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Holarrhena antidysenterica (Kutaja)

Aegle marmelos (Bael)

Punica granatum (Anaar)

Cyperus rotundus (Nagar Motha)

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Editorial

Dear Readers,

I am happy to present this 23rd issue of University Journal of Phytochemistry and Ayurvedic Heights (UJPAH) to be released in 16th symposium of UJPAH 2017. UJPAH is gaining popularity amongst eminent scientists, research scholars, students of the scientific institutions who contribute their research papers, articles reviews on therapeutic properties, pharmacognostic characteristics, phytochemistry, pharmacology and microbiological aspects etc. on Indian herbs.

Today antibiotics are developing immunity in microbes and whole world is looking towards newer solutions and anti-infective therapies. We are on the verge of a medical disaster, 700,000 deaths worldwide today and this number could rise to 10 million within the next few decades unless new antibiotics and alternative antimicrobial therapies are developed. Antibiotic resistance-one of the greatest threat- says WHO since 2009. The antibiotic pipeline is almost empty. During the last 30 years only 2 classes of antibiotics have been developed that reach the clinic and the world is looking for new medical solutions and innovation of new antimicrobial drugs that can face the challenge of increasing drug resistance. Herbs are the best friends, India has great potentials and Uttarakhand as a leading state in the field of Ayush can be a trendsetter. India has always been known in the world as leader in Ayurveda with its immense herbal wealth and has great potential in this area which needs to be tapped and utilized in the interest of the state. India is world largest food producer after China hence only with research on these herbs one can give alternative options to the medical fraternity which will pave way to the farmers of hills and plain to increase their income by growing herbs instead of general crops only.

I offer my best wishes to all those scientists, research scholars, students and teachers who contributed for bringing out this issue and also express my sincere gratitude to all board members who make this issue a memorable for scientific fraternity of Uttarakhand and the country.

Dr. S. Farooq
Chief Editor

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In vitro Antibacterial Activity of *Citrus limetta* Peel Extracts against Biofilm Forming MDR Gram Negative Bacterial Pathogens

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Abstract- The rapid emergence and spread of multidrug resistant (MDR) pathogens is continuously endangering the efficacy of common antibiotics. Biofilm development by bacterial pathogens further enhanced drug resistance levels to several fold higher. This has necessitated the search for novel bioactive compounds from natural products. In this study, a panel of biofilm forming MDR Gram –ve bacterial pathogens were subjected to their sensitivity against ethyl acetate, butanol and benzene fractions of peel extracts of *Citrus limetta.* Ethyl acetate fraction showed broad spectrum promising activity against all test bacteria followed by butanol fraction. Benzene fraction showed no antibacterial activity at the tested concentration. Further, effect of these extracts on production of violacein pigment and growth of *Chromobacterium violaceum* 12472 (a sensitive strain) was determined. Butanol fraction inhibited the quorum sensing controlled pigment production. On the other hand, ethyl acetate fraction inhibited the growth of bacteria but not violacein production. Thin layer chromatography analysis of active fractions reveals the presence of phenolic compounds. Further studies on identification of active compounds are needed.

Keywords: Biofilm, *Citrus limetta*, Antibacterial activity, Violacein pigment.

Introduction

Infectious diseases are still one of the leading causes of mortality and morbidity globally. Excessive and indiscriminate use of antibiotics in medical, veterinary and agricultural practices has resulted into high level of emergence and spread of multidrug resistant bacterial pathogens. This has created immense clinical problem in the treatment of infection caused by MDR bacteria (Falagas and Bliziotis, 2007; Maheshwari *et al*., 2016). The development of new antibacterial with novel mode of action or development of alternative strategies to control bacterial infection is urgently required. Medicinal plants used in traditional system of medicine are considered as a promising source of novel bioactive compounds (Aqil and Ahmad, 2007). India has rich diversity of medicinal flora which has not yet systematically explored and exploited against MDR bacteria. We have previously tested several Indian medicinal plants for their antibacterial activity against drug resistant bacteria (Ahmad *et al*., 1998; Ahmad and Aqil, 2007).

Citrus limetta, commonly known as sweet lime or sweet lemon is used for juice processing globally and also used in traditional system of Indian medicine. Peel of this fruit is an important bioresource for useful bioactive compounds before its disposal. The peel extract and oil of this plant is known for various biological activities including mosquitocidal activity (Pohlit *et al*., 2011). Flavonoids isolated from this plant (naringin and naringenin) are known for health benefits in diabetes, obesity, hypertension and metabolic syndrome (Alam *et al*., 2014). Antioxidant and anticancer activities have been reported for hydroxyl cinnamic acid (phenolic acid derivatives) isolated from peel extracts (Meyer *et al*., 1998; Kaul and Khanduja, 1998). Therapeutic property of this plant has been found to be associated with several compounds such as flavonoids, phenolic acids, limonoids, Vitamin C etc. Antimalarial activity of alcoholic extract of the peel has also been documented (Mohanty *et*

al., 2015). Few workers have shown antibacterial activity of *Citrus limetta* (Javed *et al*., 2013; Prakash *et al*., 2013) against selected bacteria. However, no study has been performed on peel extract of *C. limetta* against an array of MDR bacterial pathogens of clinical and environmental origin. Therefore, in this study we reported broad spectrum antibacterial activity of *C. limetta* peel extracts against a variety of biofilm forming MDR bacteria.

Material and Methods

Bacterial strains and chemicals

A total of 19 bacterial isolates used in the study, belonging to Gram negative bacteria such as *E. coli, Enterobacter, Klebsiella, Proteus, Pseudomonas* and *Salmonella sp*. were previously isolated from clinical and environmental source and characterized in our laboratory (Khan, 2017). Sixteen antibacterial drugs used includes: ampicillin (AMP, 2 µg/disc), cefotaxime (CE, 10 µg/disc), ceftriaxone (CTR, 30 µg/disc), ciprofloxacin (CIP, 5 µg/disc), gentamycin (HLG, 120 µg/disc), tetracycline (TE, 30 µg/disc), nitrofurantoin (NIT, 300 µg/disc), imipenem (IPM, 10 µg/disc), cefoxitin (CX, 30 µg/disc), oflaxacin (OF, 5 µg/disc), cefepime (CPM 30 µg/disc), rimapicin (R, 30 µg/disc), azithromycin (AZM, 15 µg/disc), erythromycin (E, 15 µg/disc), streptomycin (S, 10 µg/disc) and cefuroxime (CXM, 30 µg/disc) were obtained from Hi-Media Pvt. Ltd., India. Microbiological media (Nutrient broth, Mueller-Hinton agar, Luria-Bertani broth) were obtained from Hi-Media Pvt. Ltd., India. All the other chemicals and reagent used are of analytical grade.

Antibiotic susceptibility of test isolates

Antibiotic susceptibility was performed by standard disc diffusion method on Mueller-Hinton agar plates using standard method (Bauer *et al*., 1966).

Biofilm formation assay

The ability of test bacterial strain to form biofilm *in vitro* were analyze by method of O'Toole and Kolter (1998) using microtiter plates using 0.1 % crystal violet (Hi-Media) as staining dye.

Plant material and extraction method

Fruits of *Citrus limetta* were purchased from local market of Aligarh. Identification of fruit was done in Department of Botany, AMU, Aligarh and a voucher specimen (AGM-CL-2/17) was submitted in the Department of Agricultural microbiology. Peels were removed from fruits and shade dried for 10 days. 100gm of dry powder was extracted in methenol. Subsequent, liquid-liquid extraction of crude methanolic extract with benzene, ethyl acetate and butanol was done using the method described by Berahou *et al*. (2007). Extracts were dried and reconstituted in minimum amount of DMSO at a concentration of 50 mg/ml for further use.

Antibacterial activity

Antibacterial activity of the plant extracts were determined using agar well diffusion assay on Mueller-Hinton (MH) agar plates as described earlier (Ahmad *et al.* 1998). Briefly, MH agar plates were seeded with 100 µl of overnight grown cultures of test organisms and allowed to dry for 20 minutes. Subsequently, wells of diameter 8 mm were punched into the plates and loaded with 100 µl of extracts. Plates were incubated at 37 °C for 18 hrs. and zone of inhibition were recorded in mm.

Chromobacterium violaceum sensitivity assay

Effect of peel extract on violecein pigment production and growth of *Chromobacterium. violaceum*12472 was determined by the method described by Zahin *et al.* (2010). Twenty µl of extracts from DMSO stock (50 mg/ml) were impregnated on sterile discs whereas DMSO was used as control. Pigment or growth inhibition were recorded after overnight incubation at 37 °C. Zone of pigment and growth inhibition was measured and expressed in millimeters.

Thin layer chromatography

Thin layer chromatographic analysis of peel extracts was performed according to the method described by Wagner and Bladt (1996). Aluminum TLC plate, silica gel coated with fluorescent indicator F254 (Merck) were used. The solvent

mixture Toluene: Ethyl acetate: Formic acid (5:4:1) was used for elution. Developed TLC plates were visualized under UV 254 and UV 365 nm to detect the bands. Further, plates were sprayed with natural product (NP) reagent (Wagner and Bladt, 1996) to enhance the sensitivity.

Results

A total of 19 isolates were isolated from clinical and environmental sources which belong to *E. coli* (EC), *Enterobacter aerogens* (EN), *Klebsiella pneumoniae* (K), *Proteus vulgaris* (PRO), *Pseudomonas aeruginosa* (PSEU) and *Salmonella sp.* (S), based on the morphological, cultural and biochemical characteristics in accordance with the Bergey's Manual of Determinative Bacteriology. Antibiotic sensitivity of the test isolates against 16 antibiotics revealed their multi drug resistance nature as presented in (Table-1).

Table-1 Antibiotics resistance profile of bacterial isolates

Isolates	Source	Resistance profile	No. of antibiotics
EC1	Slaughter house waste water	AMP,TE,HLG,CXM,CX,CE,AZM,E,CIP,R,OF,CPM,CTR	13
EC2	Slaughter house waste water	AMP,NIT,TE,CXM,CX,AZM,E,CIP,CPM,CTR	10
EC3	Clinical	AMP,TE,CXM,CX,CE,AZM,E,OF,CPM,CTR	10
EN1	Slaughter house waste water	AMP,TE,IPM,CXM,CX,E	06
EN2	Slaughter house waste water	AMP,TE,CXM,CX,AZM,E,CTR ,CPM	08
EN3	Slaughter house waste water	NIT,TE,HLG,CXM,CX,CE,AZM,E,CIP,R,OF,CTR ,CPM	13
PRO1	Poultry waste	AMP,NIT,TE,HLG,S,CXM,CX,CE,AZM,E,R,CPM	12
PRO2	Poultry waste	AMP,NIT,TE,HLG,CXM,CX,R	07
PRO3	Poultry waste	TE,HLG,S,IPM,CXM,CX,CE,AZM,E,CIP,R,OF,CTR ,CPM	14
PSEU1	Clinical	AMP,NIT,TE,S,CXM,CX,CE,AZM,E,R,CPM	11
PSEU2	Clinical	AMP,NIT,CXM,CX,CE,AZM,E,CIP,R,CPM	10
PSEU3	Clinical	AMP,NIT,TE,HLG,S,IPM,CXM,CX,CE,AZM,E,CIP,R,CTR ,CPM	14
PSEU4	Clinical	AMP,NIT,TE,HLG,CXM,CX,CE,AZM,E,CIP,R,OF,CTR ,CPM	14
PSEU5	Clinical	NIT,HLG,S,IPM,CXM,CX,CE,AZM,E,CIP,R,OF,CTR ,CPM	14
PSEU6	Clinical	NIT,TE,HLG,IPM,CXM,CE,AZM,E,CIP,OF,CPM	11
PSEU7	Clinical	AMP,NIT,S,CXM,CX,CE,AZM,E,CIP,OF,CTR ,CPM	12
S1	Clinical	AMP,TE,IPM,CXM,CX,R,OF,CTR ,CPM	09
S2	Clinical	AMP,NIT,TE,HLG,S,CXM,CX,AZM,E,R,OF,CTR	13
K1	Clinical	AMP,NIT,TE,HLG,CXM,CE,AZM,E,CIP,R,OF,CTR ,CPM	13

The most effective antibiotics *in vitro* was imipenem followed by streptomycin and ciprofloxacin. The reason for high percentage of incidence of multiple resistance in the isolates is probably due to the selection of resistant isolates in clinical and environmental conditions. Varying level of antibiotic resistance pattern among Gram negative bacterial pathogens of environmental and clinical origin was also reported by other workers (Alam *et al*., 2013; Usha *et al*., 2013; Maheshwari *et al*., 2016). The rapid emergence of resistant bacteria is globally reported which are endangering the efficacy of antibiotics. The reasons for antimicrobial resistance increase are complex, but through the studies, it has become evident that the excessive use of antibiotic is linked to the selection and emergence of resistance. This increase and spread can also be associated to the host population structure, which mainly occurs due to the interaction of the hospital and community (Molton *et al*., 2013). The findings from this study suggest that clinical as well as environmental strains of Gram –ve bacteria commonly harboured multidrug resistance determinants/plasmids. Therefore, indiscriminate use of antibiotics in medical, veterinary and agricultural practices should be strictly monitored and regulated. Interestingly, these isolates showed varying levels of biofilm forming ability *in vitro* (Table-2). Formation of biofilm is a mechanism of bacterial protection against antibiotics and other stress condition (Fux *et al*., 2004). Biofilm formation by pathogenic bacteria have also been reported by other workers (Sánchez *et al.,* 2013; Liu *et al.,* 2014).

Table-2 Biofilm forming ability of bacterial isolates by crystal violet method

Isolates	Optical density at 595 nm	Biofilm forming ability
EC1	1.359±0.533	+++
EC2	1.165±0.373	+++
EC3	2.280±0.265	+++
EN1	1.200±0.382	+++
EN2	2.432±0.273	+++
EN3	2.702±0.116	+++
K1	1.594±0.210	+++
PRO1	0.668±0.085	++
PRO2	0.344±0.054	++
PRO3	0.518±0.023	++
PSEU1	0.385±0.007	++
PSEU2	0.489±0.033	++
PSEU3	0.478±0.102	++
PSEU4	0.264±0.046	+
PSEU5	0.240±0.051	+
PSEU6	0.391±0.036	++
PSEU7	0.488±0.080	++
S1	0.261±0.049	+
S2	0.430±0.014	++

+++: indicates strong, ++: indicates moderate and +: indicates weak biofilm development

Ethyl acetate (CE) and butanol (CnB) fractions of the peel extract showed antibacterial activity against all test bacteria, while benzene fraction (CB) was found inactive at tested concentration. On the basis of the present investigation it can be highlighted that ethyl acetate (CE) fraction showed promising broad spectrum antibacterial activity. Several components from *C. limetta* have been reported to possess biological activity. For instance, certain flavonoids, volatiles and phenolic acids have been reported from peel of *C. limetta* with antibacterial activity (Barbieri *et al*., 2017).

Table-3 Antibacterial efficacy of different fraction of *Citrus limetta* against bacterial isolates.

ISOLATES	Antibacterial activity		
	Fractions of *Citrus limetta*		
	Ethyl acetate	Butanol	Benzene
	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
Escherichia coli (EC1)	28	16	-
EC2	26	16	-
EC3	28	16	-
Enterobacter aerogens (EN1)	26	14	-
EN2	22	12	-
EN3	26	16	-
Klebsiella pneumoniae (K1)	18	16	-
Proteus vulgaris (PRO1)	26	14	-
PRO2	24	14	-
PRO3	22	16	-
Psedomonas aeruginosa (PSEU1)	22	14	-
PSEU2	26	12	-
PSEU3	18	16	-
PSEU4	28	16	-
PSEU5	22	14	-
PSEU6	22	16	-
PSEU7	24	16	-
Salmonella sp. (S1)	22	12	-
S2	20	14	-

'-' indicates no inhibition

Further in this study, *C. limetta* extracts also exhibited growth inhibition as well as quorum sensing regulated pigment production in *Chromobacterium violaceum*-12472. Ethyl acetate fraction (CE) inhibited the growth of bacteria but showed no effect on pigment production at the tested concentration. Whereas, CnB, showed only pigment inhibitory effect at the tested concentration. Benzene fraction (CB) neither inhibited growth nor the pigment production in *C. violaceum* (**Table-4** and **Figure-1 A and B**). These finding suggested that peel extract of *C. limetta* (butanol fraction) has potential anti quorum sensing activity hence further detailed exploration of the plant extract for anti-infective/anti-QS property is needed along with identification and characterization of active constituents.

Table-4 Sensitivity of Chromobacterium violaceum against *Citrus limetta* fractions

Fractions of *Citrus limetta*	*Chromobacterium violaceum* 12472	
	Growth inhibition(mm)	Pigment inhibition(mm)
Ethyl acetate	+(18)	Nil
Benzene	Nil	Nil
Butanol	Nil	+(12)

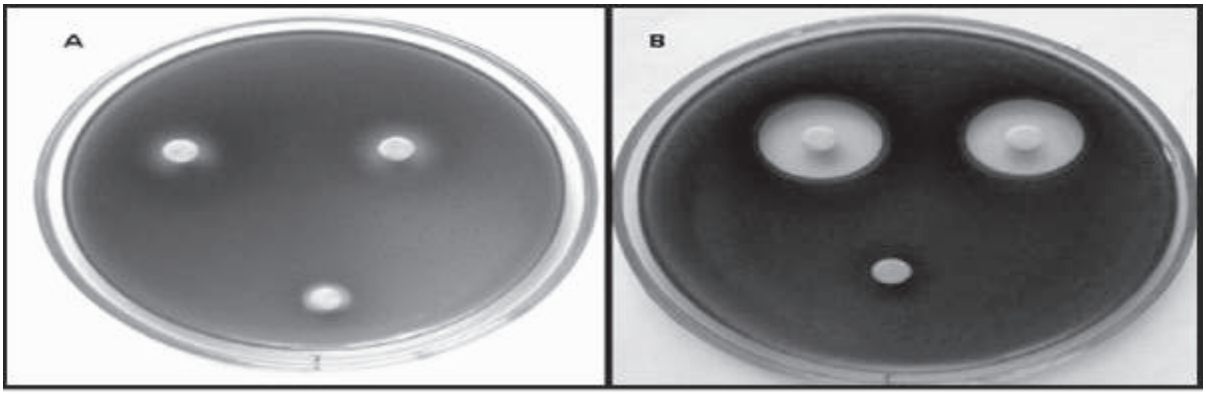

Figure-1 Plates showing sensitivity of Chromobacterium violaceum against different fractions of *Citrus limetta* A) Butanol fraction showing zone of pigment inhibition and B) Ethyl acetate fraction showing zone of growth inhibition.

