.grandis*) also, incorporation of BAP and NAA into the medium was found to improve the incidence of bud break and promote multiple shoot formation. For Eucalyptus F_1 hybrids, the bud break and number of shoots obtained were significantly influenced by different BAP and NAA treatments. The frequency of bud break on untreated control was very low or nil. FRI-6 maximum bud break (50-55%) was obtained on 1.5 mg/l BAP +0.1 mg/l NAA (Table-3). These results compare favorably with those of other workers, indicating the

Table-2 Effect of steriliant on the percent survival of explant δuring establishment phase.

Steriliant	Conc.	Duration (min)	% survival of uncontaminateδ
NaOCl	10%	10	0 ± 0
	20%	12	9.72 ± 1.39
	30%	15	30.55 ± 3.68
$HgCl_2$	.05%	10	59.72 ± 6.05
	.10%	12	95.83 ± 4.17
	.15%	15	100.00 ± 0
H_2O_2	10%	10	15.27 ± 3.68
	20%	12	33.33 ± 7.22
	30%	15	56.94 ± 2.78
Significance			***
CD			5.63

Table-3 Effect of combination of Cytokinin (BAP +NAA) on axillary buδ inδuction in MS Meδium using noδal segments.

Hormonal concentration (mg/l) BAP+NAA	Number of replicates inoculateδ	Response %
0.1 + 0.1	24	-
0.1 + 0.5	24	-
0.1 + 1.0	24	-
0.1 + 1.5	24	-
0.5 + 0.1	24	-
0.5 + 0.5	24	4.39 ± 0.31
0.5 + 1.0	24	-
0.5 + 1.5	24	-
1.0 + 0.1	24	4.39 ± 0.31
1.0 + 0.5	24	12.50 ± 0.29
1.0 + 1.0	24	16.50 ± 0.30
1.0 + 1.5	24	8.34 ± 0.20
1.5 + 0.1	24	58.43 ± 0.30
1.5 + 0.5	24	45.61 ± 0.31
1.5 + 1.0	24	33.44 ± 0.29
1.5 + 1.5	24	16.57 ± 0.30
Significance		***
CD		0.63

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

efficiency of BAP and NAA for shoot culture initiation and multiplication in several Eucalyptus F1hybrids. Bisht *et al.,* (2000) used BAP and NAA for the establishment of cultures in FRI-5 (*E. camaldulensis X E. tereticornis*). Similarly, Joshi *et al.,* (2003) obtained single bud and multiple shoot in *E.tereticornis X E.grandis* on medium containing 1.0 mg/l BAP+1.0 mg/l NAA respectively. Kapoor and Chauhan (1992) reported that incorporation of BAP and NAA is essential in case of Eucalyptus hybrid (FRI-14).

MS medium proved to be the best medium for the establishment of shoot cultures in both 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 (Bisht *et al*., 2000a and 2000b; Joshi *et al*., 2003; Kapoor and Chauhan 1992; Gupta *et al*., 1981).

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Aleurites moluccana Seeδs: A Rich Source of Linolenic Aciδ

Rashmi anδ Sapna Bharδwaj
Chemistry Division, Forest Research Institute,
P.O. New Forest-248006, Dehradun, India

Abstract - *Aleurites moluccana* (kukui tree) belongs to family *Euphorbiaceae* and native to Indo-Malayan region. Kukui tree, like other oilseeds, is a potentially valuable source of fatty acid which is rich in alpha-linolenic acid (ALA), linoleic acid (LA) and oleic acid (OC). The fatty oil content in the kernels of *Aleurites moluccana* was found to be 58.15% on air dried basis and 60.68% on the moisture free basis. GLC analysis of kukui seed oil shows the presences of eleven fatty acids and linolenic (49.55%) was identified to be the major constituent of its seed oil. Linolenic acid is also reported as an essential omega-3 fatty acid and needed for normal human growth and development.

Keywords: *Aleurites moluccana,* Seedoil, GLC, Linolenic acid.

Introduction

Aleurites moluccana (L) Wild (Family-*Euphorbiaceae*), commonly known as the kukui tree, jungle akrot, candlenut and wild walnut. Kukui is one of the great domesticated multipurpose trees of the world.

It has been extensively used in folk medicine for the treatment of ulcers, headache, fevers, diarrhea and hypercholesterolemia (Niazi et al., 2010). The bark and flowers are used for asthma, sores, ulcers and tumors, and as laxative. The fruit is tonic and found to be useful in diseases of heart and blood. It is carminative and expectorant, used in piles, hydrophobia and ring warm. The oil is purgative and sometimes used like castor oil. Kernels of *A. moluccana* are laxative, stimulant and sudorific. The oil is rubbed on scalp as a hair stimulant. Malayans apply boiled leaves to the temples for headache and to the pubes for gonorrhea (Anon, **1985)**. The candlenut stem bark is traditionally used for diarrhea (Wiart, 2006). It is also used in East Borneo to cure typhoid. They have been screened for their potential uses as alternative medicines for many infectious diseases in other countries (Acharyya et al., 2010). In Japan, the stem bark was used for tumors while in Malaysia; decoction of the leaves was used in treating coughs, diarrhea, and pain in the chest. Boiled leaves were used for headaches, fever, ulcers, and gonorrhea. The candlenut fresh sap was used to treat thrush and candidiasis (Krisnawati et al., 2011; Scott, 2000). The fruit juice was squeezed into the mouths of new born babies to make them vomit and to clear their throats (Han, 1998). *Aleurites moluccana* seeds contain glycerides, linoleic, palmitic, stearic, myristic acid, oil, protein, vitamin B1 while the stem bark contains alkaloids, polyphenols, flavonoids, coumarins, tannins, steroids and triterpenoids (Silva, et,al., 1997; Samah,et,al., 2010).