Development of TLC plates with NP reagent results into enhancement of band's fluorescent as well as revealing new fluorescent bands which predominantly indicates towards presence of different derivatives of phenolic acids (Figure 2). Thin layer chromatographic profile of the fractions showed the presence of one or more different phytochemicals as evident from different bands. The eluent used for development of TLC plates separates different phenolic compounds from mixture. Quenching (dark) bands observed in different fraction at 254 nm represents phytocompounds having conjugated double bonds such as flavonoids, phenols, coumarins etc. Fluorescent bands at 365 nm, also indicates flavonoids but predominantly phenol carboxylic acids such cinnamic acid, chlorogenic acid etc. Further development of TLC plates with Natural

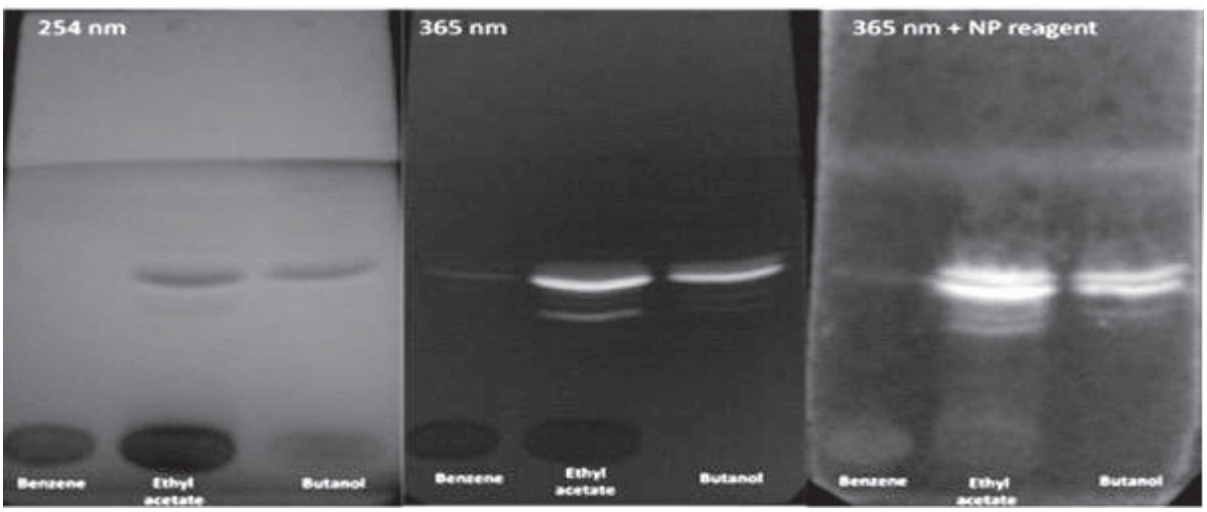

Figure-2 Phytochemical profile of different fractions of *Citrus limetta* on TLC F254 plates at different wavelengths (254 and 365 nm) and at 365 nm with natural product (NP) reagent.

Product (NP) reagent revealed new bands with intense blue fluorescent at 365 nm. These blue fluorescent bands are strong indicative of phenol carboxylic acids. However further identification and confirmation is required.

Conclusion

On the basis of the present study, it is concluded that the *C. limetta* peel extracts are promising source of antibacterial compounds against MDR bacteria and could be exploited for herbal preparations against infectious diseases. Further, inhibition of quorum sensing regulated production of pigment by butanol extract highlights its anti quorum sensing activity and needs further investigation to identify active principle.

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References

- Ahmad, I. and Aqil, F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiological Research,* 2007 Jul 27, 162(3): 264-75.
- Ahmad, I.; Mehmood, Z. and Mohammad, F. Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of ethnopharmacology*, 1998 Sep 30, 62(2): 183-93.
- Alam, M. A.; Subhan, N.; Rahman, M. M.; Uddin, S. J.; Reza, H. M. and Sarker, S. D. Effect of *citrus* flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. *Advances in Nutrition: An International Review Journal*, 2014 Jul 1, 5(4): 404-17.
- Alam, M. Z.; Aqil, F.; Ahmad, I. and Ahmad, S. Incidence and transferability of antibiotic resistance in the enteric bacteria isolated from hospital wastewater. *Brazilian Journal of Microbiology,* 2013 Sep, 44(3): 799-806.
- Aqil, F. and Ahmad, I. Antibacterial properties of traditionally used Indian medicinal plants. Methods and findings in *Experimental and clinical pharmacology,* 2007 Mar 1, 29(2): 79-92.
- Barbieri, R.; Coppo, E.; Marchese, A.; Daglia, M.; Sobarzo-Sánchez, E.; Nabavi, S. F. and Nabavi, S. M. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological Research,* 2017 Mar 31, 196: 44-68.
- Bauer, A. W.; Kirby, W. M.; Sherris, J. C. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* 1966 Apr, 45(4): 493-6.
- Berahou, A.; Auhmani, A.; Fdil, N.; Benharref, A.; Jana, M. and Gadhi, C. A. Antibacterial activity of Quercus ilex bark's extracts. *Journal of ethnopharmacology,* 2007, 112(3): 426-9.
- Eloff, J. N. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta medica*, 1998 Dec, 64(08): 711-3.
- Falagas, M. E. and Bliziotis, I. A. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era. *International journal of antimicrobial agents,* 2007 Jun 30, 29(6): 630-6.
- Fux, C. A.; Costerton, J. W; Stewart, P. S. and Stoodley, P. Survival strategies of infectious biofilms. *Trends in microbiology,* 2005 Jan 1, 13 (1): 34-40.
- Javed, S.; Ahmad, R.; Shahzad, K.; Nawaz, S.; Saeed, S. and Saleem, Y. Chemical constituents, antimicrobial and antioxidant activity of essential oil of *Citrus limetta* var. Mitha (sweet lime) peel in Pakistan. *African Journal of Microbiology Research,* 2013 Jun 30, 7(24): 3071-7.
- Kaul, A. and Khanduja, L. Polyphenols inhibit promotional phase of tumorigenesis:

relevance of superoxide radicals. *Nutrition and Cancer,* 1998 Aug, 32(2): 81-85.

- Khan, M. A. Antibiotic resistance, biofilm formation and sensitivity to selected natural products among Gram negative bacteria isolated from clinical and environmental origin (M.Sc. Dissertation). Department of Ag. Microbiology, Aligarh Muslim University, 2017.
- Liu, N. T.; Nou, X.; Lefcourt, A. M.; Shelton, D. R. and Lo, Y. M. Dual-species biofilm formation by *Escherichia coli* O157: H7 and environmental bacteria isolated from fresh-cut processing facilities. *International journal of food microbiology,* 2014 Feb 3, 171: 15-20.
- Maheshwari, M.; Yaser, N. H.; Naz, S.; Fatima, M. and Ahmad, I. Emergence of ciprofloxacin-resistant extended-spectrum β-lactamase-producing enteric bacteria in hospital wastewater and clinical sources. *Journal of global antimicrobial resistance*, 2016 Jun 30, 5: 22-5.
- Meyer, A. S.; Donovan, J. L.; Pearson, D. A.; Waterhouse, A. L. and Frankel, E. N. Fruit hydroxycinnamic acids inhibit human low-density lipoprotein oxidation in vitro. *Journal of Agricultural and Food Chemistry,* 1998 May 18, 46(5): 1783-7.
- Mohanty, S. K.; Maurya, A.; Saxena, A.; Shanker, K.; Pal, A. U. and Bawankule, D. Flavonoids rich fraction of *Citrus limetta* fruit peels reduces proinflammatory cytokine production and attenuates malaria pathogenesis. *Current pharmaceutical biotechnology,* 2015 Jun 1,16(6): 544-52.
- Molton, J. S.; Tambyah, P. A.; Ang, B. S; Ling, M. L and Fisher, D. A. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. *Clinical infectious diseases,* 2013 Jan 18, 56(9): 1310-8.
- O'toole, G. A. and Kolter, R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Molecular microbiology*, 1998 Oct 1, 30(2): 295-304.
- Pohlit, A. M.; Lopes, N. P.; Gama, R. A.; Tadei, W. P. and de Andrade Neto, V. F. Patent literature on mosquito repellent inventions which contain plant essential oils–a review. *Planta Medica*, 2011 Apr, 77(06): 598-617.
- Prakash, A.; Mathur, K.; Vishwakarma, A.; Vuppu, S. and Mishra, B. Comparative assay of antioxidant and antibacterial properties of Indian culinary seasonal fruit peel extracts obtained from Vellore, Tamilnadu. *Int. J. Pharm. Sci. Rev. Res.*, 2013 Mar, 19(1): 131-5.
- Sánchez, C. J.; Mende, K.; Beckius, M. L.; Akers, K. S.; Romano, D. R.; Wenke, J. C. and Murray, C. K. Biofilm formation by clinical isolates and the implications in chronic infections. *BMC infectious diseases,* 2013 Jan 29, 13(1): 47.
- Usha, K.; Kumar, E. and Gopal, D. S. Occurrence of various beta-lactamase producing Gram negative bacilli in the hospital effluent. *Asian J Pharm Clin Res.*, 2013, 6(3): 42-6.
- Wagner, H. and Bladt, S. Plant drug analysis: a thin layer chromatography atlas. *Springer Science and Business Media,* 1996.
- Zahin, M.; Hasan, S.; Aqil, F.; Khan, M.; Ahmad, S.; Husain, F. M and Ahmad, I. Screening of certain medicinal plants from India for their anti-quorum sensing activity. *Indian Journal of Experimental Biology,* 2010 Dec., 48(12): 1219-24.

Insecticidal Efficacy of *Plumbago zeylanica* (Chitrak) against *Clostera cupreata* (Lepidoptera: Notodontidae)

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Abstract- *Plumbago zeylanica* Linn (Plumbaginaceae) is a perennial herb commonly distributed in forest of the Uttarakhand, India, and cultivated in the gardens as well throughout India.literature survey revel as that it has insecticidal properties. Therefore, a study was undertaken to evaluate the insecticidal efficacy of different extracts of *P. zeylanica against Clostera cupreata* (Lepidoptera: Notodontidae), a major defoliator of poplar.

Out of four extracts (PZPE, PZA, PZM and PZW) of *Plumbago zeylanica*, two extracts viz. PZM and PZA were found effective for the control of poplar defoliator - *Clostera cupreata*. Bioassay experiments of effective extracts were also carried out with 0.0625, 0.125, 0.25, 0.50, 1.00, 1.50 and 2.00% concentrations. It was found that extracts, PZM and PZA caused 66.67±0.75% and 60.00±0.0% larval mortality of *C. cupreata* at 2.00% concentration after 72 hrs. of exposure respectively. The details of study are being presented here in the paper.

Key words: *Plumbago zeylanica, Clostera cupreata,* Lepidoptera, Notodontidae

Introduction

Populus spp. is distributed in the states of Jammu and Kashmir, Punjab, Haryana, Uttar Pradesh, Himachal Pradesh and Arunachal Pradesh (Mathur and Sharma, 1983). There are six species of poplars viz. *Populus alba, P. ciliata, P. euphratica, P. gamblei, P. jacquemontiana va.r glauca* and *P. aurifolia,* indigenous to Himalayan region of India. *P. deltoides* is a fast growing exotic tree species which has been extensively planted in India (Lohani, 1979). Poplar is very susceptible for insects attack. Over 108 insect species of varying nature of damage have so far been recorded causing infestation to the poplar of different dimensions (Beeson, 1941; Chaterjee and Thapa, 1964; Tiwari 1993). Poplar defoliator-*Clostera cupreata* is a major defoliator of poplar, which appears in out breaks and caused severe loss, reduced the productivity and also quality of the timber. In northern India, poplar defoliator is controlled by unlimited use of insecticides leading to several health and environmental hazards. These insecticides are not target specific, broad spectrum and develop resistance to insecticides.With a greater awareness of hazards associated with the use of synthetic organic insecticides, there has been an urgent need to explore suitable alternative products for pest control. Plant based medicines have played an important role in primary health care needs of human as well as animals. Variety of plants exhibit antimicrobial, larvicidal, anti- inflammatory and antioxidant activities due to the presence of some active compounds like essential oils, flavonoids, terpenoids, tri-terpenoids, glycosides, alkaloids (Gupta and Joshi, 1995). Therefore, the present work was initiated to study the insecticidal effectiveness of different extracts of *Plumbago zeylanica* for the control of poplar defoliator.

Material and Methods

Survey and Collection

Field surveys of different forest areas were conducted for the collection of different stages of *C. cuptreata* a major defoliator of poplar. The areas visited, include Barkot, Lachhiwala, Jhajra, Kalsi ranges of Dehradun Forest Division; Chhichrauli and Yamunanagar (Haryana); Bahadrabad, Biharigarh (Haridwar) and FRI campus Dehradun. Collection of larvae was carried in the morning hours by hand picking in

plastic containers, open end covered with muslin cloth tied with rubber band. The collected immature and mature stages of defoliator brought from the field in the laboratory for rearing and to maintain the laboratory culture to lay down a series of experiments.

Rearing of insect

Larvae of *C. cupreata* were reared in glass chimney and wooden cages with fresh leaves of poplar. The pupae when formed were sorted out and kept separately in glass jars covered with muslin cloth till the emergence occurred. The emerged moths of *C. cupreata* were released in wooden glass cages (60x60x90 cm) having fresh foliage of poplar for egg laying. Cotton soaked in water solution of honey/sugar was supplied as a food.

Collection, drying and grinding of plants material

Leaves of *P. zeylanica* were collected from FRI campus, Dehradun. The collected leaves were air dried and powdered for extraction in different solvents. Powdered plant material of *P. zeylanica (*400g) was extracted with different solvents viz. petroleum ether, acetone, methanol and water sequentially by using soxhlet apparatus. The yield percentage was determined on moisture free basis (table-1). The extracted extracts were coded as PZPE, PZA, PZM and PZW.

PZPE mean leaves of *P. zeylanica* extracted in petroleum ether, PZA stands for *P. zeylanica* extracted in acetone, PZM means leaves of *P. zeylanica* extracted in methanol while PZW means leaves of *P. zeylanica* extracted in water.

Testing of Extracts against *Clostera cupreata*

Experiments were carried out to evaluate the larval mortality of different extracts -PZPE, PZA, PZM and PZW on the 3rd instar larvae of *C. cupreata* at 1% concentration. Ten number of 3rd instar larvae of *C. cupreata* were taken from the culture and released in glass jars and fresh leaves of poplar treated with 1% of above extracts were given for feeding. Observations on the mortality of larvae were recorded after 24, 48 and 72 hrs. of exposure. The moribund larvae were considered as dead. The percent mortality of larvae was calculated by using the formula:

$$\text{Percent mortality} = \frac{\text{No. of larvae dead}}{\text{No. of larvae released}} \text{ X } 100$$

Results and Discussion

Observations in Table-2 showed that at 1% concentration of PZPE extract against *C. cupreata*, after 24 hrs showed the mortality 3.33±1.82%. No further larval mortality happened after 48 and 72 hrs. The average larval mortality was calculated 3.33±1.82%% and was taken as non effective. 1% concentration of PZA (*P. zeylanica* in Acetone) extract after 24 hrs. gave 23.33±1.30% larval mortality. After 48 hrs. the mortality status was increased to 46.67±0.81% which remained the same after 72 hrs. of observation and was taken as effective extract. PZM (*P. zeylanica* in methanol) extract at 1% concentration gave 30% larval mortality after 24 hrs. After 48 hrs the larval mortality raised to 56.67±0.74%. There was no enhanced in larval mortality after 72 hrs. The average larval mortality after 72 hrs. was recorded as 56.67±0.74% and was taken as effective extract. The experiment with PZW (*P. zeylanica* in water) extract, no larval mortality occurred at 1% concentration up to 72 hrs. of observation. The data was recorded after every 24 hrs. In control experiment, no larval mortality was recorded.

Bioassay experiments of effective extracts (PZA & PZM) of *P. zeylanica* in acetone and methanol were carried out to test the mortality status of poplar defoliator- *C. cupreata*. In the first step PZA extract was tested by using 0.0625, 0.125, 0.25, 0.50, 1.00, 1.50 and 2.00% concentration. After 72 hrs. the larval mortality was found 0.0, 6.67±1.83, 10.00±0.0, 30.00±0.0, 46.67±0.82, 53.33±0.82 and 60.00±0.0% respectively. Similarly testing of PZM extract was also carried out with the same concentrations pattern. The respective larval mortality after 72 hrs. was

recorded as 0.0, 6.67±1.83, 10.00±0.0, 30.00±0.0, 53.33±0.75, 56.67±0.75 and 66.67±0.75% respectively. In control experiments, no larval mortality was observed (Table-3). It was also concluded that PZM at 2% concentration after 72 hrs. provided maximum larval mortality (66.67±0.75%) and considered the most effective extract for the control of larvae of *C. cupreata*.

Similar type of work was carried out by various workers. Singh K.P. and Yousuf M. (2016) tested the extracts of *Tagetes minuta* for the control of *Clostera cupreata* and it was reported that, the extract (TMM), extracted in methanol caused 50% larval mortality at 2% concentration after 72 hrs whereas the extract (TMA), extracted in acetone provided 46.66% larval mortality. Bhandari, *et.al.* (1988) observed that methanol extractives of neem seed found effective against poplar defoliator, *P. cupreata* for their antifeedant activity. Ahmad, *et.al.* (1991) recorded that extract of *Acorus calamus*, *Lantana camara* var. *aculeata, Adhatoda vesica* and *Melia azedarach* were effective in killing *Ailanthus* web worm, *Atteva fabriciella*. Meshram (2000) tested crude extracts fresh leaves of 14 plants against larvae of *Dalbergia sissoo* to evaluate their antifeedent and insecticidal activity and it was observed that *Melia azadarach* followed by *Eucalyptus hybrid* and *Pongamia pinnata* were found effective in decreasing order to control the damage due to larvae of *Plecoptera reflexa*.

Table-1 Yield % of *Plumbago zeylanica* in different solvents.

Sl. No	Total weight	Name of solvents	Yield (%)
1	400 gms	**Petroleum ether**	1.64
2		**Acetone**	2.35
3		**Methanol**	3.45
4		**Water or aqueous**	10.71

Table-2 Larval mortality of *C. cupreata* at 1% concentration of *P. zeylanica* extracts.

Chemical extract		Mortality after			Effective or not effective
		24 hrs	48 hrs	72 hrs	
PZPE	Avg.	3.33	3.33	3.33	Not effective
	SEM±	1.82	1.82	1.82	
	Control	0.00	0.00	0.00	
PZA	Avg.	23.33	46.67	46.67	effective
	SEM±	1.30	0.81	0.81	
	Control	0.00	0.00	0.00	
PZM	Avg.	30.00	56.67	56.67	effective
	SEM±	0.00	0.74	0.74	
	Control	0.00	0.00	0.00	
PZW	Avg.	0.00	0.00	0.00	Not effective
	SEM±	0.00	0.00	0.00	
	Control	0.00	0.00	0.00	

Table-3 Bioassay of effective extracts of *P. zeylanica* against the larvae of *C. cupreata.*

Effective extracts		Extract concentrations						
		0.0625	**0.125**	**0.25**	**0.50**	**1.00**	**1.50**	**2.00**
PZA	Avg.	0.00	6.67	10.00	30.00	46.67	53.33	60.00
	SEM±	0.00	1.83	0.00	0.00	0.82	0.82	0.00
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PZM	Avg.	0.00	6.67	10.00	30.00	53.33	56.67	66.67
	SEM±	0.00	1.83	0.00	0.00	0.75	0.75	0.75
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

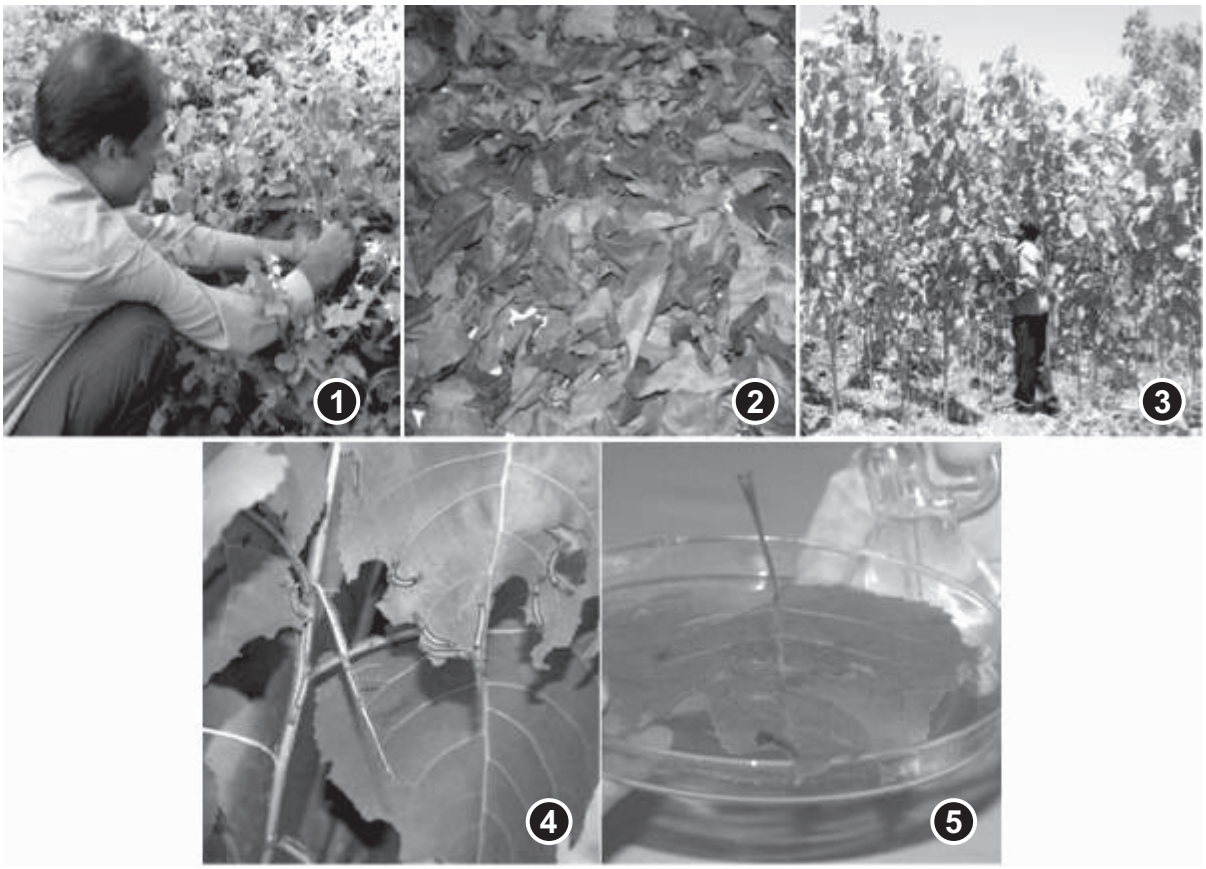

Figure-1 Collection of plants material of *P. zeylanica.*
Figure-2 Collected plant materials.
Figure-3 Collection mature and immature stages of poplar defoliator.
Figure-4 Rearing of *C. cupreata.*
Figure-5 Exposure of different concentrations of extracts on 3rd insrar larvae.

References

- Ahmad, M.; Gupta, B. K. and Bhandari, R. S. Efficacy of some plant extracts against *Ailanthus* Webworm, *Atteva fabriciella*. *Ind. Journal of Forestry*, 1991, 14 (1): 5-7.
- Beeson, C. F. C. The ecology and control of

the forest insects in India and neighboring countries. Govt. of India. Publ., 1941, Pp. 767.

- Bhandari, R.S.; Lal, Jia.; Ayyar, K. S. and Singh, Pratap. Effect of neem seed extractives on poplar defoliator *Pygaera cupreata* in Laboratory. *Indian Forester*, 1988, 114 (11):790-795.
- Chatterjee, P. N. and Thapa, R. S. Insect pests of fast growing species. Preliminary investigation on Poplar stem and root borer *Apriona cinerea* Chevrolet in New forest. *Indian Forester*, 1964, 90 (11): 777-781.
- Gupta, B. N. and Joshi, K. C. Evaluation of some biopesticides against forest insect pests. *Journal of Tropical Forestry*, 1995, 11 (1): 51-57.
- Lohani, D. N. Performance of exotic poplars in U.P. symposium papers: Silviculture, Management and Utilization of poplars, 1979, October 15-18, Srinagar (J and K), India.
- Mathur, R. S. and K. K. Sharma. Poplars in India. *Indian Forester*, 1983, 09 (9): 591-631.
- Meshram, P. B. Antifeedant and insecticide activity of some medicinal plant extracts against *Dulbergia sissoo* defoliator *Plecoptera reflexa* Guenee (Lepidoptera: Noctudae). *Indian Forester*, 2000, 126 (9): 961-965.
- Singh K. P. and Yousuf M. Efficacy of extracts of *Tagetes minuta* against *Plecoptera reflexa* (Lepidoptera: Noctuidae) – A major defoliator of shisham. *Ann.For*., 2015, 23 (1): 33-37
- Tiwari, D. N. A monograph of Poplar. International Book Distributors, 1993, Pp. 333.

Antibacterial Activity of *Andrographis paniculata* against *Salmonella typhi*

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Abstract-Typhoid is a major public health problem in tropical and subtropical countries including India. It is caused by the species of genus *Salmonella serover typhi* and *paratyphi*. It has caused significant morbidity and mortality in an endemic region. Development of drug resistance in *Salmonella* to the existing antibiotic is a major obstacle to control the disease in an endemic region. The discovery of new drugs by screening medicinal plants to discover new bioactive molecules is needed. The present study was aimed to screen medicinal plant *Andrographis paniculata* to know about the anti-*salmonella* activity. In this study, laboratory adopted *S. typhi* isolates were used and further characterization of isolates was performed. The antibiotic sensitivity and screening of medicinal plant extracts against all *Salmonella* isolates was done. The microbiological and biochemical analysis confirmed that all isolates were *S. typhi*. Antibacterial activity of β-lactams, quinolones, aminoglycosides and sulphonamides classes of antibiotics was determined against the isolates. The antibacterial activity of methanol, ethanol and acetone leaf extracts of *Andrographis paniculata* in different concentrations were screened against *S. typhi* isolates. The isolates were found resistant for trimethoprim, co-trimoxazole, sulphanilamide drugs and sensitive for levofloxacin, amikacin, amoxicillin and penicillin-G. In the present study, we have observed the potent antibacterial activity of methanol extract in comparison to other extracts. The study suggests that the methanol leaf extract of *A. Paniculata* have bioactive molecules, which possess antimicrobial activity against S. typhiand may be used to develop strong antibiotics. Being a preliminary study, and we can not make any conclusion on the basis of this study, further study is being undertaken.

Keywords: *S. typhi*, Antibacterial activity, *Andrographis paniculata*

Introduction

Typhoid is an acute systemic infection caused by *Salmonella enteric subsp. enterica serotype typhi* and *paratyphi*. Typhoid continues to be a global public health problem with an estimated 22 million cases annually, which result in 2 lakhs deaths worldwide every year (Curtis and Wheeler, 2006).It was observed that approximately 884 million person worldwide lack access to safe water and nearly 2.5 billion persons do not have access to adequate sanitation (WHO, 2012).Antimicrobial therapy reduces the morbidity and mortality but despite the use of antibiotics and development of newer antibacterial drugs, enteric fever has continued to be a major health problem. Multidrug resistant strain of *S. typhi* is of great concern not only because of its resistance to antibiotics that results in high death rate but also it's potential for epidemic outbreak which may be difficult to manage.

The resistance problem demands a renewed effort to screen various medicinal plants for their potential antimicrobial traits. Medicinal herbs are rich source of antimicrobial agents (Karinge *et. al*., 2006). Recently, *Andrographi spaniculata* have been studied extensively for their pharmacological activities (Jarukamjorn and Nemoto, 2008) and it has been reported that *Andrographi spaniculata* possess antimicrobial properties against micro pathogens (Hosamani *et. al*., 2011; Sharma et al., 2011; Kumar *et al*., 2013). With the given rationale, the present study was aimed to screen the medicinal plant for antibacterial activity against *S. typhi* isolates from Solan district of Himachal Pradesh.

Material and Methods

The present study was conducted in Solan district of Himachal Pradesh. The laboratory adopted 14 isolates of *S. typhi*, (Molecular and Immuno-parasitology Research Laboratory, Shoolini University, Solan) isolated from Solan district of Himachal Pradesh, were used for the study. The standard isolate MTCC 733 of *S.typhi* used as a control, was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India.

Characterization of isolates

The laboratory adopted clinical isolates were cultured on Nutrient agar media, Mac Conkey agar media , Bismuth Sulphite agar media and Xylose Lysine Deoxycholate media (HiMedia) by streak plate method and inoculation of isolates in Selenite F broth was done.The bacterial isolates were identified on the basis of colony morphology i.e. colour, shape, margin, elevation, surface etc. that appeared on media. Gram staining and biochemical tests like Catalase test,Indole production test, Methyl red test, VP (VogesProskauer) test, Citrate utilization test, Urease test, Carbohydrate fermentation test(Glucose, Lactose, Maltose and Mannitol)and Motility test were also performed.

Drug sensitivity assay

Drug sensitivity test was performed by using disc-diffusion method (Bauer *et al*., 1966). Different antibiotics including trimethoprim (5mcg), sulfanilamide (10mg), amikacin (30mcg), amoxicillin (10mcg), co-trimoxazole (25mcg), levofloxacin (5mcg) and penicillin-G (10 units) were screened in-vitro against *S. typhi*. The experiment was performed in triplicate under strict aseptic conditions. The resistant patterns were recorded as per guidelines given by National Committee for Clinical Laboratory Standards (NCCLS, 2009) for Gram negative *S.typhi*. Control was also included in the study.

Screening of Medicinal Plant *A. Paniculata* against *S. typhi*

A. paniculata were obtained from the herbal garden of Shoolini University, Solan. The plant was verified from Y.S. Parmar Horticulture University, Solan.

The plant leaves were washed under tap water for removal of dust particles and epiphytic hosts found on soil surface. They were further air-dried on filter paper at room temperature and powdered with the help of sterile mortar and pestle under aseptic conditions. Dry crushed material of leaves was subjected to extraction in a soxhlet apparatus using solvents, methanol, ethanol and acetone. The extracted material was evaporated to dryness under reduced pressure and different dilutions i.e. 25mg/ml, 50mg/ml and 100mg/ml were prepared in Dimethyl sulfoxide (DMSO). Extracts were stored at 4°C for further use.

The agar well diffusion method was used to test the antibacterial activity of different extracts of *A. paniculata* leaves against *S. typhi*.

The petriplates containing Muller Hinton Agar (HiMedia) were spread with *S. typhi* strains. The agar was punctured with an agar borer in five different sites for making wells and 50 µl of each plant extracts in acetone, methanol and ethanol was added into each well. The organic solvent used for extract preparation was used as negative control while trimethoprim (10µg/ml) was used as positive control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The experiment was performed in triplicate under strict aseptic conditions (NCCLS, 1993).

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of methanol extract of *A.paniculata* was checked by Microtitre broth dilution method (CLSI, 2012). The standard strain MTCC 733 of *S. typhi* was used as a control. 50mg of methanol extract was dissolved in 1ml of DMSO. Then,100µl of nutrient broth was added in the wells of microtitre plate. 100µl of methanol extract was added in 1st well and further 1:1 dilutions were prepared. The

dilutions, going from least concentrated to most concentrated, were inoculated with 5µl of *S. typhis* strains. Positive and negative controls were also included. The microtiter plate was incubated at 37±1°C for 24 hrs and observed. Turbidity indicated the growth of the microorganism and the MIC taken was the lowest concentration, where no growth was visually observed.

Results

All the 14 isolates of Salmonella showed characteristic large, thick, greyish white, moist, dome-shaped and smooth colonies on Nutrient agar media, black coloured colonies on Bismuth sulfite agar media, pink coloured colonies with black center on XLD media and colourless or pale colonies on MacConkey agar media.Change in colour of Selenite F Brothalso confirmed the isolates as Salmonella.The Biochemical analysis again confirmed that the isolates used is *Salmonella* (Table).

Table: Biochemical characterization of the *S. typhi* isolates

Strain No.	Indole	MR	VP	Citrate	Urease	Glucose	Lactose	Maltose	Mannitol	Motility
S*	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-3	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-4	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-5	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-6	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-7	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-9	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-10	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-11	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-12	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-13	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-14	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-15	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-16	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve
S-17	-ve	+ve	-ve	-ve	-ve	A	NF	A	A	+ve

Where, S* = Standard strain, -ve = Negative, +ve = Positive, A = Acid production and
NF = Non-fermenting (neither acid, nor gas production)

Drug sensitivity assay

All 14 isolates of *S. typhi* showed resistance to trimethoprim (100%), sulfanilamide (100%) and co-trimoxazole (100%) and were found sensitive to levofloxacin (100%), amikacin (100%), amoxicillin (78%), penicillin-G (71%) (Figure-3a, b and c).

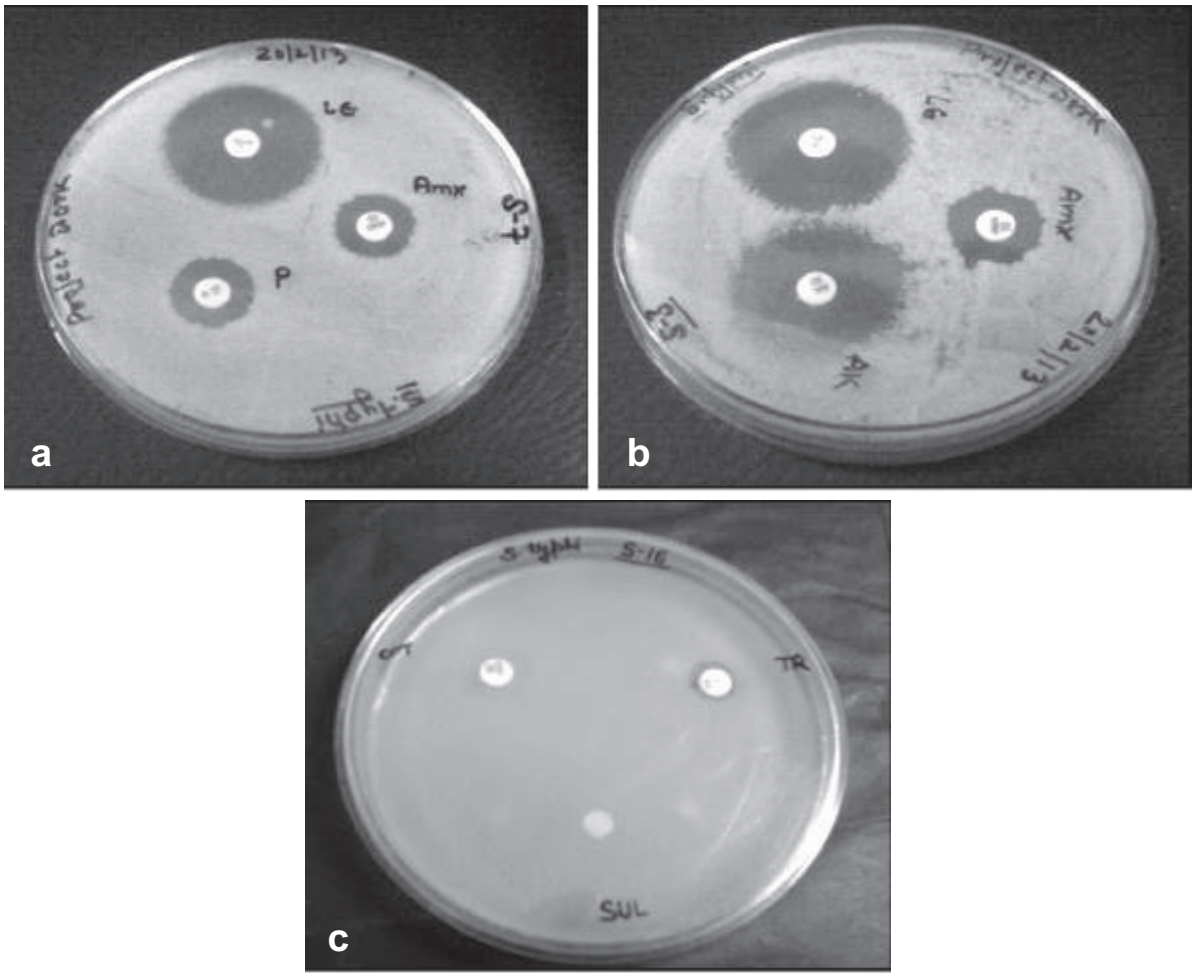

Figure-3 a-*S. typhi* strain showing sensitivity to Levofloxacin, Penicillin and Amoxycillin
b-*S. typhi* strain showing sensitivity to Amoxycillin, Levofloxacin and Amikacin
c-*S. typhi* showing resistance to Co-trimoxazole, Trimethoprim and Sulfanilamide

Antibacterial activity of *A. paniculata*

Methanol, ethanol and acetone extracts of leaf of *A. paniculata* plant was screened for antibacterial activity. 25mg/ml dose of all extracts (methanol, ethanol and acetone) was found inactive while 50mg/ml and 100mg/ml dose of all extracts showed zones of inhibition against *S. typhiisolates*. The methanol extract showed potent antibacterial activity than ethanol and acetone extracts (Figure. 4a, b, c). This indicates that the methanol extract of *A. paniculata* have higher activity against *S. typhi*. No zones were observed for positive control (trimethoprim) and negative control. MIC value of methanol extract was observed as 50mg/mlagainst *S. typhi* strains.