A number of secondary metabolites have been isolated and characterized from different parts of *A. moluccana.* Three novels 3, 4-seco-podocarpane-type trinorditerpenoids, moluccanic acid, moluccanic acid Methylester, and 6, 7-dehydromoluccanic acid were isolated from the twigs and leaves (Liu, *et.al*. 2008). A new phorbol diester, 13-O-myristyl-20-O-acetyl-12-deoxyphorbol was isolated from the benzene extract of the heartwood. In addition, hentriacontane, 6, 7-dimethoxycoumarin, 5, 6, 7-trimethoxycoumarin and β-sitosterol have been reported for the first time from this species (Satyanarayana *et al.,* 2001). Two compounds swertisin and a flavonoid C-glycoside from leaves and bark have been isolated and characterized. A new coumarinolignoid moluccanin (I) was isolated from stem chips of *A. moluccana* (Shamsuddin, *et.al*., 1988). Kukui nut oil acts in synergy as an adjunct to the therapeutic agent to promote the alleviation of the medical condition

being treated. The oil is extracted on a large scale in China and in Philippines, but it does not appear to be expressed anywhere in India. The kukui nuts are being used for constipation or cleansing. The oil is starting to become a source of ingredients for cosmetics preparation and quicker drying than linseed oil. It is used as a wood preservative, in varnishes and paint oil, as an illuminant, for soap making, water proofing paper, rubber substitutes and insulating material. Oil is also painted on bottom of small crafts to protect against marine borers. Oil is also used topically to stimulate hair growth in Fiji (Darmstadt, et.al, 2002). Linolenic acid is principal unsaturated fatty acids in plants and an essential fatty acid in mammals that cannot be synthesized by tissues and must be obtained in the diet. It helps in reducing inflammation and prevents chronic diseases, such as heart disease and arthritis. In the body, it is particularly important for cognitive and behavioral health as well as normal growth and development (Angerer, *et.al,* 2007). Therefore, seeds of *A. moluccana* were explored chemically.

Material and Methods

The seeds of *Aleurites moluccana* were collected from the campus of Forest Research Institute, Dehradun. The kernels from the seeds were removed and crushed to obtain a coarse powder. The powdered seeds were extracted separately with petroleum ether (60^0-80ºC) by using a soxhlet apparatus. The solvent was removed under reduced pressure to recover fatty oil (Sawhney, 2005). The oil was saponified with 0.5N alcoholic potassium hydroxide (KOH) for two hours and the mixture of fatty acids and unsaponifiable matter of the oil was separated by using routine methodology (Hilditich. 1956). Fatty acid methyl esters were prepared by refluxing the mixture of fatty acid with 1% sulphuric acid /MeOH on water bath for 4 hr, cooled and usual work up yielded methyl esters.

The fatty acid methyl esters were analyzed using Agilent 6850 Gas chromatograph unit equipped with FID. A non-bonded cyanosilicone column SP-2330 (30 m x 25 mm, id x 0.20 mm, film thickness) was used for the purpose. The oven temperature was programmed from 170 to 220ºC at 5ºC/min and the flow rate of carrier gas (N2) was 1.5 mL/min. The injector and detectors were maintained at 230 and 250ºC respectively, and the area percentages were recorded with a HP Chemstation data system.

Results

The fatty oil content obtained from the seeds of *A. moluccana* was found to be 58.15% on air dried basis and 60.68% on the moisture free basis. The oil ranges in colour from pale yellow to orange. Physico-chemical properties were determined (Table-1).

GLC analysis of a mixture of methyl esters of the fatty acids prepared from the fatty oil showed the presence of eleven fatty acids in the oil. The identified fatty acids constituted 100% of the mixture of fatty acids obtained from the fatty oil. The fatty acid composition indicated that Linolenic acid (49.55%) is the major constituent of the oil followed by arachidic acid (16.76%), palmitic acid (16.42) while oleic acid (6.02%), pelargonic acid (2.54%), capric acid (2.37%) etc. are the minor components (Table2). Literature survey reveals that in an earlier report, *A. moluccana* seed oil was reported to contain

Table- 1 Physico-chemical Characteristics of *Aleurites moluccana seed* oil.

Characteristics	*Aleurites moluccana*
Oil (wt. %)	60.68
Specific gravity (d^{19})	1.0860
Refractive Index (η_D^{20})	1.479
Acid value	6.148
Saponification value	195.508
Ester value	172.8
Unsaponifiable matter (wt. %)	0.891
Protein content	20.337

Table-2 Fatty Aciδ composition (wt %) of *Aleurites moluccana*

Fatty aciδ	Composition (wt %)
C:9	2.54
C:10	2.37
C:12	1.09
C:14	1.40
C:16:0	16.42
C:17	0.75
C:18:1	6.02
C:18:3	49.55
C:20:0	16.76
C:21:0	2.35
C:22:0	0.754

palmitic acid (5.5%), stearic acid (6.7%), oleic acid (10.5%), linoleic acid (48.5%) and linolenic acid (28.5%) (Anon, 1985) but in our study, we found linolenic acid (49.55%), arachidic acid (16.76%) and palmitic acid (16.42%) as major constituents.

Discussion

The present study revealed that *A. moluccana* seed (Kukui nut) oil is a promising source of linolenic acid. It is the major constituent of fatty oil and constitutes 49.55% which is significantly very high. As reported earlier, linolenic acid is an essential omega-3 fatty acid. In the human body, linolenic acid is used to make substances called eicosanoids, which regulate inflammation. It is also a component of cell membranes and is converted to the longer chain omega-3 fatty acids eicosapentaenoic acid, or EPA, and docosahexaenoic acid, or DHA. The omega-3 fatty acids deliver numerous health benefits, according to the National Institutes of Health Office of Dietary Supplements (Strak, et.al, 2008). Along with linolenic acid, fatty oil of kukui nut also contains some other important fatty acid like arachidic acid, palmitic acid, oleic acid and pelargonic acid in significant amount which have various therapeutic applications. Therefore, this study would lead to the exposure of *A. moluccana* as a very important and rich source of fatty acid (linolenic acid) in the category of tree born oil seeds (TBOS).

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Screening anδ Isolation of The Soil Bacteria For Ability To Proδuce Antibiotics

Afshan Tarranum, Harish Rana, Raj Kumar anδ Mona Chauhan
Department of Life Science, Beehive College of Advance Studies, Dehradun
E-mail: afshantarannum2003@gmail.com

Abstract- Antibiotics are one of the most important commercially exploited secondary metabolites produced by bacteria and employed in a wide range. Most of the antibiotics used today are from the microbes. Bacteria are easy to isolate, culture, maintain and to improve their strain. Bacillus species being the predominant soil bacteria because of their resistant endospore formation and production of vital antibiotic like bacitracin etc. are always found inhibiting the growth of the other organisms. In the present research study, soil bacteria with the antibiotic activity were screened and isolated. The media used in this research was nutrient agar medium. 1g of the soil samples were dissolved in 10ml of sterile water to make soil suspensions. Portion of the suspensions were inoculated on the nutrient agar by streaking and were incubated at 37°C for 24 hours. The inhibitory activities of the isolated microorganisms were checked against some of the important microflora like *Staphylococcus aureus* and *Pseudomonas* species.

Keyworδs: Bacterial Inhibition, Heterogeneous Soil, Antibiotic, Endospore.

Introδuction

The term soil refers to the outer loose material of the earth crust. It may be regarded as a three phase system composed of solids, liquids and gases, dispersed to form a heterogeneous matrix. The bacteria are the most abundant group usually more numerous than the four combined. Soil bacteria can be rod, (bacilli) cocci (spherical) spirilla (spirals), of these, bacillus are more numerous than the others. They are one of the major groups of soil bacteria population and are very widely distributed [Bhagabati A.,*et al*.,2004]. Antibiotics are medicines that kill bacteria or slow the growth of bacteria. They are used to cure diseases. Antibiotics were first produced in 1939. The term antibiotic was introduced by S.A Waksman in 1942. There are numbers of bacteria having potential to produce antibiotic example of which is bacillus species which produce antibiotic like bacitracin, pumulin and gramicidin which are active against Gram positive bacteria such as *Staphylococci, Streptococci, Corynebacter, Streptomyces* species which produce antibiotic like tetracycline, cloramphenicol, vancomycin, gentamycin which are active against Gram negative bacteria and Lactobacillus species which produce antibiotics like nisin which is produced by *Lactobacillus lactis* (Waites M.J. *et al., 2008*). Hawker, E.L. and Linton, H.A.., (1979) mentioned that large numbers of actinomycetes, as many as millions per gram, are present in dry worm soil and the large count on dilution plates suggest that this group is present very largely as spores. *Bacillus* species of the family *Bacillaceae* is the largest in the order. The genus contain gram positive, endospore forming, chemotherotrophic rod that are usually motile with peritrichious flagella, it is aerobic and catalase positive. Many species of *Bacillus* are of considerable importance because they produced the antibiotic [Waites, M.J., *et al*., 2008]. *Streptomyces* is very important medically, the natural habitat of most *Streptomyces* being the soil, where they may be constitute from 1to 20% of the culturable population. *Streptomyces* are best known for the synthesis of a vast range of antibiotics. *Lactobacillus* species produce many kind of antibiotic which share a full range of antimicrobial activity, example of which is nisin which is produce by *Lactobacillus lactis* and active against many gram positive organism such as *Corynebacter, Clostridia.* [Waites, M.J., *et al*.,2008].

Methoδs anδ Material

Collection of soil sample

In systematic screening program for isolation of bacteria eight soil samples were collected at different location of Selaqui, Beehive hostel, Beehive college, Pharma city, Haripur, Siwali academy, Green valley school, Rampur, ITI Jamanpur were collected from upper layer where most of the microbial activity takes place and thus where most of the bacterial population is concentrated. Soil sample were collected using some clean dry and sterile polythene bag along with sterile spatula.1gm of the soil samples were dissolved in 10ml of water to make soil suspensions.

Isolation of bacteria: 28gm of nutrient agar powder was weighted and dissolve in 1000ml and of distilled water. It was stirred vigorously and dissolved using hot plate after which was sterilized in autoclave for 15 minutes at 121^0C. It was then allowed to cool after which dispensed in Petri dishes and allowed to solidify.

Sample inoculation: Portion of the suspensions were the inoculated on the nutrient agar by streaking and were incubated at 37^0C for 24hours. After which colonies with a clear zone of inhibition were observed.

Gram's Staining

The Gram stain is used to classify bacteria on the basis of their forms, sizes, cellular morphologies, and Gram reactions. Gram stain permits the separation of all bacteria into two large groups, those which retain the primary dye (gram-positive) and those that take the color of the counterstain (gram-negative). The primary dye is crystal violet and the secondary dye is usually either Safranin O or Basic Fuchsin. Some of the more common formulations include: saturated crystal violet (approximately 1%), Hucker's crystal violet, and 2% alcoholic crystal violet.

Biochemical Tests

Inδole Test

The ability of an organism to use the amino acid tryptophan is one of the key metabolic tests used in conventional identification procedures. The metabolism of the amino acid tryptophan by organisms that possess the enzyme tryptophanase produces three major degradation products including indole resulting from the deamination of tryptophan. Indole, split from the tryptophan molecule, can be detected by the addition of *p*-dimethylaminobenzaldehyde (Ehrlich's or Kovac's reagent) to the test medium. After the addition of the *p*-dimethylaminobenzaldehyde reagent a distinct color reaction is observed. The source of tryptophan in the media is generally a peptone derived from casein.

The indole test procedure is a commonly used biochemical test for the differentiation of *Enterobacteriaceae*. The indole test is also commonly used in the identification of other bacteria.

Catalase Test

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is a potent oxidizing agent that can wreak havoc in a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. One of those ways is to make catalase.

Citrate Utilization Test

Simmon's Citrate agar slants contain sodium citrate (as the only carbon source) and ammonium ions (as the sole nitrogen source). A pH indicator, Bromothymol Blue is also included. Bromothymol Blue is **GREEN** at pH < 7.0 and **BLUE** at pH > 7.6. Organisms that utilize citrate for energy produce alkaline compounds as by-products. Thus, a positive result for citrate utilization is the formation of a **BLUE** color.

Table-1 Morphology anδ Biochemical Characterization of the Bacterial Isolates

	B1	B2	B3	B4	B5	B6	B7	B8
Gram's reaction	–	–	+	–	+		+	–
Shape	Bacilli	Bacilli	Cocci	Bacilli	Bacilli	Cocci	Bacilli	Cocci
Catalase	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	-	-	+	-
Inδole	–	+	–	+	–	–	+	–
Methylreδ	+	+	-	-	+	+	-	-
Glucose l	+	+	+	–	–	–	–	–
Lactose	+	+	+	+	–	–	–	–
Sucrose	+	+	+	+	–	–	–	–

MR-VP Test

MR-VP is a buffered Peptone-Glucose broth. Organisms that ferment dextrose will release acid into the broth. Adding Methyl Red, an indicator dye which turns red at pH 4.4 and yellow at pH 6.2, to the inoculated MR-VP medium indicates if the bacteria fermented dextrose. The Voges and Proskauer test was originally developed in 1898 by two German associates of Robert Koch. Some bacteria can be distinguished on the basis of their production of acetoin, a neutral end product, after incubation in buffered pepton-glucose media. The addition of alpha-napthol and KOH solutions will result in a pink- red color within a few minutes.