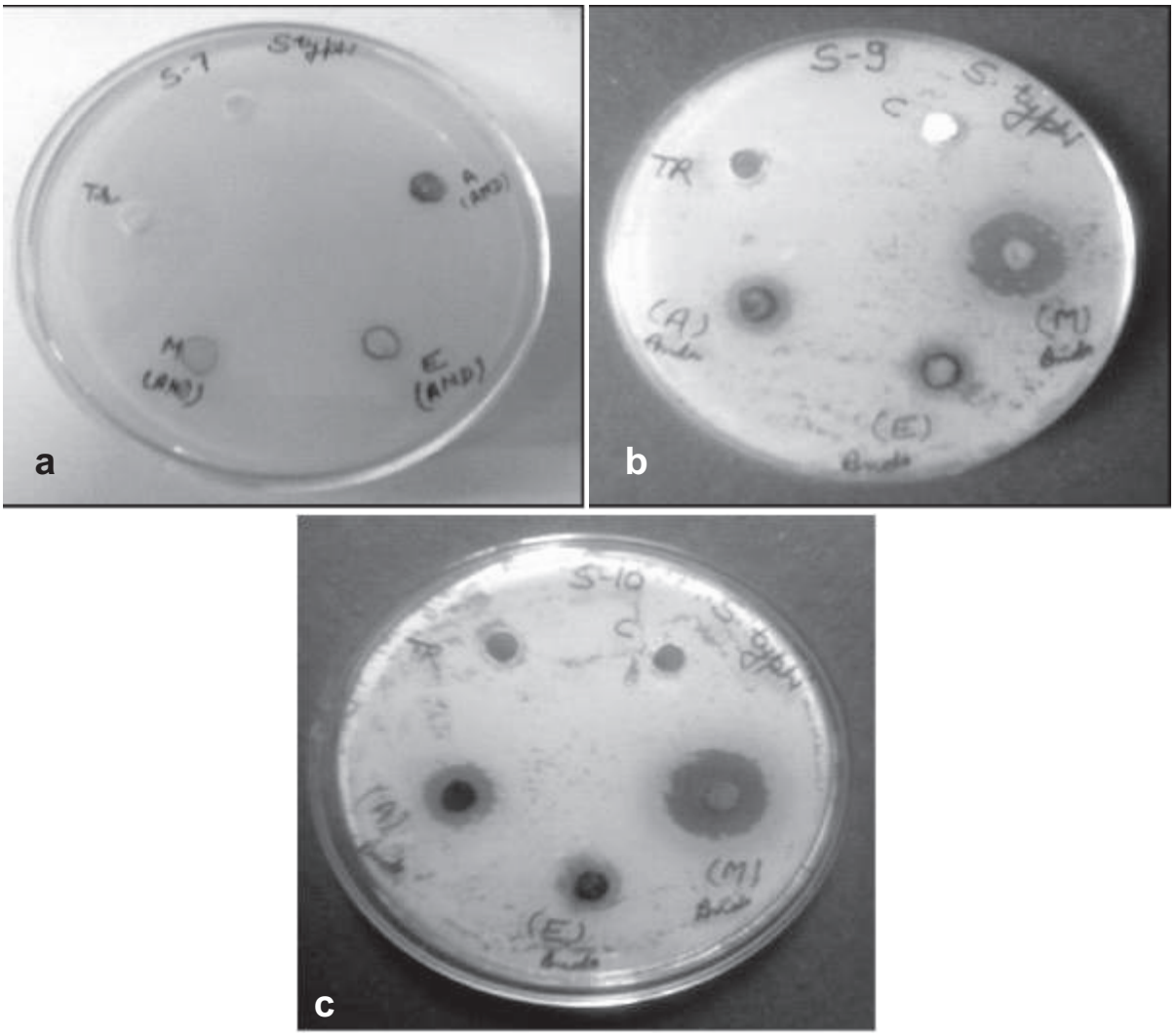

Figure-4 a- ***S. typhi*** **strain showing resistance to trimethoprim, methanol, ethanol and acetone extracts of** ***A. paniculata*** **(25mg/ml)**

b- ***S. typhi*** **strain showing high sensitivity to methanol extract, mild sensitivity toacetone extractsand resistance/least sensitivity to ethanol extracts of** ***A. paniculata*** **(50mg/ml)**

c- ***S. typhi*** **strain showing high sensitivity to methanol extract, mild sensitivity toacetone extracts and resistance/least sensitivity to ethanol extract of** ***A. paniculata*****(100mg/ml)**

Discussion

Multidrug resistant *S. typhi* is endemic and has become a public health concern in many areas around the globe, particularly in developing countries including India. This requires the use of plants as therapeutic agents for the development of new antimicrobial drugs. In the present study, we have characterized the laboratory adopted *S. typhi* isolates on the basis of microbiological and biochemical assays, which confirmed the isolates used in this study are *S. typhi*. In accordance to present study, several workers have identified *S. typhi* by using Biochemical and morphological methods (Bello, 2002; Cheesbrough, 2002;

Cowan and Steel, 2002; Perilla, 2003 and Abdullahi, 2010). In the present study, we have observed that the levofloxacin, amikacin, amoxicillin and penicillin-G drugs have shown sensitivity against *S. typhi* while trimethoprim, co-trimoxazole and sulphanilamide were found resistant/inactive against the challenge. In accordance to our findings, multidrug resistant strains of *S. typhi* against trimethoprim, streptomycin, sulfonamides and tetracycline have been reported from many developing countries, especially Pakistan (Karamat *et al*., 1990) and India (Prakash and Pillai, 1992). Due to the drug resistance problem, antibiotics such as, ampicillin, trimethoprim-sulphamethoxole and nalidixic acid, have been banned in most endemic countries including Asia and sub-Saharan Africa (Amyes and Gupta, 2002). Chand (2013) reported the activity of penicillin-G against *S. typhi* which is concordance to our study.

In the present study, we have screened different extracts of *A. paniculata* but our findings suggested the higher potential of methanol extract of *A. paniculata* as an antibacterial agent against *S. typhi*, as we observed potent anti-salmonella activity of methanolic plant extract in comparison to other. Similarly, Sule *et. al*., (2011) have found higher activity of methanol extract of *A.paniculata* whole plant, as an antimicrobial agent. Recently, It has been reported that the methanol extract of the leaves of *A. paniculata* exhibits more appreciable activity than the aqueous and ethanol extracts against *S. typhi* (Sharma and Joshi, 2011, Kumar *et. al*., 2013).

Conclusion

The present study confirmed that *Salmonella enteric serovar typhi* is associated with typhoid in Solan, which needs consideration. The study revealed that the drug resistant and sensitive *S. typhi* isolates are sensitive against methanol leaf extract of *A. paniculata*, suggesting its use as new drug against *S. typhi*. Though, the present study is a preliminary screening of *A. paniculata*, a large study may be undertaken to know the efficacy of *A. paniculata* plant extracts, which may lead to the formulation of new and potent antimicrobial drugs of natural origin.

References

- Abdullahi, M. Incidence and antimicrobial susceptibility pattern of *Salmonella species* in children attending some hospitals in Kano metropolis, Kano state–Nigeria, Medical Laboratory Science Department, School of Health Technology, 2010.
- Amyes, P. and Gupta, S. P. *Salmonella* serotypes in Utter Pradesh. *Indian Journal of Medicine*, 2002, 52: 235-240.
- Bauer, A. W.; Kirby, W. M.; Sherris, J. C and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* 1966, 45: 493-496.
- Bello, C. S. S. Laboratory manual for students of medical microbiology, Satohgraphics press, 2002, 2: 80-85.
- Chand, B. Antibacterial effect of garlic (*Allium sativum*) and ginger (*Zingiberofficinale*) against *Staphylococcus aureus*, *Salmonella typhi*. *Journal of Microbiology, Biotechnology and Food Sciences*, 2013, 2: 2481-2491.
- Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-**7**th Edition, M02-A11, 2012, 32.
- Cheesbrough, M. District laboratory practice in tropical countries, Combridge University Press, 2002, 2: 97-182.
- Cowan, S. T. and Steel, K. J. Manual for the identification of medical bacteria, Cambridge University press, 2002, 2: 51-120.
- Curtis T and Wheeler, D. J. Typhoid fever. *International Journal of Health Research*, 2006, 50: 3516-3517.
- Hosamani, P. A.; Lakshman, H. C.; Sandeepkumar, K.; Rashmi, C. And

Hosamani, C. Antimicrobial Activity of Leaf extract of *Andrographis panicula*. *WallScience Research Reporter*, 2011, 1: 92-95.

- Jarukamjorn, K. and Nemoto, N. Pharmacological aspects of *Andrographis paniculata* on Health and its major Diterpenoid Andrographolide. *Journal of Health Science*, 2008,54: 370-381.
- Karamat, K. A. Multiple drug resistant *Salmonella typhi* and ciprofloxacin, In proceedings of the 2nd Western Pacific Congress on Infectious Diseases and Chemotherapy (Jakarta, Indonesia). *Western Pacific Society of Chemotherapy,* 1990, 480.
- Karinge, J. W. A survey of traditional health remedies used by the Maasai of Southern Kaijiado District, Kenya. *Ethnobotany Research and Applications*, 2006, 4: 61-73.
- Kumar, U. A.; Amrita, K.; Phaneendra, P. and Manikumar, M.N.V.S. Preliminary investigation into the antibacterial activity of *Andrographis paniculata* leaf extract to *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Shigella flexneri. International journal of universal pharmacy and bio sciences*, 2013, 2.
- Perilla, M. J. Manual for the laboratory identification and antimicrobial testing of bacterial pathogens of public health importance in the developing world, World health organization, 2003:133-284.
- Prakash, K. and Pillai, P. K. Multidrug resistant *S. typhi* in India, Alliance for prudent use of antibiotics newsletter, 1992, 10: 1-3.
- National Committee for Clinical Laboratory Standards (NCCLS), Performance standards for antimicrobial disk and dilution susceptibility tests-7th edition: Approved Standard M2-A7. Wayne, Pennsylvania, USA, (2009).
- National Committee for Clinical Laboratory Standards (NCCLS), Performance standards for antimicrobial disk susceptibility tests, Approved standard. NCCLS document M2-A5, Wayne, Pennsylvania, USA, (1993)
- Sharma, M. and Joshi, S. Comparison of anti-oxidant activity of *Andrographis paniculata* and *Tinospora cordifolia* leaves. *J. Curr. Chem. Pharm. Sc*., 2011, 1: 1-8.
- Sule, A.; Ahmed, Q. U.; Samah, O. A. and Omar, M. N. Bacteriostatic and bactericidal activities of *Andrographis paniculata* extracts on skin diseases causing pathogenic bacteria. *J. Med. Plant Res*., 2011, 5: 7-14.
- World Health Organization, UNICEF, Progress on drinking water and sanitation–(2012) update, Geneva, Switzerland.

Phytochemical Evaluation and Antimicrobial Activity of *Zanthoxylum armatum* DC, *Solanum nigrum* and *Coriandrum sativum*

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Abstract- The present study was conducted to evaluate the phytochemical and antibacterial properties of three selected medicinal plants viz. *Zanthoxylum armatum*, *Solanum nigrum* and *Coriander sativum*. Phytochemical investigation was done for alkaloids, tannins, Carbohydrate, proteins, and saponins. All of the phytochemicals tested, were present in the leaf of *Solanum nigrum*. Antibacterial activity of three plants showed that extract of *Z. armatum*stem was active only against *S. aureus* and *Bacillus cereus,* this extract did not show any activity against the *E.coli*, *Citrobacter, Morganellaspp , Salmonella Typhi* and *Shigellaflexneri*. Leaf extract of *Solanumnigrum* showed activity against *Bacillus cereus* only and was ineffective against *E.coli*, *Citrobacter, Morganellaspp, Salmonella typhi, Shigellaflexneri* and *S. aureus.* Leaves extract of *Coriandrumsativum* showed activity only against *S. aureus* and was ineffective against *E.coli*, *Citrobacter, Morganella, Salmonella typhi*, *Shigellaflexneri* and *Bacillus cereus.*

Keywords: *Zanthoxylum armatum, Solanum nigrum, Coriander sativum***,** Phytochemicals

Introduction

The traditional system of medicine is well known medicinal system in India. Indian subcontinent has very rich biodiversity and consists of numerous medicinal plants of which some are not even explored for therapeutic properties. Bacterial species may become resistant by changing at the antibiotic binding site, inhibition of metabolic pathways, and inhibition of protein synthesis. Indiscriminate uses of antibiotic also contribute in the development of antibiotic resistance (Chandra *et al*., 2017). Antibacterial agents, such as β-lactams (like penicillins), cephalosporins or carbapenems and aminoglycosides are the compounds that inhibit the bacterial growth without being toxic to the surrounding tissue. These antibiotics are effective and mostly used as therapeutic agent to control certain type of infections. The problem associated with the antibiotic is the development of multiple drug resistant microorganisms and their associated side effects. Plant metabolites such as quinines, alkaloids, flavonoids, flavones, coumarins, essential oils terpenoids, tannins, antimicrobial peptides, lignans and glucosinolates are known to have potent antimicrobial and other medicinal properties (Chandra *et al*., 2017).

Zanthoxylum armatum is an aromatic shrub belongs to the family Rutaceae and well known for its medicinal properties in diseases like asthma, colic pain, toothache, headache, cardioprotective and antinflammatory properties (Gilani *et al*., 2010). *Solanum nigrum* belongs to the family Solanaceae, and known for its antimicrobial properties (Sridhar *et al*., 2010). *Coriander sativum* is well known herb used as spice in India and it is used as medicine also. Its medicinal properties such as antimicrobial activity were reported by Burdock and Carabin (2009).

The present study was designed to investigate the antibacterial potential of medicinal plants commonly distributed in Garhwal region. The experimental plants for the present study were *Zanthoxylum armatum, Solanum nigrum,* and *Coriander sativum.*

Material and Methods

Collection of Plant Material

The plants sample *viz.* stem of *Z. armatum* and leaves of *S. nigrum* were collected from the nursery of High Altitude Plant Physiology Research Centre (HAPPRC), leaves of *C. sativum* were collected from local market of Srinagar, Garhwal in the month of February, 2017. Fresh plant material was washed under running tap water, air dried and then homogenized to the fine powder and stored in airtight packets.

Extraction of Plant extract

Plant materials were air dried in shade at room temperature for one week and the shaded dried leaves were then powdered in the electronic grinder (Usha Pvt. Ltd). The plant powdered (25gm) were successively extracted using methanol solvent (Qualigens) in Soxhlet apparatus. All the extracts were filtered through Whatman filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get the dried extracts. The extracts were preserved in airtight screw cap vials and kept at 4-5°C in the refrigerator for further use. The dry extracts were dissolved in 20% dimethyl sulfoxide (DMSO), positive control drug (Amikacin, and Chloramphenicol) were used to represent the zone of inhibition of the control and extracted plant materials.

Determination of Phytochemical Constituent

Phytochemical constituent was determined according to the methods described in Trease and Evans (1989.)

Test for Alkaloid: 200 mg plant materials were taken in 10 ml of methanol and then filtered. After that, 2 ml filtrate was taken, added 1 % HCL and few drops of Mayer's reagent to it. It produced creamish/brown/red/orange precipitate which indicates the presence of alkaloids.

Test for Tannins: 200 g powder was boiled in 20 ml of distilled water for few min, added to it 3 drops of 5% $FeCl_3$. Brownish-green or blue black precipitate indicates the presence of tannins.

Test for Saponins: 1 g powder was boiled in 10 ml distilled water for 15 min, after cooling, the extract was shaken vigorously to record froth formation.

Test for Carbohydrate: 3-5 ml of the plant extracts in a test tube was taken and added to it a few drops of Benedict's reagent and mixed thoroughly and heated. The colour change from blue to green, yellow, orange or red indicates the presence of Carbohydrate.

Test for Protein: 3-5 ml of the plant extract in test tube was taken and added to it a few drops of ninhydrin reagent and mixed thoroughly and heated. The appearance of blue colour indicates the presence of protein.

Antibacterial activity of plant extract

0.5 ml of inoculums of test organism was mixed with sterile Muller Hinton agar Medium and shaken and poured in sterilized Petri dishes. Wells of 6 mm diameter were punched with the help of cork borer and 100ml of tested material (100mg/ml) were poured in wells. Then incubated the plates at 37°C for 24 hours (Okeke *et al.,* 2001). Zone of inhibitions were measured in millimetre (mm), manually with help of scale.

Results and Discussion

Qualitative constituents of investigated plant species are shown in Table-1. Phytochemical analysis showed that *Z. aramatum* contains alkaloids, tannins, saponins and carbohydrate but lack protein. However, in case of *C. sativum* there was presence of tannins, protein and carbohydrate but lack alkaloid and saponins. Joshi *et al.* (2012) also reported the presence of sterols, triterpenes, volatile oils, coumarins, alkaloids, flavonoids, flavonic glycosides, saponins and tannins in *Z. armatum*. The similar finding was also reported by Mukhija and Kalia (2014).

Phytochemical investigation of *S. nigrum* revealed the presence of all the phytochemicals tested. The presence of tannins, proteins, alkaloids, flavanoids and saponin were also reported by Ashrafudoulla *et al*. (2016).

Table-1 Qualitative Phytochemical analysis of Medicinal Plants

S.No.	Plant materia	Alkaloids	Tannins	Saponins	Carbohydrate	Protein
1	***Z. armatum*** **(Stem)**	+	+	+	+	-
2	***S. nigrum*** **(leaf)**	+	+	+	+	+
3	***C. sativum*** **(leaf)**	-	+	-	+	+

Effect of methanol extracts of the dried plant materials of *Z. armatum, S. nigrum* and *C. sativum* was examined for the control of seven bacteria species-Antibacterial activity (denoted in terms of inhibition zone) against *Bacillus cereus* (Gram positive), *Salmonella typhi*(Gram negative), *Shigellaflexneri* (Gram negative). Two standard antibiotics (Amikacin, and Cloramphenicol) were used as positive control for comparison of antibacterial activity of plants extracts against the same bacteria. Results and observed and summarized in Table-2, Table-3 and Table-4. The results of plant extracts and standard compared to confirm the potential of antibacterial activity of plants extract. Results of assessment of antibacterial activity revealed variability in different plants extracts against tested bacterial pathogens.

In the case of *Z.armatum*, methanolic (MeOH) extract of stem does not show any activity against the *E.coli*, *Citrobacter, Morganella , Salmonella typhi*, *Shigella flexneri* and but showed activity against *S. aureus* and *B. cereus*. The similar finding was also reported by Rafael *et al*. (2017) in which the root extracts of *Zanthoxylum spp.* have antibacterial activity against *Staphylococcus aureus* strain ATCC 25923.*S.aureus* when tested against standard antibiotics, no zone of inhibition was observed for Amikacin and Chloramphenicol, whereas methanol extract showed 15.0 ± 0.7 mm zone of inhibition, which was more active than both antibiotics tested. *B. cereus* when tested against standard antibiotics, no zone of inhibition was observed for Amikacin, whereas the methanolic extract of stem showed 14.0 ± 1.4 mm zone of inhibition which was more active than Amicacin antibiotics (Table-2).