Triple Sugar Iron Agar Test

TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for detecting carbohydrate fermentation and ferrous ammonium sulfate for detection of hydrogen sulfide production (indicated by blackening in the butt of the tube). Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. To facilitate the detection of organisms that only ferment dextrose, the dextrose concentration is one-tenth the concentration of lactose or sucrose. The small amount of acid produced in the slant of the tube during dextrose fermentation oxidizes rapidly, causing the medium to remain red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube because it is under lower oxygen tension. After depletion of the limited dextrose, organisms able to do so will begin to utilize the lactose or sucrose (Musliu Abdulkadir and Salawudeen Waliyu, 2012).

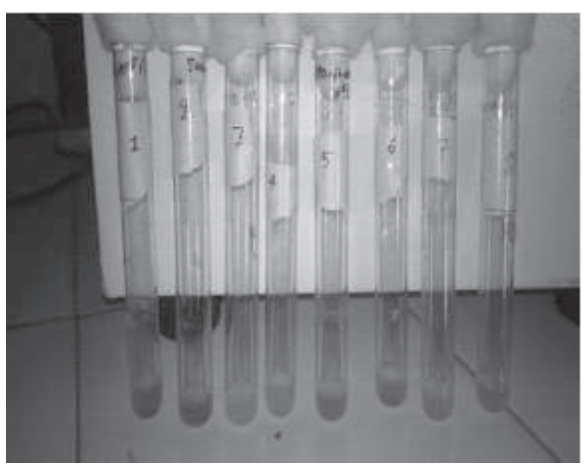

Fig. 1 Methyl Reδ Test

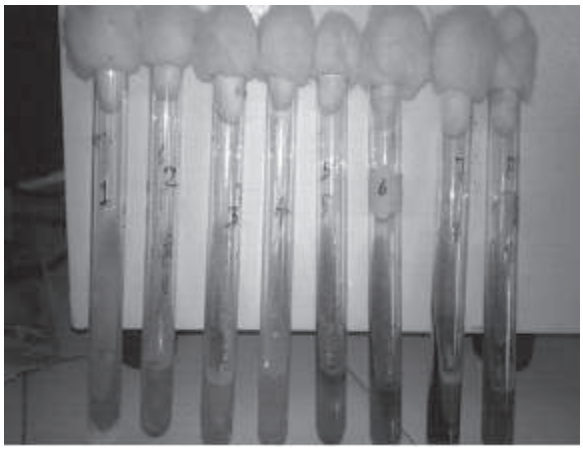

Fig. 2 TSI Test

Red, Vogues-Proskauer, and Citrate) that are used to differentiate among the Gram-Negative bacilli in the family *Enterobacteriaceae.*

Triple Sugar Iron Agar is used for the differentiation of microorganisms on the basis of dextrose, lactose, and sucrose fermentation and hydrogen sulphide production.

Out of the eight bacterial isolates two isolates show the potential of antibiotics production. The antibiotics isolates may be bactericidal or bacteriostaticin nature.

Bacterial strain no. seven has shown inhibitory activity against *Clostridium* and *S. gordini* while bacterial strain no. two inhibited the growth of *S.*

Table-2 Zone Inhibition in Millimetre (mm) of Bacteria Isolates against Test Bacteria (Bacterial isolates having potential of antibiotics proδuction)

Test organism	1	2	3	4	5	6	7	8
E.Coli	-	-	-	-	-	-	-	-
Clostridium sp.	-	-	-	-	-	-	10mm	-
Listeria sp.	-	-	-	-	-	-	-	
S. Gardoni	-	12mm	-	-	-	-	12mm	-

Results anδ DiscussioI

In searching for new antibiotics, relatively simple and rapid methods have been developed for screening microorganisms for antibiotic producing ability. In this study we isolated eight bacterial strains from different location of Selaqui. The morphological and biochemical characterization of the bacterial isolates has been given in the Table 1.

MR VP test is very useful in separating members of the family *Enterobacteriaceae* and some *Streptococcus*. Most members of the *Enterobacteriaceae* give either a positive MR or a positive VP test.

This test is among a suite of tests (Indole, Methyl-

gordini to a moderate extent. It may be concluded that these two strains isolated during the course of this research from soil samples have a potential to produce antibiotics.

As a future prospect these strains may be identified and improved by mutagenic agents to enhance their activity. Extraction and purification method can be employed for pure antibiotic production.

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Review– Nanoparticles in Ayurveδic Meδicine: Role of Bhasma anδ Plant Extract

Iqbal Ahmaδ[1*], Mohammaδ Shavez Khan[1], Mohδ. Musheer Altaf[1], S. Farooq[2]

[1]Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh-202002, India

[2]The Himalaya Drug Company, Dehradun-248001, India

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

Abstract- The concept of reduction in particle size of metals is recognized since Charak Samhita and confirmed by recent studies by various workers. The herbo-mineral formulations of Ayurveda known as 'Bhasma' are the major source of nanoparticle in Ayurvedic preparation and may be equivalent with nanotechnology witnessing the production of nanoparticles in the contemporary era. In this article, we have discussed the basics of nanoparticle detection and characterization from Bhasma and its potential application. On the other hand, a literature survey was done on the potential of Ayurvedic medicinal plants for their green synthesis of nanoparticles. Thirty-six plants belonging to twenty-seven plant family were demonstrated to synthesize silver and or gold nanoparticle. Based on present literature it can be concluded that Bhasma can be effectively used in enhancing the efficacy of Ayurvedic medicine through nanoparticles contribution. Further interaction of plant extract in contributing green synthesis of nanoparticles needs to be explored in Ayurvedic preparations containing Bhasma.

Keyworδs: Bhasma, Nanoparticles, Ayurvedic plants

Introδuction

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. Nanoparticles provide detail insight understanding the degree and variation in physiochemical properties of a voluminous material and its structures at a molecular level. Particle size often plays the insignificant role in determining the physical properties of a mass material, whereas, in the case of nanoscale material, this is not true, rather at nano levels, these same particles exhibit some of the most interesting and peculiar characteristics (Kumar et al., 2006). This variation or acquired characteristics of the nanoparticle is attributed to its very high aspect ratio, along with other explanations. This very high aspect ratio tremendously increases the biological efficacy of nanoparticles. Interaction of nanoparticle with other particles or structure also increases significantly, another important trait that explains the enhanced biological activity of nanoparticles, also attributed to its extraordinary aspect ratio. This concept of reduction of particle size of metal is prevailing since Charka Samhita. For a metallic preparation of metal based formulations, the metal is heated to very high temperature, and flaks are quenched in liquid media until a fine powder of metal is form. Nanotechnology can work at this level, to form the larger bioactive entity with a new structure of different physiochemical properties. Bhasmas are unique metallo-herbal Ayurvedic preparations are commonly suggested for treatment of multiple ailments also with other Ayurvedic medicines (Sarkar and Chaudhary, 2010).