Table-2 Antibacterial activity of *Zanthoxylum armatum* stem against bacterial pathogens

S.No	Name of Bacteria	Plant Part	Zone of inhibition (in mm)		
			Ampicillin	Chloramphenicol	MeOH extract
1	***Bacillus cereus***	stem	NA	22.0 ± 0.7	14.0 ± 1.4
2	***Salmonella typhi***		18.0 ± 1.4	28.0 ± 0.0	NA
3	***E. coli***		24.0 ± 0.0	33.0 ± 1.4	NA
4	***Morganella***		18.0 ± 0.7	30.0 ± 2.1	NA
5	***S. aureus***		NA	NA	15.0 ± 0.7
6	***Shigella flexneri***		16.0 ± 0.0	27.0 ± 1.4	NA
7	***Citrobacter***		14.0 ± 0.7	27.0 ± 1.4	NA

In case of *S. nigrum* methanolic leaf extract does not show any activity against the *E.coli*, *Citrobacter, Morganella* spp., *Salmonella* Typhi, *Shigella flexneri*, *S. aureus* and showed antibacterial activity against *Bacillus cereus* which when tested against standard antibiotics, no zone of inhibition was observed for Amikacin and Chloramphenicol, whereas the methanolic leaf extract showed 10.0 ± 1.4 mm zone of inhibition (Table-3). Antibacterial activity against *B. subtilis*, *B megaterium*, *Proteus vulgaris*, *Staphylococcus aureus*, *Klebsiella pneumonia*e and *E.coli* of different parts of *S. nigrum* was also reported by various researchers (Patil *et al*., 2009 ; Sridhar *et al*., 2011).

Table-3 Antibacterial activity of *Solanum nigrum* against bacterial pathogens

S.No	Name of Bacteria	Plant Part	Zone of inhibition (in mm)		
			Ampicillin	Chloramphenicol	MeOH extract
1	***Bacillus cereus***	Leaf	NA	NA	10.0 ± 1.4
2	***Salmonella* Typhi**		18.0 ± 1.4	28.0 ± 0.0	NA
3	***E. coli***		24.0 ± 0.0	33.0 ± 1.4	NA
4	***Morganella***		18.0 ± 0.7	30.0 ± 2.1	NA
5	***S. aureus***		NA	NA	NA
6	***Shigella flexneri***		16.0 ± 0.0	27.0 ± 1.4	NA
7	***Citrobacter***		14.0 ± 0.7	27.0 ± 1.4	NA

NA- No Activity

In case of *C. sativum*, extract of leaves does not show any activity against the *E.coli*, *Citrobacter, Morganella, Salmonella Typhi*, *Shigella flexneri*, *Bacillus cereus* and only shows activity against *S. aureus.* which when tested against standard antibiotics, no zone of inhibition was observed for Amikacin whereas in case of methanolic extract of *C. sativum* showed 8.0 ± 1.2 mm zone of inihibiion , which was more active than both antibiotics (Table-4). The similar finding was also reported by Rathabai and Kanimozhi (2012). Cao, *et al* (2012) also reported the antibacterial activity of water extract against *E. coli* and *Bacillus subtilis* but shown no activity against *Saccharomyces cerevisiae* and *Penicillium spp.*

Table-4 Antibacterial activity of *Coriandrum sativum* leaves against bacterial pathogens with methanol extracts.

S.No	Name of Bacteria	Plant Part	Zone of inhibition (in mm)		
			Ampicillin	Chloramphenicol	MeOH extract
1	***Bacillus cereus***	Leaf	NA	22.0 ± 0.7	NA
2	***Salmonella* Typhi**		18.0 ± 1.4	28.0 ± 0.0	NA
3	***E. coli***		24.0 ± 0.0	33.0 ± 1.4	NA
4	***Morganella***		18.0 ± 0.7	30.0 ± 2.1	NA
5	***S. aureus***		NA	NA	8.0 ± 1.2
6	***Shigella flexneri***		16.0 ± 0.0	27.0 ± 1.4	NA
7	***Citrobacter***		14.0 ± 0.7	27.0 ± 1.4	NA

NA- No Activity

The findings of the present study support the conventional usage of the plant for the use to treat the infection caused by *S. aureus* and *B. cereus*. It is suggested that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for wound healing and for the preparation of antibacterial ointments. According to WHO, medicinal plants are the best source to obtain a variety of drugs. Therefore, such plants need further investigations to better understand their mechanism of action, and other then medicinal properties such as antioxidant, antitumor activity etc.

The study has revealed that many secondary metabolites are present in these medicinal plants (*Z.armatum, S.nigrum* and *C. sativum*). *Z. armatum* are known to possess a remedy for dental carries, skin infection, urinary tract infection, and stomach ulcers caused by the pathogens. The antibacterial activity of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The traditional use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. These findings support the traditional knowledge of local users. Awareness of

local community should be enhanced by incorporating the traditional knowledge with scientific findings.

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References

- Burdock, G. A. and Carabin, I. G. Safety assessment of Coriander (*Coriander sativum* L.) essential oil as a food ingredient. *Food Chemical Toxicology*, 2009, 47:22-34.
- Cao, Xin-Zhi; You, Jian-Ming; Li, Shen-Xin and Zhang, You-Liang. Antimicrobial activity of the extracts from Coriandrum sativum. *International Journal of Food Nutrition and Safety*, 2012, 1(2): 54-59.
- Chandra, H.; Bishnoi, P.; Yadav, A.; Patni, B.; Mishra, A. P. and Nautiyal, A. R. Antimicrobial resistance and the alternative resources with special emphasis on plant based antimicrobials- A review, 2017, 6:16.
- Gilani, S.N.; Khan, A. and Gilani, A. H. Pharmacological basis for the medicinal use of Zanthoxylum armatum in gut, airways and cardiovascular disorders. *Phytotheraphy Research*, 2010, 24: 553-558.
- Joshi, S. (Mulmi) and Gyawali, A. Phytochemicals and Biological Studies on Zanthoxylum Armatum of Nepal. *J. Nepal Chem. Soc.*,2012, 30:71-77.
- Mukhija, M. and Kalia, A. N. Antioxidant potential and total phenolic content of Zanthoxylum alatum stem. *J. App. Pharm*., 2014, 6 (4): 388-397.
- Okeke, M. I.; Iroegbu, C. U.; Eze, E. N.; Okoli, A. S. and Esimone, C. A. Evaluation of extracts of roots of Landophia for antibacterial activity. *J. of Ethnopharmac.,* 2001, 78-80.
- Patil Suhas; Joshi Vijaya; Prasanna Sutar and Sambrekar Sudhir. Screening of whole plant extract of *Solanum surattense* for antibacterial activity. *International Journal of pharmaceutical Sciences*, 2009, 1(1):110-114.
- Rafael, S. C.; Manuela, O. L.; Mireille, L. H.; Tânia, F. B. and Eudes, S. V. In vitro antibacterial effects of *Zanthoxylum tingoassuiba* root stem extracts and two of its alkaloids against multiresistant Staphylococcus aureus. *Brazilian Journal of Pharmacognosy,* 2017, 27: 195–198.
- Rathabai, V. and Kanimozhi, D. Evaluation of antimicrobial activity of *Coriandrum sativum*. *International Journal of Scientific Research and Revie*w, 2012, 1(3): 1-110.
- Sridhar, T. M.; Josthna, P. and Naidu, C. V. In Vitro Antibacterial Activity and Phytochemical Analysis of *Solanum nigrum* (Linn.) - An Important Antiulcer Medicinal Plant. *Journal of Experimental Sciences*, 2011, 2(8): 24-29.
- Trease, G.E and Evans, W.C. A text book of Pharmacognosy, 16th edition, *Elsevier*, 1989, Pp. 133-148.

Inhibitory Activity of Herbs on Diarrhoea Causing Pathogens

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Abstract- Infections of the gastrointestinal tract produce a variety of symptoms and can be due to a large number of different infective agents. The most common symptom is diarrhoea, which leads to considerable morbidity and mortality worldwide. Underlying medical conditions, such as autoimmune diseases and patients receiving immunosuppressive drugs including corticosteroids, may influence the response to infections. The main stay of managing diarrhoeal diseases is the recognition and correction of water and electrolyte depletion, anti-diarrhoeal agents and antimicrobial chemotherapy. A herbomineral formulation diarex eliminates common micro-organisms responsible for acute and chronic infectious diarrhea contains a host of natural herbal ingredients were evaluated for its *in vitro* antibacterial activity against the common pathogens. The most active extract was found to be the Methanol extract of *Holarrhena antidysenterica* showing the maximum zone of inhibition of 32 mm against *Staphylococcus aureus* followed *Punica granatum 28mm and Aegle marmelos(27mm)*. While *Salmonella enterica* and *E.coli* were the next most susceptible test organisms after *Staph.aureus*.

Keywords: Herbomineral formulation, Diarex, antimicrobial activity, Diarrhoeal diseases

Introduction

The ancient Indian system of medicine has long been using herbs and minerals to cure various disorders ailing human being. Ayurveda has described remedies well known for their efficacy in treating diarrhoeal disorders of both chronic and acute origin. Modern research has proved the efficacy of these herbal drugs.

Diarex, a phytopharmaceutical formulation of Himalaya Drug Company, is recommended for the treatment of acute and chronic diarrhoea relieves post prandial abdominal discomfort and regularizes bowel movement.

Himalaya Diarex - A perfect herbal solution to keep away Diarrhoea and Dysentery. It ensures gastro-intestinal health. As an anti-oxidant, Diarex restores GI health. Diarex's anti-inflammatory and demulcent properties facilitate healing of the intestinal mucosa, and its anti-spasmodic action alleviates abdominal colic associated with bowel infection.

Diarex is an herbal supplement that helps relieve abdominal pain and intestinal discomfort. The complex formula is an amebicidal agent found effective in treating short and long-term amebic infections as well. Other uses of Diarex include maintenance of normal stool consistency and regulating normal stool passing frequency and amount. The intestinal mucosa is in a better state due to the demulcent and anti-inflammatory characteristics of the supplement. Diarex has antispasmodic mechanism that aid abdominal colic and bowel infection.

Diarex consists of five herbs that have been used in ancient Indian medicine for several years in order to treat bowel and intestinal disorders. Each of the herbs in Ayurvedic medicine are considered to be already effective, so combining them have resulted to a more potent and useful formula. Included are bael tree fruit and nut grass which are known anti-diarrhoea agents, conessi tree and guduchi stem which are highly effective in ridding *Entamoeba histolytica* parasites and pomegranate fruit which is also highly effective controlling the infections. The gastrointestinal tract is then regulated and relaxed by the anti-inflammatory and antispasmodic actions of the herbal formula. In the end, fluid secretion in the intestine is normalized and stool consistency and frequency is improved. The *in vitro* antibacterial activity of diarex and its herbal ingredients were undertaken

to validate the inhibitory activity of this formulation on infection causing organisms.

There are many published reports on the effectiveness of traditional herbs against pathogenic bacteria, viruses and fungi and as a result plants are still recognized as the bedrock for modern medicine to treat infectious diseases.

Keeping in view of the importance of herbs traditionally used for the treatment of infectious diseases, this study is designed to evaluate the antimicrobial activity of *herbs* used in the Indian system of medicine for the treatment of manifestations caused by microorganisms.

Material and Methods

Collection of Test material

The Diarex tablets and herbs were collected from the market and the Himalaya Drug Company Dehradun (Uttarakhand state, India) respectively and authenticated by pharmacognist. The tablets sample as well as the plant material was crushed, powdered and used for extraction.

Test microorganisms

The antibacterial activity of the extracts was 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* and *Salmonella enterica MTCC 3858.*

Preparation of aqueous extracts

The finely powdered 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 extracts

100 grams of the powdered material was extracted with 500ml of methanol &Hexane separately for 24hrs. The extracts were filtered with sterile whatman filter paper No.1 into clean conical flasks. 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 4°C 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.

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 flavonoids.

Test for Sterols and Triterpenoids (Salkowski's test)

The extract was treated with chloroform, few drops of concentrated H2SO4 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 H2SO4 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, Salmonella enterica* and *Pseudomonas aeruginosa* 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 µL 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 & Hexane was tested for each organism. The inoculated plates were kept in refrigerator for 2 hours to allow the extracts to diffuse into the agar. The agar plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 30 µg of standard antibiotic Ciprofloxacin was used as positive control and respective solvents as negative controls.

Results and Discussion

The efficacy of hexane, methanol and aqueous extracts of these medicinal plants against pathogenic bacteria showed varied level of inhibition (Table 1, 2 and 3). It was revealed from the result that the herbs showed different degrees of inhibition against different microorganisms.

The maximum zone of inhibition was observed in the case of *Holarrhena antidysenterica* against *Staphylococcus aureus (32 mm)* followed by *Salmonella enterica* and *E.coli* (30 mm) as depicted in Table-1,while methanol extract of *Punica granatum* showed (28mm) zone against Staph aureus followed by *E.coli* (27mm) and *Salmonella enterica* (26mm) revelaed in Table-2. Methanol extract of *Aegle marmelos* showed maximum zone of inhibition against Salmonella enteric(26mm) followed by *E.coli* (25mm) as in Table-3. While the aqueous extract also showed good antibacterial activity against the test pathogens.

Phytochemical screening on the crude methanol extracts of Diarex was done. The results (Table- 4) revealed the presence of secondary metabolites such as saponins, tannins, alkaloids, flavonoids and glycosides.

Hexane extracts showed very low activity against *the* pathogens. 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 bacteria. The highest antibacterial activity as seen with methanol extract might be due to the presence of alkaloids and tannins (Okemo, 1996). Broad spectrum activity of methanol extract tended to show that the active ingredients 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 and 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 and Adesanya, 2005).

Aegle marmelos which is proved to be an excellent and effective remedy in controlling acute diarrhoea (Beg and Khan, 1993). It contains a large amount of tannins which effectively control nonspecific diarrhoeas (Singh *et. al.,* 1993). *Holarrhena antidysenterica* along with *Aegle marmelos* and other herbs is known to help control diarrhoea (Kohli *et. al.,* 1993). *H. antidysenterica* has been found to be more effective in treating *Entamoeba histolytica* positive patients (Ghosh and Shaw, 1992). A combination of *Aegle marmelos, Punica granatum, Tinospora cordifolia* along with other herbs is known to have potential antispasmodic activity (Srivastava and Bhatt, 1993). In addition, *Punica granatum* has shown anthelmintic activity in *in vitro* studies (Hukkeri *et. al.,* 1990) and antimicrobial activity against *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* (Navarro *et. al*., 1996). *Tinospora cordifolia* is known to be active against *Entamoeba histolytica* (Sohni and Bhatt, 1996).

The results of this study suggest that Diarex is effective in controlling not only non-specific diarrhoea but may also resolve bacterial and amoebic dysentery and diarrhoea. The abolition of ascariasis infection enhances the broad-spectrum activity of this drug.

Table-1 Antibacterial activity of *Holarrhena antidysenterica*

Test organism	Diameter of zone of inhibition (mm)			
	Hexane extract	Methanol extract	Aqueous extract	Ciprofloxacin
E.coli	18	30	22	34
Staph. aureus	19	32	24	35
Salomenella enterica	17	30	20	30
Pseudomonas aeruginosa	18	25	17	25

Table-2 Antibacterial activity of *Punica granatum*

Test organism	Diameter of zone of inhibition (mm)			
	Hexane extract	Methanol extract	Aqueous extract	Ciprofloxacin
E.coli	12	27	18	34
Staph. aureus	14	28	16	35
Salomenella enteric	14	26	16	30
Pseudomonas aeruginosa	12	20	12	25

Table-3 Activity of *Aegle marmelos* against test pathogens

Test organism	Diameter of zone of inhibition (mm)			
	Hexane extract	Methanol extract	Aqueous extract	Ciprofloxacin
E.coli	12	25	18	34
Staph. aureus	14	27	20	35
Salomenella enteric	13	26	19	30
Pseudomonas aeruginosa	16	22	13	25

Table-4 Phytochemical evaluation of Diarex Tablets

Phytochemical analysis of Diarex tablets				
Alkaloids	**Flavonoids**	**Tannins**	**Glycosides**	**Saponins**
+	+	+	+	+

+; Present

References

- Beg, M. Z. and Khan N. H. Med. and Aromatic Plants Abstr., 1993; 15:5, 470. "Effect of Aegle marmelos Corr. Syrup in acute diarrhoea and dysentery cases." Proc. 1 st National Seminar on Ilmul advia, Bennapara, Abstr. England, 1993, 23:23-25.
- Singh, A. K. Abhimanyu Kumar, Sharma, K.K. Shriphal powder: Antidysenteric action. Sachitra Ayurved, Hin., 1993, V: 46(8), 574.
- Kohli, K. R.; Gharge, S. and Nail, S. An Ayurvedic drug for its antidiarrhoeal property. *Ind. J. Int. Med.,* England , 1993;V:3(7),199.
- Ghosh, R. B. and Shaw, B. P. Effect of mushakani (Hemigraphis birta) on clinical amoebiasis. International Seminar, Traditional Medicines, Calcutta, 7-9 November, 1992; 142. (Eng).
- Srivastava, D. N. and Bhatt, K.R. Effect of Neblon on transit time of ingesta in and out of albino rats. *Ind. J. Indigenous Med*., 1993, V: 10(1), 23.
- Hukkeri, V. I.; Kalyani, G. A.; Hatapaki, B. C. and Manvi, F. V. In vitro anthelmintic activity of aqueous extract of rind of *Punica granatum*. (Abstract). Proceedings of 42, Indian Pharmaceutical Congress, Manipal, E08, 1990, 39. (Eng).
- Navarro, V.; Villarreal, M. L.; Rojas, G. and Lozoya, X. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *J. Ethnopharmacol*., 1996, 53(3):143.
- Sohni, Y. R. and Bhatt, R. M. Activity of a crude extract formulation in experimental hepatic amoebiasis and in immunomodulation studies. *J. Ethnopharmacol*., 1996, 54(2/3): 119.
- Atata, R.; Sani, A.and Ajewole, S.M. Effect of stem back extracts of *Enantia chloranta*on some clinical isolates.*Biokemistri*, 2003, 15(2): 84-92, 16.
- Bonjar, G.H. Evaluation of antibacterial properties of Iranian medicinal plants against *Micrococcus aureus, Serratia marcescens*, *Klebsiella pneumoniae* and *Bordella bronchoseptica*. *Asian J. Sci.,* 2004, 3(1): 82-86.

- Atata, R.; Sani, A.and Ajewole, S.M. Effect of stem back extracts of *Enantia chloranta*on some clinical isolates.*Biokemistri*, 2003, 15(2): 84-92, 16.
- Bonjar, G.H. Evaluation of antibacterial properties of Iranian medicinal plants against *Micrococcus aureus, Serratia marcescens*, *Klebsiella pneumoniae* and *Bordella bronchoseptica*. *Asian J. Sci.,* 2004, 3(1): 82-86.

- Okemo, P. O. Antimicrobial efficacy of selected medicinal plants used by Kenyan Herbal doctors. *Ph.D. thesis,* Kenyatta University of Nairobi. 1996: 173 –190.
- Aqil, F. and Ahmad, I. Broad spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World I Micro. Biotech.*, 2003, 19(6):653-657.
- Kannan, P.; Ramadevi, S. R. and Hopper, W.Antibacterial activity of *Terminalia chebula* fruit extract. *Afr. J. Microbiol. Res.*, 2009, 3: 180-184.

- Sampathkumar, P.; Dheeba ,B.;Vidhyasagar, V.; Arulprakash, T.and Vinothkannan, R. Potential antimicrobial activity of various extracts of *Bacopa monnieri* (Linn.). *Int. J. Pharmacol*., 2008, 4:230-232.
- Iwu, M. M. In: '*Hand-book of African Medicinal Plants*.' Published by CRC Press, 1993.
- Adesanya, S. A. In: *Theories and realities.Inaugural lecture series*. Published by Obafemi Awolowo University Press Ltd. Ile-Ife, Osun State Nigeria, 2005,181.
- Harbourne, J. B. *Phytochemical methods*. A guide to modern techniques of plant analysis. London, Chapman and Hall, 1973: 221- 232.
- Khandelwal, K. R. *Practical Pharmacognosy* Technique and experiments, 9th ed., Nirali publications, Pune, 2000: 149 – 156.

Phytochemical, Antioxidant and Antibacterial Activity of Chloroform and Methanolic Fruit Extracts of *Kigelia africana*

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Abstract- Phytochemicals present in plants probably explain the various uses of plants for traditional medicine. In this study *Kigelia africana* fruit was selected for assessing the level of various Phytochemicals, enzymatic and non-enzymatic antioxidants and antimicrobial activity. Fruits of the plants were taken, dried, ground to a powder and then its chloroform and methanolic extracts were prepared. The antimicrobial activity of these extracts was then studied using agar well plate method. The results of the study showed that chloroform extract of *Kigelia africana* has a significant amount of phytochemicals and antioxidant enzymes useful to prevent chronic diseases related to oxidative stress in the human body. Antimicrobial activity of chloroform extract was found to be more than the methanolic extract.

Keywords: *Kigelia africana*, Phytochemicals, Antioxidants, Antimicrobial activity.

Introduction

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

Material and Methods

Collection of plant materials

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

Extraction of plant material

The plant material taken for the study was stored under refrigerated condition till use. The extracts were prepared by using Sonicator and soxhlet apparatus and evaporated on a water bath, then crude extracts were obtained and stored at the temperature of 4°C till use for investigation.

Phytochemical investigations

Tests for Alkaloids and Flavonoids

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

Test for Saponins

Foam test: 1ml of chloroform extract was diluted separately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Test for Tannins

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

Test for Glycoside

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

Test for Terpenoid

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

Test for Reducing Sugar

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

Antioxidant Activity

Catalase activity was assayed by the method of Sinha *et al.*[9].

Peroxidase activity was carried out by the method of Addy and Goodman *et. al.*[10].

Ascorbate Oxidase activity was carried out by the method of Vines and Oberbacher *et. al.*[11]. The ascorbic acid activity was carried out by the method of Sadasivam S *et. al.*[12].

Determination of Antibacterial Activity

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

Results and Discussion

Phytochemical investigation of *Kigelia africana*

Qualitative analysis carried out of these plant extracts showed the presence of phytochemical constituents and the results are summarized in Table-1.

Results showed the presence of Alkaloids, Glycosides, Terpenoids and Flavonoids, Tannins and Reducing Sugars. The phytochemical screening done revealed that the *Kigelia africana* fruit extract contain the following active constituents, alkaloids, flavonoids, tannins, cardiac glycosides, cyanogenic glycosides, anthraquinone glycoside, saponins, anthocyanosides (anthocyanin pigment) and reducing compounds[13]. Terpenoids reduce sugar levels in the blood hence *Kigelia africana* shows anti diabetic activity[14].

Antioxidant Activity of Methanolic and Chloroform extract of *Kigelia africana*: Enzymatic antioxidants

The levels of Enzymatic Antioxidant assessed in different extracts of *Kigelia africana* were collectively represented in Table-2.

The Superoxide Dismutase activity was observed to be maximum in chloroform extract (2.40 units/mg protein) and the minimum in the methanolic extract of *Kigelia africana* (2.27 units/mg protein). Among the two extracts of *Kigelia africana*, the highest activity of catalase

was observed in chloroform extract (3.267 mg protein) and lowest in the methanolic extract (1.802 units/mg protein). Peroxidase activity was found to be very high in the methanolic extract ($2.942x10^6$ units/mg protein/min). In plants, antioxidant enzymes namely catalase[15] and peroxidase have been shown to increase when subjected to stress conditions. The ascorbate oxidase activity was highest in chloroform extract (0.0315 units/mg protein) and lowest in methanolic extract 0.0123 units/mg protein.

Non-enzymatic antioxidants

The concentration of different non-enzymatic antioxidants in *Kigelia africana* extracts was also assessed and the results are represented in Table-3.

Vitamin C content was low in methanolic extract (0.144 mg/ g tissue) whereas high in methanolic extract it is (0.427 mg/ g tissue). Ascorbate has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells and shown to function as a reductant for many free radicals[16].

Flavonoids The antioxidant properties of tocopherol are the result of its ability to quench both singlet oxygen and peroxides[17].

Antibacterial Activity of Methanolic and Chloroform extract of *Kigelia africana*

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

Table-1 Phytochemical analysis of both the extracts of *Kigelia africana*.

Plant extracts	Alkaloids	Glycosides	Terpenoids	Flavonoids	Tannins	Reducing Sugars	Saponins
Methanolic Extract of ***Kigelia africana***	+	+	+	+	+	+	-
Chloroform Extract of ***Kigelia africana***	+	+	+	+	+	+	+

Table-2 Enzymatic antioxidant analysis of both the extracts of *Kigelia africana.*

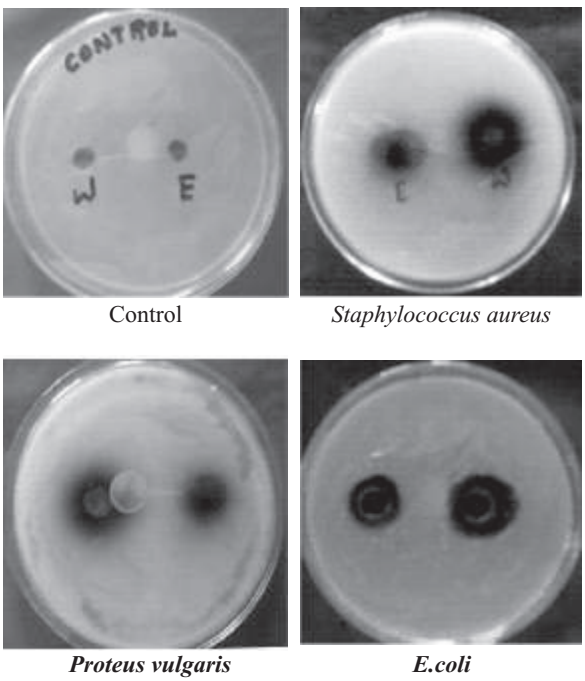
Samples	Catalase µ/moles of H_2O_2 decomposed/min/g protein	Peroxidase IU/L	Ascorbate oxidase µ mole/ml
Methanolic extract of *Kigelia Africana*	1.802	$2.942x10^6$	0.0123
Chloroform extract of *Kigelia Africana*	3.267	$2.904x10^6$	0.0315
	1 unit = µ/moles of H_2O_2 decomposed/min/g protein	1 unit = µ moles pyrogallol oxidized/min	1 unit = 0.01 O.D change/min

Table-3 Non-enzymatic antioxidant activity (Ascorbic acid)

Samples	Vitamin C (mg/g)
Methanolic extract	0.144
Chloroform extract	0.427

Table-4 Zone of inhibition of Methanolic and Chloroform extracts of *Kigelia africana* against bacterial species, i.e. *Proteus vulgaris*, *E.coli* and *Staphylococcus aureus*

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



Control *Staphylococcus aureus*

Proteus vulgaris ***E.coli***

Figure-1 Zone of inhibition of methanolic and chloroform extracts of *Kigelia africana*

Plants extracts are usually more active against gram positive bacteria than gram negative bacteria[18]. According to Abu-Shanab *et. al.*[19] gram negative bacteria are more resistant to plants extracts as compared to gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism and this is in support of the present finding which showed that both the extracts of *Kigelia africana* were effective on the growth of *S.aureus* which is gram positive bacterial species. Further studies are needed to isolate the exact active component, which are responsible for antimicrobial activities. So from the present study it has been concluded that chloroform extract of *Kigelia Africana* possesses a significant amount of phytochemicals, antioxidant enzymes and antimicrobial agents as compared to the methanolic extract.

Conclusion

From the present study, it has been concluded that chloroform extract of *Kigelia africana* possesses significant amount of phytochemicals and antibacterial agents. The results showed that the methanolic and chloroform extracts of *Kigelia africana* have significant amounts of antimicrobial activity. It is hoped that this report will serve as a basis of information for future project to be embarked on in order to evaluate the potentials of *K. pinnata* (Lam) Benth as a strong medicinal plant in improving human health status.

Conflict of interest statement

We declare that we have no conflict of interest.

References

1. Graham, J. G.; Quinn, M. L.; Fabricant, D. S. and Farnsworth N R. Plants used against

cancer– an extension of the work of Jonathan Hartwell. 2000.

2. Singh, M. *International Journal of Drug Research and Technology*, 2012, 2(2): 203-207.
3. Soulsby, J. Resistance to antimicrobials in humans and animals. *Braz. J. Med*., 2005, 331: 1219-1220.
4. Burt, S. Essential oils: their antimicrobial properties and potential application in foods-A review. *International Journal of Food Microbiology*, 2004, 94: 223-253.
5. Khan, M.; Kihara, M. and Omoloso, A. Antimicrobial activity of the alkaloidal constituents of the root bark of Eupamatia laurina. *Pharmaceut. Biol,.* 2003, 41: 277-280.
6. Aburjai, T.; Darwish. M.; Al-Khalil, S.; Mahajznh, A. and A1-Abbadi, A. Screening of antibiotic resistance inhibitors from local plant materials against two Abu-Shanab B, different strains of Pseudomonas aeruginosa. *J. Ethnopharmacol,.* 2001, 76: 39-44.
7. Harborne, J. B. Phytochemical methods, London. Chapman and Hall, Ltd. 1973, p. 49-188.
8. Siddiqui, A. A. and Ali, M. Practical Pharmaceutical chemistry. CBS Publishers and Distributors, New Delhi, First Edition. 1997, Pp. 126-131.
9. Sinha, A. K. Colorimetric assay of catalase. *Anal. Biochem*., 1972, 47:389-394.
10. Addy, S. K. and Goodman, R. N. Polyphenols oxidase and Peroxidase in apple leaves inoculated with a virulent or an avirulent strain for Ervinia amylovor. *Ind. phytopath*., 1972, 25: 575-579.
11. Vines, H. M. and Oberbacher, M. F. Response of oxidation and phosphorylation in citrus mitochondria to arsenate. *Nature*, 1965, 206:319-320.
12. Sadasivam, S. and Manickam, A. Vitamins, In Biochemical methods. Eds, A. New Age International (P) Limited, New Delhi, 2nd Eds. 1997, 85-186.
13. Azu, O. O.; Duru, F. I. O.; Osinubi, A. A.; Noronha, C. C.; Elesha, S. O. and Okanlawon, A. Preliminary study on the antioxidant effect of Kigelia africana fruit extract (Bignoniacieae) in male Sprague-Dawley rats. *African Journal of Biotechnology*, 2010, 9 (9):1374-1381.
14. Nyarko, A. K.; Okine, L. K. N.; Wedzi, R. K.; Addo, P. A. and Ofosuhene, M. Subchronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat:absence of organ toxicity and modulation of cytochrome P450.*J Ethnopharmacol.,* 2005, 97 (2): 319-325.
15. Hertwig, B.; Steb, P. and Feierabend, J. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant physiol*., 1999, 100: 1547-1553.
16. Foyer, C. Ascorbic acid. In: Antioxidants in Higher plants. Eds. Alscher, R.G. and Hess, J.L. CRL Press, Boca Raton. 1993, Pp. 31-58.
17. Freyer, M. J. The antioxidant effect of thylakoid vitamin E (α-tocopherol). *Plant cell Environ.,* 1992, 15:381-392.
18. Basri, D. F. and Fan, S. The potential of chloroform and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian J. Pharmacol.,* 2005, 37(1): 26-29.
19. Adwan, G.; Abu Safiya, D.; Jarrar, N.and Adwan, K. Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turk. J. Biol*., 2004, 28: 99-102.

Antibacterial Studies in Plant Extract of Curry Patta, Garlic and Ginger

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Abstract- Herbal plants have some active compounds due to which they have important pharmacological activities. Their extracts can perform important biological functions. They can defend against attack from insects, fungi and herbivorous mammals.

The inhibitory effects *in vitro* of these selected plant extracts were studied against selected six bacteria (Three gram-negative, *E. coli, Kleibsella spp.* and *Proteus spp.,* and three gram-positive, *S. aureus, B. cereus* and *Micrococcus spp.*). The plants juice extracts showed higher inhibitory effects against gram positive bacteria than the gram-negative. Fungi are strongly inhibited by the garlic essence as compared to ginger and curry patta. Garlic and curry patta have shown better antibiotic effect than the standard reference antibiotic (Choloroamphenicol).

Keyword: Herb, *Allium sativum*, *Murraya koenigii*, *Zingiber officinale*, Essential oils, Extract.

Introduction

Herbal plants have very important pharmacological properties due to which they are being used and can be used further for curing many diseases. Herbal extracts have so much active biological compounds which produce effective result on microbes as compared to synthetic antibiotics. Some time synthetic antibiotics fail to give effect on virus or microbes but many selected herbs give effective result. Garlic, ginger, curry patta, onion, cinnamon are very common herbal plants which are used in Indian kitchens (Singh, A., 2008). For example, quinine from the cinchona, morphine and codeine from the poppy are used in making drugs. (Augusti K. T., 1973*)*. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. *(Brown Deni, 1985).* Naturally occurring microbial or bacterial inhibitors have been recovered from a wide variety of foods including garlic, onion, ginger, cloves,fruits, vegetables, cereals and spices (Ajay Singh *et. al.,* 2007). Many of these antimicrobials contribute to the food stuff natural resistance to deterioration. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value (Siegers C. P., *et. al.,* 1992).

Garlic bulbs contain pectin, garlicin, volatile oils, allin and allistatins I and II due to which it shows bactericidal, antibiotic, high hypoglycemic and fungicidal activities *(Lai PK, Roy J, Roy. June, 2004).* It can even be effective as a natural mosquito repellent. (Amagase H., Milner J., 1993).The body does not appear to build up resistance to the garlic, so its positive health benefits continue over time. Also, garlic could potentially disrupt anti-coagulants, so it is best avoided before surgery. (Block E., 1985).

Curry patta leave extract has Linalool (32.83%), Elemol (7.44%), Geranyl acetate (6.18%), Myrcene (6.12%), Allo-Ocimene (5.02), α-Terpinene (4.9%), and (E)-β-Ocimene (3.68%) and Neryl acetate (3.45%) which are responsible for its biological properties like antixodant and antibacterial properties.(*Mini Priya, Rajendran et. al.,* 2014). The extract showed hypotensive, analgesic sedative and antileptazol properties. (Julseth. R. M. and Deibel; 1974).It also contains the minerals potassium, phosphorus, calcium , magnesium, sodium and selenium (Jandke, J., *et. al.,* 1987). Lemon *(Citru limon) Peel Oil* consists mainly of terpenes, particularly limonene, also gamma terpinene and beta-phellandrene. There

are small amounts of sesquiterpenes and aldehydes, citral (Sies, H., 1995). Ginger-*Zingiber officinal* is a flowering plant in the family Zingiberaceae whose rhizome and root is widely used as a spice . Today, India is the largest producer of ginger *(Wood, C., 1988).* The characteristic odor and flavor of ginger is caused by a mixture compound of zingerone, shogaols and gingerols which are volatile oils that compose one to three percent of the weight of fresh ginger. Gingerols can inhibit growth of ovarian cancer cells. Ginger contains about 3.1 % of a fragrant essential oil whose main constituents are sesquiterpenoids, with (“)-zingiberene as the main component.

Material and Methods

Sampling: In this study we have taken green parts i.e leaves of the selected plants (garlic, curry patta and ginger) .Extract/essential oils were extracted by using standard methods.

Processing of extracts: Essential oils/extracts of curry patta, ginger and garlic plants were taken after purification. Purification of extracts were done by using rotary evaporator.

Combination of juice extracts: The extracts of garlic, ginger and curry patta are mixed in double combinations also for assaying properties.

Preparation of antibiotics solutions: The stock solution of 10mg/ml was made first and further diluted to make concentration 1mg/ml using sterile distilled water.

Procedure for antibacterial activity of plant sample juices Antibacterial assay

The extracts of selected plant samples were screened for its antimicrobial activity against certain selected bacterial strains, *Escherichia coli* (EC 536), *Kleibsella spp.* (Isolate), *Staphylococcus aureus* (Isolate), *Proteus spp.* (Isolate), *Bacillus cereus* (Isolate) and *Micrococcus spp.* (Isolate). The cultures were obtained from the standard cultures maintained in the Microbiology Lab of Uttaranchal University, Dehradun. These cultures were maintained on nutrient agar slants at first being incubated at 37º C for about 18 – 24 hours and then stored at 4º C as stock cultures for further antibacterial activity. Fresh cultures were obtained by transferring a loopful of culture into nutrient broth and then incubated at 37º C overnight. To test antibacterial activity, the Cup well diffusion method was used.

Culture media Preparation: The microbiological media were prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were MHA, NA and NB. These were prepared and sterilized at 121º C at 15 lbs for 15 – 30 minutes in autoclave.

Plate preparation: 25ml of pre autoclaved Muller Hinton agar (MHA) was poured into 90mm diameter pre sterilized petriplates and was allowed to solidify at room temperature.

Cup or hole well diffusion plate method

After the plates solidified, the freshly prepared 24hrs microbial broth culture suspensions about 0.1ml was spreaded over MHA media using L-shaped sterilized glass spreader separately under aseptic conditions using Laminar Air Flow (LAF). Then four wells were made in each plate with the help of borer of 8mm diameter. In these wells, about 0.1ml of each plant sample juice extracts were loaded and the antibiotics solutions 1mg/ml (0.1ml) were also loaded in the wells as reference. All tests were made in triplicate set. This method depends upon the diffusion of the antibiotics or tested material (juices) from a vertical hole through, the solidified agar layer of a Petri dish to such an extent that growth of the added microorganism is prevented entirely in a circular area or zone around the hole containing a solution of tested material or antibiotics (Rios *et. al.,* 1988). 0.1ml of diluted inoculums (10^{8} CFU/ml) of test organism was mixed in Muller Hinton agar media, shaken and poured in sterilized Petri plates. Wells of 8mm diameter were punched into the agar medium and filled with 50µl (200mg/ml) of the plants extracts (juices), solvent for blank or negative control.