In recent years, there is a tremendous interest shown by the scientific community in nanoparticles and development of nanotechnology and nanomedicine with great expectation and potential applications in health and medicine. Research in the traditional medicine has also been accelerated in the recent past due to its global recognition. The role of nanoparticles in Ayurvedic system of medicine was recognized since long. However, scientific evaluation and scientific data are less known. On the other hand, the use of metals and its oxides in traditional medicine is often considered a matter of toxicity points of view as Bhasma contains

inorganic elements such as Arsenic, Mercury, Lead, etc. (Weber 2004; Chan 2003). However, data on non-toxic nature of Bhasmas were also reported indicating their antioxidant and other biological activities (Kumar et al., 2006; Chan, 2003; Pattanaik et al., 2003).

In this article, we have tried to present two dimensions of nanoparticles in Ayurveda, first, Bhasma as a source of nanoparticle and second is the potential of Ayurvedic plant extracts to synthesis nanoparticles.

Bhasma as Source of Nanoparticles

Bhasmas are near to nanocrystalline materials, which are solid crystallites with size less than 100nm at least in one dimension. Bhasmas means "ash" derived from metals, their oxides combined with different herbs. Herbs and other adjuvants act as carrier moiety for the mineral part, promotes proper assimilation into the human body and also eliminate the harmful or toxic potential of metals (Savrikar, 2004; Svoboda, 1996). This unique combination of metals with organic macromolecules obtained from repeated incineration at the very high temperature that eventually led to the significant decrease in overall particle size (Kumar et al., 2006). Due to its small size basic character gets changed. It is mainly due to change in electrical, thermal, inorganic, optical, chemical and biological behavior. However, recent findings suggested that due to repetitive calcination of the chasm as leading to agglomeration of particles that result into increase particle size (Paul and Sharma, 2011). Therefore, it is apparent that the steps involve in the processing of bhasmas play a significant role in determining the particle size and subsequently the overall efficacy of the drug.

Recently, several Bhasmas have been investigated using modern analytical tools aiming at the structural characterization of these drugs and their mode of action inside the living bodies via in vivo tests in different animals (Krishnamachary et al., 2012; Bhatia, et al., 2012; Panyala et al., 2009). Bhasmas used in Ayurvedic medicines do not adversely affect normal physiological functions of the body when properly used as the medicament.

Recently, Tarakeshwara Rasa, combinations of different Bhasmas and prescribed for the management of diabetes mellitus was prepared using standard protocols, and physiochemical characterization was done employing different analytical techniques. Based on XRD and energy dispersive X-ray (EDX) analyzes, the presence of different elements such as tin, iron, aluminum, mercury, calcium, manganese, and magnesium in combined forms was confirmed in this drug. Scanning Electron Microscopy (SEM) study revealed that the compound comprises the agglomerated particles, 0.5–2 μm in diameter. The nanostructural basis of Bhasma medicine can be illustrated further by Mandura Bhasma, an iron-based preparation (Mulik and Jha, 2009). The chemical analysis showed that the Mandura in the raw form contained Fe_2SiO_4 while the Bhasma contained Fe_2O_3 and SiO_2 with the grains 200–300 nm in diameter organized in a uniform fashion, much smaller than the micron-sized particles in the raw Mandura. Similar results were obtained confirming the presence of nano-size complex particles in different bhasmas and bhasmas based Ayurvedic formulations in recent studies (Jagtap et al., 2012; Unni et al., 2013; Adhikari, 2014).

Preparations anδ Characterization of Bhasma

Reduction of metallic or nonmetallic substances into ashes involving the process of repetitive incineration constitutes the basic step in Bhasma preparation. In Ayurveda, bhasmas are prepared by metals and plant/animals products involving specific processes and subsequently calcinated in closed crucibles. The steps are involved in the preparation of bhasma includes Shodhana (purification) and Marana (calcination) as described elsewhere (Pal et al., 2014). Bhasmas prepared are then characterized for physical parameters like color, lusterless, lightness, fitness and tactile sensation. While chemical characterization includes "Apunarbhavata" and

"Niruttha" tests to ensure the chemical stability of bhasmas (Rawat and Verma, 2011).

Nanoparticles in bhasma are detected and characterized by various techniques based on XRD and energy dispersive X-ray (EDX) analyzes; the presence of different elements such as tin, iron, aluminum, mercury, calcium, manganese, and magnesium in combined forms was confirmed in the bhasma drug. SEM study revealed that the compound comprises the agglomerated particles of nano dimensions. Methodologies used to test nanoparticles are environmental microscope, electron microscope, cryo-TEM, fast freeze fracture, confocal laser scanning microscope, fluorescence optical microscopy, quasi-elastic light scattering, energy dispersive x-ray analysis, inductive coupled plasma, atomic absorption spectroscopy, X-ray-induced photoelectron spectroscopy, enzyme expression etc. (Sarkar and Chaudhry, 2010). Various types of nanoparticles detected and characterized in bhasma have been documented along with its Ayurvedic uses for treatment of various ailments (Table-1).