Incubation: The Petri plates were incubated for 24 hours at 37º C in the incubator.

Measurement of zone of inhibition: After the incubation, the diameter of clear zone of inhibition produced around the well (or hole) were measured in mm and the diameter of inhibition by the juices extracts were compared with the reference antibiotics.

Determination of Minimum inhibitory concentration (MIC) of the juice extracts and the antibiotics

Minimum inhibitory concentration (MIC) level of any antimicrobial substance is the lowest concentration of the drug inhibiting the bacterial growth. The MIC value of those microorganisms against a particular fraction is considered which exhibit a maximum activity in preliminary screening process either by disc diffusion or by serial dilution. The MIC of the juices and extracts were estimated by serial dilution method and compared with the MIC of the antibiotic taken as reference. The confirmatory test to find out the MIC, the two border lines dilution of visible and non-visible growth were sub-cultured in Nutrient agar media to observe the growth after incubation.

Observations

Antibacterial activity of plant juice extracts against selected microbes are given in table-1

Table-1 Mean Diameter (mm) and SD of zone of inhibition by extracts against the bacteria.

Name of bacteria	G	Cp	Gi	GGi	GCp	GiCp	C	Most Pot.
E. coli	26.3 ± 0.5	24 ± 0.2	20.3± 0.6	24.8 ± 0.2	21.3± 0.6	23.6 ± 0.2	21.5± 0.5	**G,**
Kleibsella spp	24.2 ±0.3	26.5± 0.4	21.6± 0.1	22.6± 0.1	25.6± 0.2	22.5± 0.4	24.3± 0.2	**Cp**
Proteus spp	19.2 ±0.4	20.3±0.4	21.3±0.4	22.3±0.4	21.6±0.4	20.1±0.2	22.1±0.4	**GGi**
S. aureus	25.5 ±0.2	22.1±0.3	23.1±0.2	23.2±0.3	23.4±0.2	25.1±0.3	14.3±0.4	**G**
B. cereus	26.8 ±.5	20.6±0.8	22.6±0.1	21.6±0.2	21.6±0.4	23.8±0.4	24.6±0.3	**C**
Micrococcus spp	24.9 ±0.2	26± 0.8	24.2± 0.8	22.2± 0.4	24.6± 0.2	24.2± 0.5	23± 0.3	**GCp**
Most Sensitive bacteria	**B, 26.8 ± 0.5**	**K, 24.5± 0.4**	**M, 24.2± 0.8**	**E, 24.8 ± 0.2**	**K, 25.6± 0.2**	**S, 25.1±0.3**	**B, 24.6±0.3**	

G= Garlic, Cp= Curry patta, Gi= Ginger C= Chloroamphenicol (reference), SD=Standard deviation, B= B. cereus, S= S. aureus, M= Micrococcus sp, E= E. coli Pot- Potent

Results and Discussion

Extracts obtained from selected plants were assessed as singly as well as in combination for the inhibitory effects against selected bacterial pathogens namely *E. coli, Kleibsella spp, Proteus spp,, S. aureus Bacillus cereus and Micrococcus spp* .The antibiotic Chloroamphenicol was taken as reference for comparison.Data is shown in table-1.

Garlic juice extracts singly produced the highest zone of inhibition of *B. cereus* (26.8 ±0.5 mm) followed by *E. coli* (26.3 ±0.5mm) and *S. aureus* (25.5 ±0.2 mm).The order of bacteria which were inhibited more strongly by the garlic juice extracts:

B. cereus> E. coli > S. aureus >Micrococcus spp> Kleibsella spp> Proteus spp

From the observation, *B. cereus* was found to be the most sensitive bacteria to garlic juice and the garlic extract .Its extract showed greater potency

than Chloroamphenicol which was used as references against the bacteria. The author Cavallito C. J and Bailey J. H (1951) described that garlic contains the antimicrobial agent; allicin (sulphur containing compound), inhibits the growth of both gram negative and gram positive bacteria.

Ginger juice extracts singly produced the highest zone of inhibition of *Micrococcus spp followed by Micrococcus spp.* and *S. aureus* .

Curry Patta essential oil has shown best antimicrobial potential against the bacteria *Kleibsella spp. (26.5 mm)* which is better than the standard antibiotic(24.3 mm) taken in the study.

Minimum inhibitory concentration

MIC values for different extract combinations were also determined in this study. 40 µl/ml MIC value was determined for garlic against most potent bacteria (*E. coli).Similarly* 70 µl/ml MIC value was determined for ginger against most potent bacteria(*Micrococcus spp*) and 65 µl/ml MIC value was determined for curry patta against most potent bacteria(*Kleibsella spp*)while for standard reference antibiotic chloroamphenicol, it was found to be about 95 µg/ml against *E. coli,* which is much higher than that of garlic, so it can be concluded that these plant extract have better antibiotic property.

Discussion of Antibacterial activity of plant extracts applied in combination

The garlic and ginger extract combination (GGi) produced the highest zone of inhibition of *Micrococcus spp.* (41.33±0.11mm) followed by *B. cereus* (36±0.0mm), the reference, chloroamphenicol showed the higher inhibitory effect than the combination against the *B. cereus.* The order of bacteria which were inhibited more strongly by the GO combination:

Micrococcus spp.> B. cereus> S. aureus> E. coli> Kleibsella spp.> Proteus spp.

From the observation it can be seen that GGi combination inhibits *Micrococcus spp.* more strongly than the *B. cereus* although *B. cereus* was inhibited strongly by garlic when applied singly.

The garlic and curry patta extract combination (GCp) showed the highest zone of inhibition of *Kleibsella spp. followed by Micrococcus spp.* followed by *S. aureus.*

In GiCp combination (ginger and curry patta) has shown best antibacterial potential against *S. aureus followed by Micrococcus spp*.. In the combination of extracts we found that GGi and GCp combinations have shown much better antibacterial potential which is better than standard reference antibiotic chloroamphenicol taken. In this way, extracts of these selected plants have strong antibacterial potential.

References

- Ajay Singh and Harish Chandra. Comparative studies on the antimicrobial activities of garlic, onion and lemon: individual and combined. *NPAIJ*, 2011, 7(3): 101-109.
- Singh, A.; Awasthi, G.; Deepak; Chandra, H. and Rawat, M. S. M. Studies on Antioxidant Activities of Garlic *(Allium Sativum),* Onion *(Allium Cepa)* and Lemon *(Citrus Limon).* Universities Journal of Phytochemistry and Ayurvedic Heights, 2007, 2(3):45-48.
- Augusti, K. T. and Mathew, P. T. Effect of long-term feeding of the aqueous extracts of onion (Allium sepa linn.) and garlic (Allium sativum linn.) on normal rats. *Indian J. Exp. Biol.*, 1973, 11: 239-241
- Azzouz, M. A. and Bullerman, L. R. Comparative antimycotic effects of selected herbs and spices, plant components and commercial antifungal agents. *J. Food Protect*., 1982, 45: 1248-1301.
- Bown, Deni. Encyclopedia of herbs and their uses. Dorling Kindersley. *ISBN 0-7894-0184-3, 1995.*
- Cavallito, C. J. and Bailey, J. H. Allicin the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and

antibacterial action. *J. Am. Chem. Soc.*, 1944, 66:1950-1951.

- Chandra Harish and Singh Ajay. Antibacterial Activity of *Allium sativum* (L.) Against Bacteria Isolated from Upper Respiratory Tract, *IUP Journal of Life Sciences*, Nov 2010, Vol. 4: 43-49.
- J.M. Robertson *et. al.* "A Possible Role for Vitamins C and E in Cataract Prevention," *Am. J. Clin. Nutr.,* January 1991, 53 (1 Suppl.): 346S-351S.
- Lawson, L. D. Bioactive organo sulfur compounds of garlic and garlic products. Kinghorn A. D. Balandrin M. F. eds. ACS Symposium Series 534, Human Medicinal Agents from Plants. American Chemical Society, Washington, DC, 1993:306-330.
- Lai, P. K; Roy, J. and Roy. "Antimicrobial and chemo preventive properties of herbs and spices". *Curr. Med. Chem., 2004, 11 (11): 1451–60.*
- Mini Priya and Rajendran *et, al.* Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* (L.) leaves. *Avcine J. Phytomed.*, 2014 May-Jun, 4(3): 200-214
- Mishra, S. and Palanivelu, K. "The effect of curcumin (turmeric) on Alzheimer's disease: An overview". *Ann. Indian Acad. Neurol., 2008, 11 (1): 13–9.*
- Pafumi, J. Assessment of microbiological quality of spices and herbs. *J. Food Protect*, 1986 49:958-963
- Sawhney, S .K. and Singh, R. Introductory practical Biochemistry, Narosa Publishing House, India, 2001.
- Singh, Ajay *et. al.* Studies on antimicrobial and antioxidant activities of *Allium sativum*, *Allium cepa* and *Citrus limon*, Phytochemicals: a therapeutant for critical disease management, 2008:277-292.
- Siegers, C. P.; De Smet. P. A. G. M.; Keller, K.; Hänsel, R. and Chandler, R. F. eds. Adverse Effects of Herbal Drugs, 1992:73-77 Springer-Verlag Berlin, Germany.
- Wood, C. Comparison of efficacy of ginger with various antimotion sickness drugs. *Clin. Res. Pr. Drug Regul. Aff., 6 (2): 129–36.*

Fragrant Profile of Two *Citrus* Species Peels from Dehradun Region, Uttarakhand, India

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Abstract- *Citrus spp.* fruits are among the most popular fruits worldwide and have a very long history of usage. Recent advancement has begun to develop industrial technologies in order to convert *Citrus spp.* fruits into commercial products. The present study is aimed to isolate the essential oil of *Citrus sinensis* and *Citrus pseudoliomon* in order to analyze their chemical composition. Results revealed that the yield of essential oil was found maximum in *Citrus sinensis* (0.97%) as compared to *C. pseudolimon*. Both the species were dominated by monoterpene hydrocarbons. The predominant monoterpene was limonene 85.68% in *Citrus sinensis* and *C. pseudoliomon*, both similar. Presence of essential oil in the peel generally considered a waste product is an indicative that the same can be used for bioprospecting.

Keywords: *Citrus*, Oils, Essences, GC-MS, Limonene, Bioprospecting.

Introduction

Genus *Citrus* (*Rutaceae*) is the third most important tropical fruit crop of India after mango and banana with an area of 712.4 thousand hectares and 5996.9 thousand metric tonnes of production. The most important Citrus growing states in India are Andhra Pradesh, Maharashtra, Orissa, Gujarat and Uttarakhand. In Uttarakhand, *Citrus spp.* occupies about 14.14 percent (26410 ha) of total fruit area[1]. It is an ancient crop with records of human cultivation extending back to at least 2100 BC[2]. *Citrus* fruits are mainly used for dessert, juice and jam production. The food and agro-food processing industry yields considerable amount of waste or by-products such as peels, seeds and pulps which represent 50% of the raw processed fruit[3]. These by-products are considered as valuable sources of functional ingredients namely flavonoids, dietary fibers and essential oils[4]. Essential oil is one of the by-products attracting interest as a value added product[5]. Citrus essential oil has been identified in different parts of fruits (preferably in fruit flavedo) as well as in leaves, peels, which shows that limonene, β-myrcene, α-pinene, sabinene, Δ-3-carene, α-terpinolene, etc. are the major aromatic compounds[6,7,.....,15].These aromatic compounds are relatively inexpensive and abundant in raw materials and can be used in flavour and food industries[16]. Also, they can serve as an excellent starting material in the synthesis of fine chemicals and of new fragrances for the cosmetic industry[17]. Moreover, citrus essential oils have been recognized as safe due to their wide spectrum biological activities such as antimicrobial, antioxidant anti-inflammatory and antixiolytic[18, 19, 20] Peels of species considered as waste product are available in large quantities[21]. Due to their great demand in nutraceutical and economic importance, they are candidate for investigation. The present study designs for identification of peels of the two *Citrus* species fragrant composition was conducted.

Material and Methods

Collection of plant material

The fruits of *Citrus sinesis* and *Citrus pseudolimon* commonly known as Malta and Galgal respectively in Uttarakhand were collected from Doiwala, Dehradun, Uttarakhand, India. The taxonomic identification of the plant material was confirmed by Botanical Survey of India, Northern circle, Dehradun. Voucher specimens have been deposited in the University for future references.

Isolation of the essential oils

The fresh fruit peels (1 kg) of *Citrus sinesis* and *C. pseudolimon* were hydro-distilled in glass Clevenger apparatus. The colorless volatile oil was dried over anhydrous sodium sulphate and stored at 4°C in the dark. The yield was calculated as a mean of three independent extractions, 0.97-0.30 % based on the weight of the fresh fruit peels.

Gas Chromatography

The gas chromatograph (GC) analyses of the volatiles oils were carried out using an Agilent Technology 7890 gas chromatograph equipped with a FID detector and a HP-5 fused silica column (30m x 0.32 mm x 0.2µm film thickness). Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210 °C and 230 °C respectively. The column oven temperature was programmed from 60 °C to 220 °C with an increase in rate of 3°C/min. The injection volume was 0.02 µL.

Gas Chromatography-Mass Spectrometry

The GC-MS analysis of the volatiles oils was performed out on a Agilent Technology mass spectrometer (Model 7890) coupled to a gas chromatograph with a 60 m x 0.32 mm x o.2µm film thickness column (HP-5). Helium was used as the carrier gas (flow rate 1ml/min). The oven temperature was programmed in the range from 60°C to 220°C at 3°C/min. Other conditions were the same as described for gas chromatography. The mass spectrum was recorded using a mass range of 40-600 Daltons. The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, C9-C24 under experimental conditions, co-injection with standards (Aldrich and Fluka), MS library search (NIST and WILEY), and by comparing with the MS literature data[22]. The relative amounts of the individual components were calculated based on the GC peak area (FID response) without correction factors.

Results and Discussion

The essential oil content from peels of *Citrus sinesis* and *Citrus pseudolemon* vary among the species and found 0.97 % in *C.sinesis* and 0.30% in *C. psedolemon*. The yield of essential oil of *C. sinesis* was higher as compared to *C. psedolemon.* The chemical composition of *C. sinenis* and *C. psedolemon* peels oil are presented in Table-1. A total of 11 compounds were identified in 95. 6 % of essential oil in *C. sinenis* while 14 compounds accounting 96.5 % of the oil in *C. psedolemon*. As far as volatile constituents of the *C. Sinenis* is concerned, limonene was detected as a major components (85.7 %), followed by linalool (3.6 %) as a second major components, and β- myrcene covering (3.0 %) total identified constituents of the oil. Including other minor constituents like α-pinene, sabnine, octanol, 1- octanol, α- terpineol and deconol constitutes accounting only 3.4 % of the oil. These are found in very trace amounts in peel oil sample. In case of *C. pseudolimon* oil, limonene (86.9%) was found as major constituents which is slightly higher than *C. sinenis* peel oil. Mycenae (3.07%) detected as a second major component whereas as linalool (1.76%), α-pinene(0.41%), sabinene(0.14%), trans- ocimene (1.2%), cis-ocimene (0.39%) Z-citral (0.27%), E-citral (0.40%, trans–alpha–bergamotone (0.62%),and β-basabolene (0.81%). It is very interesting to note that trans- ocimene, cis-ocimene, citral, trans – alpha – bergamotone and basabolene are completely absent in *C. sinenis* peels oil. Earlier study reported from oil of the *C. sinenis* peels Argentina, India and Libya limonene as a major component in tune with our finding[23], but our result, on limonene quantity is formed higher as compared to previous reports. It is well

documented that limonene found as a potent fungicides agents, fungal pathogen of sugar[24]. A large portion of this production is addressed to the industrial extraction of citrus juice which leads to huge amounts of residues, including peel and segment membranes. The world production of citrus is 122.09 million tons during 2008[25]. Peels represent between 50 to 65% of total weight of the fruits and remain as the primary by product. If not processed further, it becomes waste and produce odor, soil pollution, harborage for insects and can give rise to serious environmental pollution[26, 27]. Keeping in view of the potential of peels used, establishing aroma based small scale industry in hilly state of Uttrakhand will give way for upliftment of rural economy. The present study based on the above investigation on valuable recourses to turn for isolation of natural limonene available in peels oil in very high amount in both the species of citrus. The potential of volatile oil present in fruit peels can be useful for treatment of skin disorder and/or in aroma therapy, can be incorporated into cosmetic formulations as well as income generation for rural inhabitants of Uttarakhand region.

Table-1 Essential oil composition of Two Citrus Cultivar (*C. sinesis* & *C. pseudolimon*) from Uttarakhand Himalaya.

S.N.	Components	RI	Percentage (%)	
			Citrus sinesis	*C. pseudolimon*
1.	**α- pinene**	939	0.58	0.47
2.	**sabinene**	975	0.80	0.12
3.	**β- myrcene**	991	2.96	3.53
4.	**octanal**	999	0.69	0.04
5.	**Limonene**	1029	85.68	85.68
6.	**cis ocimene**	1037	-	0.39
7.	**trans- ocimene**	1050	-	1.23
8.	**1-octanol**	1068	0.69	-
9.	**linalool**	1097	3.56	1.76
10.	**α- terpineol**	1189	0.21	tr
11.	**decanal**	1202	0.457	tr
12.	**Z- cital**	1318	tr	0.27
13.	**E- citral**	1341	tr	0.40
14.	**trans- α- bergamotene**	1435	-	0.62
15.	**β- bisabolene**	1506	-	0.81
	Total identified		95.62	96.49

Aberration used: RI = arithmetic retention indices based on alkenes on a HP-5 column; tr< 0.10. - Absent

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References

1. Pandey, D.; Kumar, A and Singh, R. Marketing of sweet orange (Malta) in Kumaun region of Uttarakhand. *Journal of Rec. Adv. in App. Sci.*, 2011, 26: 6-11.
2. Moore, G. A. Oranges and lemons: clues to the taxonomy of Citrus from molecular markers. *Trends Genet*, 2001, 17(9): 536-540.
3. Anwar, F.; Naseer, R.; Bhanger, M. I.; Ashraf, S.; Talpur, F. N. and Aladedunye, A. F. Physico-chemical characteristics of *citrus* seeds and seed oils from Pakistan. *J. Am. Oil. Chem. Soc.*, 2008, 85: 321–330.
4. Senevirathne, M.; Jeon, Y. J and Kim, S. H. Effective drying of citrus by-product by high speed drying: A novel drying technique and their antioxidant activity, *J. Food Engineering*, 2009, 92: 157- 163.