Green Synthesis of Nanoparticles

The term 'Green' nanoparticle describe the concept of synthesizing nanoparticles from metal salts by using the reducing property of biological compounds. Different biologically active compounds are derived from a microorganism, herbal extracts, animal extracts, etc. Most of the biologically active compounds which possess the reducing capability, are biologically compatible. They are used to produce metal nanoparticles with effective therapeutic potential. From the Ayurvedic point of view, it can be stated that metal nanoparticles (as in bhasmas), in combination with herbs may serve as a potent therapeutics. Active compounds present in herbs can reduce nanoparticle, stabilize it, and in combination they can possess a better therapeutic potential (Gomes, A., et al., 2014). Recently, biological entities have been reported as serving as both reducing and stabilizing agents for green synthesis of metallic nanoparticles. Polysaccharides and phytochemicals widely present in plants act as the reducing and stabilizing agents in the green synthesis of gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs). Since ancient times, both primary and secondary metabolites of plants (phytochemicals) have demonstrated their importance in human health applications. Traditional Chinese medicine, very popular in Asian countries, actively investigates the role and biological activities of phytochemicals. For example, tea is known to contain powerful antioxidant compounds such as polyphenols (Wang and Ho, 2009). Tea leaf extracts have been recently used for the green synthesis of AuNPs and AgNPs (Begum et al., 2009; Nune et al., 2009). Nanoparticles prepared by biologically active polysaccharides and phytochemicals might exert synergistic effects by combining their

Table-1 Nanoparticles δetecteδ in various types of Bhasma

Bhasma	Nanoparticle Detecteδ	Therapeutic uses of Bhasma	Reference
Jasada Bhasma	Zinc	Diabetes, anemia, cough, ulcers, depression, ophthalmic problems,	Bhowmick, T. K., *et al*., 2009; Umrani and Paknikar, 2011,2015
Lauha bhasma	Iron oxide	Anaemia, eye disorders, skin diseases	Singh and Reddy, 2011; Krishnamachary, B., *et al*., 2012;
Tarakeshwara Rasa	Iron oxide	Diabetes mellitus	Virupaksha and Kumar, 2012
Naga Bhasma	Lead oxide	Diabetes mellitus, diarrhea, skin and Spleen diseases.	Nagarajan, S., *et al*., 2012
Vanga Bhasma	Tin	Urinary diseases, loss of appetite, inflammatory	Vishwakarma, S. K., *et al*., 2012

Table 2: List of Ayurveδic plants inδicating green synthesis of nanoparticle anδ their ethnomeδicinal uses

Plant Name	Family	Part Use	Ayurveδic Name	Ethnomeδicinal Use*	Nanoparticle Type	Biological Activities	Reference
Abelmoschus esculentus	Malvaceae	Seed	Bhaandi/Bhindaka	Antispasmodic	Gold	Antifungal	Jayaseelan, C., *et al*., 2013
Artemisia nilagirica	Asteraceae	Leaves	Damanaka/ Pushpachaamara,	Emmenagogue, menstrual regulator, nervine, stomachic (in anorexia and dyspepsia), anthelmintic, choleretic, diaphoretic	Silver	Antibacterial	Vijayakumar, M., *et al*., 2013
Artocarpus heterophyllus	Moraceae	Seed	Panasa/Kantakiphala,	Diuretic	Silver	Antibacterial	Jagtap and Bapat, 2013
Boerhavia diffusa	Nyctaginaceae	Whole plant	Punarnavaa	Diuretic, anti-inflammatory, antiarthritic, spasmolytic	Silver	Antibacterial	Kumar, P. V., *et al*., 2014
Caesalpinia coriaria	Caesalpiniaceae	Leaves	Kodivelam	Astringent	Silver	Antibacterial	Jeeva, K., *et al*., 2014
Catharanthus roseus	Apocynaceae	Leaves	Sadaabahaar/ Nayantaaraa	Astringent, anti-haemorrhagic	Silver	Antiplasmodial activity	Ponarulselvam, S., *et al*., 2012
Coleus aromaticus	Lamiaceae	Leaves	Parna-yavaani	Urinary diseases, vaginal discharge, colic and dyspepsia	Silver	Antibacterial	Vanaja and Annadurai, 2012
Dioscorea bulbifera	Dioscoreaceae	Tuber	Vaaraahi	swellings, boils, ulcer, dysentery, piles, venereal sores	Silver	Antibacterial	Ghosh, S., *et al*., 2012
Eclipta prostrata	Asteraceae	Leaves	Bhringaraaja	Deobstruent, antihepatotoxic, anticatarrhal, febrifuge	Silver	Anti filariasis and malarial vectors	Rajakumar and Rahuman, 2011
Euphorbia hirta	Euphorbiaceae	Leaves	Dudhi	Pectoral, antiasthmatic, antispasmodic	Gold	Antibacterial	Annamalai, A., *et al*., 2013
Ficus benghalensis	Moraceae	Leaves	Vata/ Nyagrodha	Abscesses and wounds for promoting suppuration	Silver	Antibacterial	Saxena, A., *et al*., 2012
Ficus racemosa	Moraceae	Bark	Udumbara/ Sadaaphala	Skin diseases, inflammations, boils and ulcers.	Silver	Larvicidal	Velayutham, K., *et al*., 2013
Garcinia mangostana	Guttiferae	Leaves	Mangustaan	Anti-inflammatory, antiimmunosuppressive	Silver	Antibacterial	Veerasamy, R., *et al*., 2011
Hovenia dulcis	Rhamnaceae	Fruit	Sikkaa	Diuretic	Gold	Antioxidant,	Basavegowda,
Iresine herbstii	Amaranthaceae	Leaves	Shveta Apaamaarga.	Astringent, diuretic, spasmolytic	Silver	Antioxidant, cytotoxity	Dipankar and Murugan, 2012
Jatropha gossypifolia	Euphorbiaceae	Latex	Rakta-Vyaaghrairanda	Purgative	Silver	Antibacterial	Patil, S. V., *et al*., 2012
Lawsonia inermis	Lythraceae	Leaves	Madayanti/ Mendika	Dysuria, jaundice, bleeding disorders, ulcers, prurigo and other obstinate skin diseases	Silver	Antibacterial, Antifungal	Gupta, A., *et al*., 2014
Leptadenia reticulata	Asclepiadaceae	Leaves	Jivanti	Stimulant, restorative, skin diseases	Silver	Antioxidant, antibacterial, cytotoxic	Swamy, M. K., *et al*., 2015

Memecylon umbellatum	Melastomataceae	Leaves	Anjani	Astringent, antileucorrhoeic, spasmolytic, hypoglycaemic	Gold/Silver	Antimicrobial	Arunachalam, K. D., *et al*., 2013
Mentha piperita	Lamiaceae	Leaves	Vilaayati Pudinaa.	Dyspepsia, flatulence, intestinal colic, and biliary disorders.	Gold/Silver	Antibacterial	MubarakAli, D., *et al*., 2011
Mimusops elengi	Sapotaceae	Leaves	Bakula/Keshara	Chronic dysentry	Silver	Antibacterial	Prakash, P., *et al*., 2013
Musa paradisiaca	Musaceae	Leaves	Kadali/ Rambhaa	Mild laxative, diarrhoea and dysentery	Silver	Ant parasitic activity	Jayaseelan, C., *et al*., 2012
Ocimum tenuiflorum	Lamiaceae	Leaves	Tulasi	Carminative, stomachic, antispasmodic, antiasthmatic, antirheumatic, expectorant, stimulant, hepatoprotective, antiperiodic, antipyretic and diaphoretic.	Silver	Antibacterial	Patil, R. S., *et al*., 2012
Pergularia daemia	Asclepiadaceae	Latex	Uttamaarani	Uterine stimulant, tones up urinary bladder, stimulates gastric secretion, expectorant, emetic	Silver	Larvicidal	Patil, C. D., *et al*., 2012
Piper longum	Piperaceae	Fruit	Pippali/Maagadhi	Respiratory tract disorder, sedative, cholagogue, emmenagogue, digestive, appetizer and carminative	Silver	Antioxidant, antibacterial, cytotoxic	Reddy, N. J. *et al*., 2014
Polyalthia longifolia	Annonaceae	Leaves	Devadaari	Febrifuge	Silver	Antibacterial	Kaviya, S., *et al*., 2011
Prosopis juliflora	Mimosaceae	Leaves	Khejaraa	Anti-dermatophytes	Silver	Antibacterial	Raja, K. *et al*., 2012
Rhinacanthus nasutus	Acanthaceae	Leaves	Yuuthiparni/ Yuuthikaparni	Skin diseases	Silver	Antibacterial, antifungal	Pasupuleti, V. R., *et al*., 2012
Sesbania grandiflora	Fabaceae	Leaves	Agastya	Catarrh, cough, consumption, glandular enlargement	Silver	Antibacterial	Das, J., *et al*., 2014
Solanum torvum	Solanaceae	Fruit	Brihati	Liver and spleen Enlargement, cough	Gold/Silver	Antioxidant, antibacterial	Ramamurthy, C. H., *et al*., 2013
Solanum xanthocarpum	Solanaceae	Berry	Kantakaari	Stimulant, expectorant, diuretic, laxative, febrifuge	Silver	Antibacterial	Amin, M., *et al*., 2012
Terminalia chebula	Combretaceae	Fruit	Haritaki/ Kaayasthaa	Gentle purgative, astringent, stomachic, antibilious	Silver	Methylene blue reduction catalysis	Edison and Sethuraman, 2012
Trianthema decandra	Aizoaceae	Root	Varshaabhu	Deobstruent	Gold/Silver	Antibacterial, antifungal	Geethalakshmi and Sarada, 2012
Tribulus terrestris	Zygophyllaceae	Fruit	Gokshura	Diuretic, demulcent, anti-inflammatory, anabolic, spasmolytic, muscle relaxant, hypotensive, hypoglycaemic	Silver	Antibacterial	Gopinath, V., *et al*., 2012
Trigonella foenum-graecum	Fabaceae	Seed	Methikaa	Appetite, flatulence, dyspepsia, colic; diarrhoea, dysentery	Gold	Catalysis	Aromal and Philip, 2012
Vitex negundo	Verbenaceae	Leaves	Nirgundi/ Shephaalikaa	Anti-inflammatory, analgesic, removes foetid discharges and worms from ulcers	Silver	Anti-cancer	Prabhu, D., *et al*., 2013

biological activities with those of nanoparticles. The preliminary studies of the synergistic properties and potential applications of these polysaccharides and phytochemicals metal nanocomposites are well documented (Park et al., 2011). Gardea-Torresdey et al., reported the synthesis of gold and silver nanoparticle by using herbal extracts (Gardea-Torresdey, 2002; 2003). Vitamins, sugars, plant extracts, microorganisms, biodegradable polymers, etc. are being used to synthesize green metal nanoparticles. Plant extracts seemed to be the best-reducing agents due to the capability of large-scale production of metal nanoparticles (Iravani, 2011). The key active compound in plant extracts is polyphenol. Polyphenols have reducing potential, and their side chain groups (mostly –OH group) are engaged in capping and stabilizing nanoparticles. Recently, some workers employ various Ayurvedic preparation and methods for the synthesis of monodispersed nanocrystal. Aroma et al., use of four different Ayurvedic arishtams (Dasamoolarishtam, Ashokarishtam, Jirakarishtam and Amrutharishtam) which is the combination of medicinal herbs as putative candidate source for the synthesis of gold nanoparticles. Nanoparticles synthesized using these aristham have shown enhanced biological activity along with comparatively lesser toxicity issue (Aromal et al., 2012). Cost effective Iron oxide nanoparticles were successfully synthesized employing the modified Ayurvedic method for the preparation of bhasmas as described by Pavani et al., (2013; 2015). A literature survey was conducted during 2011 to 2015 on above aspects (Table 2). It was found that thirty-six plants belonging to twenty-seven plants family could synthesize silver and or gold nanoparticle.

Conclusion anδ Future Prospects

The recent investigations on Bhasmas have undoubtedly confirmed their nanoscopic structural features. However, much has to be done to upgrade the efficiency of Bhasmas to that of modern nano drugs. More investigations are desirable that explore the correlation between the structural nature and mechanisms associated with their therapeutic activity. Parallel scientific studies on all the bhasmas described in Ayurvedic texts are needed. Pharmacological validation studies and potential toxicity issues should also assess for its efficacy and safety.

On the other hand, large number of plants used in Ayurvedic preparation has a capability to a green synthesis of nanoparticles. Therefore, role of plant extracts in contributing nanoparticle in herbal preparation with bhasma needs to be explored and understood.

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About Ginger *(Zingiber officinale)*





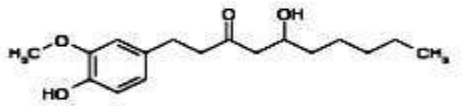

Dry Ginger (Sonth)

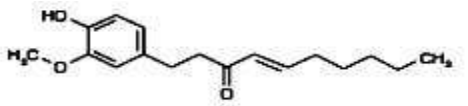
Latin Names : *Zingiber officinale*
Rosc. (Zingiberaceae)
English Names : Ginger
Sanskrit Name : *Sunthi, Srungavera, Ardraka, Vishvabheshaja, Nagara*
Hinδi Name : *Adrak, Sonth*

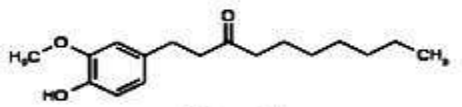
History: Ginger was known in China as early as 400 B.C. The Greeks and the Romans thought it was of Arabian origin because it was sent from India through the Red Sea. They used it as a spice.
Inδications: Ginger is valued in medicine as a carminative and stimulant to the gastro-intestinal tract. It is much in vogue as a household remedy for flatulence and colic. Ginger is reported to contain an anti-histaminic factor. It is included among anti-depressants and it forms an ingredient of some anti-narcotic preparations.

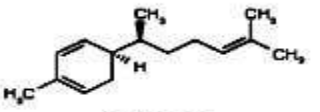
Major chemical constituents: The rhizome contains 1–4% essential oil and an oleoresin. The composition of the essential oil varies as a function of geographical origin, but the chief constituent sesquiterpene hydrocarbons (responsible for the aroma) seem to remain constant. These compounds include (-)-zingiberene, (+)-*ar*-curcumene, (-)-β- sesquiphellandrene, and β-bisabolene. Monoterpene aldehydes and alcohols are also present.

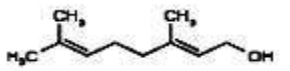
Representative structures are presented below.

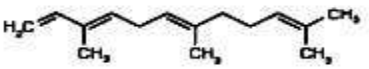

[6]-Gingerol

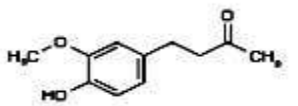

[6]-Shogaol


[6]-Paradol


Zingiberene


Geraniol


Farnesene


Zingerone

These actives are responsible for Anti-cancer effects, Anticoagulant effects, Antiemetic effects, Anti-inflammatory effects, Antinociceptive effects, Antioxidant effects, Antitussive effects, Cardiovascular effects, Gastrointestinal effects, Immunomodulatory effects, Lipid effects and Weight loss effects:
As per Ayurvedic Pharmacopeia Purity and Strength

Foreign matter:	Not more than 1 per cent,	Appendix 2.2.2.
Total Ash:	Not more than 6 per cent,	Appendix 2.2.3.
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.2.4.
Alcohol-soluble extractive:	Not less than 3 per cent,	Appendix 2.2.6.
Water-soluble extractive:	Not less than 10 per cent,	Appendix 2.2.7.

Ginger in Ayurveδa

Ginger is amongst important herbs described in Ayurveda. It is named as 'Mahaushadhi', which means great medicine. General properties of this useful herb are: sweet, pungent, warm, kapha, vata alleviating.

Ginger is good appetizer, relishing, aphrodisiac, light, and beneficial for heart and can be used for various health problems.

Dry ginger is recommended in skin diseases, anaemia, painful and burning urination, bleeding diseases, ulcers, fever, burning sensation, during summer and autumn.

Some simple and useful formulations of Ginger

1. Chewing of fresh ginger with a small quantity of salt and lemon juice before meals is a good appetizer; it enhances taste perception and purifies the tongue and throat.
2. Dry ginger powder 2-5g taken with warm water is beneficial for rheumatic patients.
3. In cough it should be given with honey in the form of paste.
4. Warm juice of fresh ginger should be dropped in the ear for earache.
5. In piles, dry ginger powder should be administered with chitrak.
6. In abdominal diseases ginger juice can be taken with equal quantity of milk.

Forth Coming Events

- 2nd International Conference and Exhibition on Pharmacology and Ethnopharmacology May 02-04, 2016 Chicago, Illinois, USA
  (http:// ethnopharmacology.pharmaceuticalconferences.com/)
- International Conference on Industrial Chemistry June 27-28, 2016 new Orleans, Los angeles, USA
  Submit Abstract (http://industrialchemistry.conferenceseries/abstract-submission.php)
- 2nd International Conference on Green Chemistry and Sustainable Engineering
  Topics: Green Chemistry, Process Chemistry, Chemical Engineering, **Keywordδs**:
  Date: /20/21/22/ July 2016, Rome, Italy, Europe
  Web Site, **Contact:** info@scienceknowconferences.info
- International Pharmacy Conference July 14-15, 2016 Philadelphia, Pennsylvania, USA
  (http://pharmacy.pharmaceuticalconferences.com/)
- Bioactive Natural Products: Translating Promise into Practice
  Topics: Biotechnology, Biochemistry, Medicinal Chemistry, Organic Chemistry, Agricultural Chemistry,
  Keywords: see below
  Date: /11/12/13/ July 2016, Oxford, United Kingdom, Europe
  Contact: maggi@maggichurchouseevents.co.uk
- International Conference and Exhibition on Marune Drugs and Natural Products July 25-27, 2016 Melbourne, Australia (http//naturalproducts.pharmaceuticalsconferences.com)
- International Conference on Organic Chemistry August 10-11,2016 Las Vagas, USA
  (http://industrialchemistry.conferenceseries.com/recommended-globalconferences. php)
- 3rd World Congress on Pharmacology August 08-10, 2016 Birmingham, UK (http://pharmacology.pharmaceuticalconferences.com/)
- 4th International Conference Pharmacognosy, Phytochemistry & Natural Products August 29-31, 2016 Sao Paulo, Brazil.
  Submit Abstract(. com/abstract-submission.php)
- 3rd International Conference on Past and Present Research Systems of Green Inernational Conference on Pharmaceutical Chemistry September 08-10, 2016 Frankfurt, Germany
  (http://industrialchemistry.conferenceseries.com/recommended-globalconferences. php)
- 7th International Conference and Exhibition on Analytical & Bioanalytical Techniques September 28-30, 2016 Miami, USA
  (http://industrialchemistry.conferenceseries.com/recommended-globalconferences. php)
- Chemistry September 19-21, 2016 Las Vegas, USA
  (http://greenchemistry.conferenceseries.com/)
- 5th Global Summit on Toxicology and Applied Pharmacology October 17-19, 2016 Houston, USA
  (products.pharmaceuticalconferences.com)
- World Pharma Congress Nov 07-Nov 09, 2016 Las Vegas, USA
  (http://world.pharmaceuticalconferences.com/)
- 3rd International Conference on Clinical Pharmacy December 07-09, 2015 Atlanta, USA
  (http://clinicapharmacy.pharmaceuticalconferences.com/)
- 5th International Conference Medicinal Chemistry & Computer Aided Drug Designing
  December 05-07, 2016 Phoenix, Arizon, USA
  Submit Abstract (http://medicinalchemistry.pharmaceuticalconferences.com/abstract-submission.php)
  USA & Americas

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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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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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