5. Njoroge, S.M.; Koaze, H.; Karanja, P. N. and Sawamura, M. Volatile constituents of red blush grapefruit (*Citrus paradisi*) and pummel (*Citrus grandis*) peel essential oils from Kenya. *J. Agric Food Chem*., 2005, 53: 9790-9794.

6. Stashenko, E. E.; Martinez, R.; Pinzdn, M. H. and Ramfrez, J. changes in chemical composition of catalytically hydrogenated orange oil (*Citrus sinensis*). *J. Chromatography*, 1996, 752: 217-222.

7. Caccioni, D. R; Guizzardi, M.; Biondi, D. M.; Renda, A. and Ruberto, G. Relationship between volatile component of citrus fruit essential oils and antimicrobial action *Penicillium digitatum and Penicillium italicum*. *J. Food Microbiol*., 1988, 43: 73-79.

8. Lota, M.; de Rocca Serra, D.; Tomi, F. and Casanova. J. Chemical variability of peel and leaf essential oils of 15 species of mandarins from *Citrus reticulata* Blanco. *Biochem. Syst. Ecol.*, 2001, 29 (1): 77-104.

9. Lota, M. L. Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco, *Biochemical Syst. Ecol*., 2000, 28: 61–78.

10. Minh, N. T.; Thanh, L. X.; Une, A.; Ukeda, H. and Sawamura, M. Volatile constituents of Vietnamese pummel, orange, tangrene and lime oils. *Flavour Fragr. J.,* 2002, 17: 169-174.

11. Vekiari, S. A.; Protopapadaki, E. E.; Papadopoulou, P.; Papanicolaou, D.; Panou, C. and Vamvakias, M. Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety, *J. Agric. Food Chem.*, 2002, 50(1): 147-53.

12. Buettner, A.; Mestres, M.; Guasch; A. F. and Schieberle, P. Evaluation of the most odour-active compounds in the peel oil of clementines (*Citrus reticulata* Blanco cv. clementine). *Eur. Food Res. Technol*., 2003, 216: 11-14.

13. Merle, H.; Moron, M.; Blazquez, M. A. and Boira, H. Taxonomical contribution of essential oils in mandarins cultivars, *Biochem. Syst. Ecol.*, 2004, 32: 491-497.

14. Pultrini, A. M.; Galindo, L. A. and Costa, M. Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice, *Life Sci.* 2006, 78(15): 1720-1725.

15. Sharma, N. and Tripathi, A. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiol. Res*., 2008, 163(3): 337-344.

16. Reische, D. W.; Lillard, D. A. and Eitenmiller, R. Chemistry Nutrition and Biotechnology. Marcel Dekker, New York, 1998.

17. Lis-Balchin, M. and Hart, S. Studies on the mode of action of the essential oil of lavender (*Lavandula angustifolia* P. Miller). *Phytother. Res*. 1999, 13 (6): 540-542.

18. Fisher, K. and Phillips, C. Potential antimicrobial uses of essential oils in food: Is citrus the answer. *Trends in Food Sci. Technol.,* 2008, 19:156–164.

19. Chutia, M.; Bhuyan, P. D.; Pathak, M. G.; Sharma, T. C. and Boruah, P. Antifungal activity and chemical composition of Citrus reticulata Blanco essential oil against phytopathogens from North East India. *Food Sci. Technol.*, 2009, 42: 777-780.

20. Rehman, Z. Citrus peels extract: A natural source of antioxidant. *Food Chem.,* 2006, 99: 450-454.

21. Murray, K. D.; Groden, E.; Drummond, F. A.; Alford, A. R.; Conley, S.; Storch, R. H. and Bentley, M. D. *Citrus* limonoid effects on Colorado potato beetle (Coleoptera: Chrysomelidae) colonization and ovipositor. *Environ. Entomol.*, 1995, 24(5): 1275-1283.

22. Adams, R. P. Identification of essential oil components by Gas chromatography/ Quadrupole Mass spectrometry. Carol Stream, USA. Allured Publishing Corporation, 2004.

23. Singh, G. and Singh, O. P. Chemistry of Essential oils of *Citrus species Natural Product Radiance*, 2002, September-October: 8- 21.

24. Nedorostova, L.; Kloucek, P.; Kokoska, L.; Stolcova, M. and Pulkrabek J. Antimicrobial properties of selected essential oils in vapor phase against food borne bacteria. *Food Control*, 2009, 20, 157-160.

25. FAO http://faostat.fao.org/ Roma, 2010.

26. Mandalari, G.; Bennett, R. N.; Bisignano, G.; Saija, A.; Dugo, G.; Lo Curto, R. B.; Faulds, C. B. and Waldron, K. W. Characterization of flavonoids and pectin from bergamot (*Citrus bergamia* Risso) peel, a major byproduct of essential oil extraction. *J. Agric. and Food Chem.*, 2006, 54: 197-203.

27. Manthey, J. A. and Grohmann, K. Concentrations of hesperidins and other orange peel flavonoids in citrus processing byproducts. *J. Agric. Food Chem.*, 1996, 44: 811-814.

Acetone Insoluble Solid (AIS) Yield (%) from Fruit Pericarp at Different Stages of Fruit Ripening from *Diospyros peregrina*

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Abstract- *Diospyros peregrina* species is medium sized spreading evergreen ornamental tree, about 15 m in height with dark grey or black bark, young parts silky and covered with grey tomentum, bark black with greenish tinge flaking off in thick scales; branchlets glabrous. Leaves are simple, elliptic, ovate, rounded at base, 13.5 cm long and 4.5 cm broad, alternate, exstipulate, coriacious, glabrous, reticulation prominent, petiolate; petiole upto 1 cm long. The tree is indigenous to India and Bangladesh, also found in many other countries of Asia and America. The present investigation aims to determine the yield of Acetone Insoluble Solid (AIS) from *Diospyros peregrina* fruit pericarp at four different stages of fruit ripening. It was observed that AIS (% yield) was different in different stages with the ripening. The AIS yield from fruit pulp first increased from immature green (9.87±1.14%) to mature green stage (12.25±0.43%) and thereafter, it decreased and was minimum (3.19±0.33%) in fully ripe stage.

Keywords: Ebenaceae, *Diospyros peregrina*, Acetone Insoluble Solid (AIS).

Introduction

Diospyros peregrina is distributed all over India especially in the plains of coastal regions and river banks. The tree is indigenous to India and Bangladesh and is also found in many other countries of Asia and America (Kirtikarand Basu, 1935). Some plant species is found in Thailand and North Malaysia (Wiart, 2006) and introduced as an ornamental tree in Pakistan (Anon, 2009). The species' name means strange or foreign, probably an exotic in the country from which the species was described.

Pectin is the general term for a group of polysaccharides that occur as structural materials in all land growing plants and their fruit. It is a heterogeneous complex polysaccharide that belong to the interrupted chain sequences family, being characterized by linear 1, 4-linked α-D-galactopyranosyluronic acid sequences separated by α-L-rhamnopyranosyl residues (Rolin and De Vries, 1990; Renard *et. al.,* 1991). They are found in the primary cell walls and intercellular layers in land plants, and in some ways, seems to correspond to the hyaluronic acid of the ground substance of animal tissue. It is thus part of the natural diet of man.

This multifunctionality of pectin is due to their functional groups which can be enhanced or even changed by introducing new functional groups onto the pectin polymer backbone using either chemical or enzymatic methods (Pappas *et. al.*, 2004). Chemical modifications of pectin can lead to new products with significant physicochemical and biological properties.

An introduction of non-polar residues increases hydrophobic character of pectin macromolecules. The hydrophilicity–lipophilicity relationship of such polymers depends on the degree of substitution, i.e. the content of non-polar substituents attached to the original polar macromolecule (Synytsya *et. al.*, 2004). At small degree of substitution, the polymer is soluble in water like the hydrophilic precursor. Slightly substituted polymers have surface-active properties and can be valorized in various

applications. Synytsya et al. (2004) prepared a number of N-alkylamides of highly methylated pectin used as bioavailable sorbents and drug delivery systems.

The main object is to study on AIS from fruit pericarp (*Diospyros peregrina*) in four stages, mature green stage, ripe stage, immature green stage and colour initiation stage, no such work has been done so far on this subject.

Material and Methods

Plant material

The fruits of *Diospyros peregrina* used in the study were collected at different stages (four stages vis. immature green, mature green, colour initiation and full ripe) of fruit ripening from the Forest Research Institute. All the chemicals used were of analytical grade. At different fruit ripening stages, different parameters were recognised as shown in Table-2 and Figure-1

Preparation of Acetone Insoluble Solid (AIS)

Acetone insoluble solids (AIS) were prepared as described by Seymour *et al*., (1987a). A known weight of frozen fruit of *Diospyros peregrine* was homogenized using a polytron (Kinematica Gmbtl, Luzern, Switzerland) in 4 volumes of cold (-20°C) absolute acetone. This homogenate was filtered under vacuum through miracloth and the retained residue washed with 10 volumes of 80 % acetone (v/v). The residue was then washed with 10 volumes of cold absolute acetone. The residual powder was then either dried over P2O5 *in vacuo* (untreated) or suspended with constant stirring for 15 minutes in phenol:acetic acid:water (PAW) in ratio 2:1:1 (w/v/v) at a concentration of 10 ml per gram of original tissue at 4°C to remove the endogenous enzyme activity (Seymour *et al*., 1987a). The summary of AIS Treatment is shown in Table-1.

Table-1 Summary of AIS Treatment

Phenol Acetic acid Water (PAW) Treatment Preparation	Volume (ml)
80 % Phenol solution in water containing 200 gm Phenol and 60 ml water	300
Acetic acid	120
Water	60

Table-2 AIS Yield (%) at different stages of fruit ripening

AIS from fruit pericarp (% Yield)	Immature Green (1st stage)	Mature Green (2nd stage)	Colour Initiation (3rd stage)	Fully Ripe (4th stage)
Wt. of AIS obtained from fruits	9.87 ± 1.14%	12.25 ± 0.43%	9.07 ± 0.52%	3.19 ± 0.33%

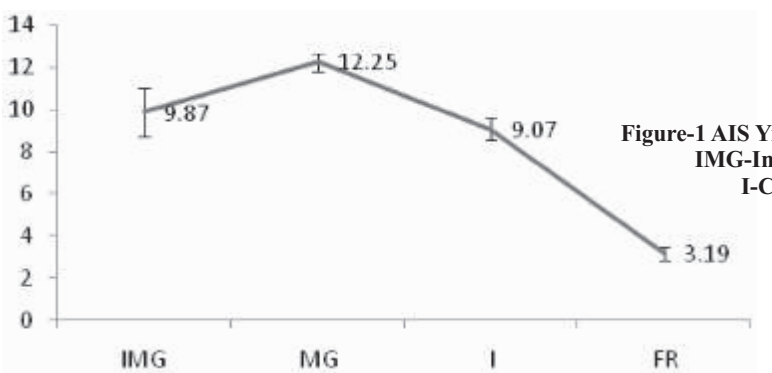

Figure-1 AIS Yield (%) at different stages of fruit ripening IMG-Immature Green, MG-Mature Green, I-Colour Initiation, FR-Fully Ripe

Results and Discussion

The % yield of AIS from fruit pericarp in four stages of fruit ripening Immature Green, Mature Green, Colour Initiation and Fully Ripe is given in Table-2. Generally pectins are soluble in water representing no or little binding to the other cell wall components. It is believed that pectins which are bound together by calcium bridges and form complex compounds with calcium ions. Therefore, pectin can be extracted by using different chelating agents such as; oxalates, hexametaphosphate, EDTA, CDTA, EGTA, etc. At normal condition (room temperature and at neutral pH), the action of chelating agent i.e. CDTA removes all the Ca-bridges from the pectins rendering its solubilization.

Generally, AIS are found abundantly in fruit, moderately in leafy vegetables. AIS solubilized more pectin from ripe fruit and these observations are conclusive about the fact that higher dissolution of pectin-rich middle lamella occur during ripening. AIS have pectin and represented by high galacturonic acid content along with relatively high galactose and arabinose residues. Acetone Insoluble Solids (AIS) samples were prepared for different stages of fruit ripening. AIS in four stages was maximum 12.25 ± 0.43% in mature green stage and minimum 3.19 ± 0.33% in fully ripe stage, it 9.87 ± 1.14% and 9.07 ± 0.52% in immature green stage & colour initiation stage respectively. The AIS % yield showed sharp initial increase from immature green to mature green stage and it decreased further from mature green to colour initiation step, and it finally decreased from colour initiation to fully ripe stage due to initial formation due to activity of pectinesterase enzyme, and finally there was decrease from colour initiation stage to fully ripe stage and this decrease is attributed to pectin degradation.

The results showed that AIS are the major polysaccharides that undergo drastic degradation during ripening, already has been reported for other fruits. The AIS yield % was majorly affecting the fruit texture where textural loss and pectin content are related to each other. Ripening involves the breakdown of tightly bound insoluble protopectin to soluble polyuronides, which are loosely bound to the cell wall, appears to influence the changes in cell wall polysaccharides. The highly branched pectic polysaccharides in the primary cell wall are available for hydration and degradation, and as a result their solubilization leads to marked decrease in neutral sugar side chains as well as textural softening. The AIS yield % showed sharp initial increase from immature green to mature green stage and then it decreased from mature green to colour initiation stage and to fully ripe stage, this may be due to its initial formation up to mature green stage and then degradation from mature green stage to fully ripe stage due to activity of pectin degrading enzyme.

The results showed that AIS are major polysaccharides that undergo degradation during ripening (already been reported for other fruits). The AIS yield % from fruit pericarp in four stages of fruit ripening was maximum 12.25 ± 0.43% in mature green stage and minimum 9.07 ± 0.52% in colour initiation stage, and 9.87 ± 1.14% and 3.19 ± 0.33% in immature green stage and fully ripe stage respectively, this may be due to formation in initial stages of fruit growth.

This research suggests that the rupture of main pectic chains contributes significantly to *Diospyros peregrina* fruit ripening. The new research found out that during ripening in four stages from the fruits of *Diospyros peregrina*, decrease in cell wall polysaccharides as acetone insoluble solids (AIS) with loss of both acidic and neutral sugar residues was observed. It also suggests, in *Diospyros peregrina* with fruit ripening, the loss in tissue firmness, increased activity of pectic enzymes, and extensive hydrolysis of pectic polysaccharides, show a clear correlation between each other.

Conclusion

In Table-2, the AIS from fruit pericarp (% Yield) in four stages (Immature Green, Mature Green, Colour Initiator and Fully Ripe), is maximum to 12.25±0.43% in mature green stage and minimum

to 3.19±0.33% in fully ripe stage, 9.87±1.14% and 9.07±0.52% in immature green stage and colour initiation stage. These AIS (% yield) was different in different stages with the ripening. In Figure-1, with ripening the AIS yield from fruit pulp first increased and was maximum 12.25±0.43% in mature green stage and thereafter, it decreased and was minimum 3.19±0.33 % in fully ripe stage, results into loss in tissue firmness, loss of cellular integrity and extensive hydrolysis of pectic polysaccharides.

References

- Anon. Annual rainfall, relative humidity and temperature of Lahore, 2004-2009. Pakistan Metrological Department Jail Road Lahore, Pakistan, 2009.
- Kirtikar, K. R. and Basu, B. D. Indian medicinal plants, Edn 2, Vol-I. Lalit mohan Basu Allahabad, India, 1935, 785-788.
- Wiart, C. Medicinal Plants of Asia and Pacific. Taylor and Francis group, LLC. CRC Press, USA, 2006, 75-76.
- Rolin, C. and De Vries, J. Food Gels, Harris, P. (Ed.), *Elsevier Applied Science,* Barking, U.K., 1990: 401.
- Pappas, C. S.; Malovikova, A.; Hromadkova, Z.; Tarantilis, P. A.; Ebringerova, A. and Polissiou, M. G. Determination of the degree of esterification of pectinates with decyl and benzyl ester groups by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and curve-fitting deconvolution method. *Carbohydrate Polymers*, 2004, 56: 465-469.
- Synytsya, A.; Copíková, J.; Marounek, M.; Mlcochová, P.; Sihelníková, L. and Skoblya, S. *et. al.* N-octadecylpectinamide, a hydrophobic sorbent based on modification of highly methoxylated citrus pectin. *Carbohydrate Polymers,* 2004, 56: 169–179.
- Seymour, G. B.; Lasslett, Y. and Tucker, G. A. Differential effects of pectolytic enzymes on tomato polyuronides in vivo and in vitro. *Phytochemistry,* 1987, 26: 3137–3139, 12: 247–251.

Permeability Enhancement of Diclofenac in Gel Formulation Using Lemon Grass Oil

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Abstract- Topical dosages are formulation of choice for the anti-inflammatory and dermatological disease. Less side effects, high site specificity and patient compliance are the unique features of topical delivery system. But ionic nature and high molecular weight drugs are difficult candidates for topical preparations as these are unable to cross skin and reach in systemic circulation. The effect of lemon grass essential oil on drug penetration rate across membrane is investigated in the present study where the diffusibility of diclofenac gels with or without containing essential oils were analysed using Franz diffusion cell. It has been observed that highest membrane permeability was obtained in the gel formulation containing lemon grass essential oil. This can be attributed due to the permeability enhancement effect of lemon grass oil.

Key Words: Lemon grass, Essential oil, Franz diffusion cell, Membrane permeability.

Introduction

Diclofenac, an important drug of category NSAIDS, belongs to chemical groups 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 adopted which include increasing of drugs, incorporate of permeability enhancers, fluidization of skin etc.[1,2] In the present study, gel of diclofenac using lemon grass essential oil were formulated . These formulations were studied for *in-vitro* membrane permeability using Franz diffusion cell method.

Material and Methods

Extraction of Essential Oil

The samples of wild growing lemon grass herb (Cymbopogan winterianus Jowitt) plant were collected during the month of February from Balawala, Dehradun 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. The leaves of lemon grass deprived from wooden parts (100 g) were subjected to hydro-distillation, using Clevenger-type apparatus for 3 h, according to the standard procedure[3-5]. 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 oils were combined and were 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).

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

Additives	Wight (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	Preservatives
Methyl Peraben	0.02	Preservative
Purified Water	q.s.	Solvent

In-vitro Membrane Permeability Study

The in vitro penetration studies were performed for diclofenac gel 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 and the receptor fluid was stirred with a magnetic bar at 200 rpm. Fluid (1ml) was withdrawn from receptor cell at regular interval 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 and Discussion

Gel was prepared by dispersing specific amount of drugs and propyl peraben in sufficient amount of water by heating ,then sodium carboxymethyl cellulose mixed with glycerine and Castrol oil in a glass mixture poured into propyl peraben solution and stirred. 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 up to 150min and concentration of the drug was determined using UV-spectrophotometer. The result of samples were determined from calibration curve plotted between different concentration and absorbance of standard solution (2,4,6,8,10 PPM) of pure diclofenac sodium (Table-2).

Table-2 Concentration vs absorbance data for standard solutions

Concentration (µg/ml)	Absorbance
2	0.06
4	0.11
6	0.166
8	0.21
10	0.256

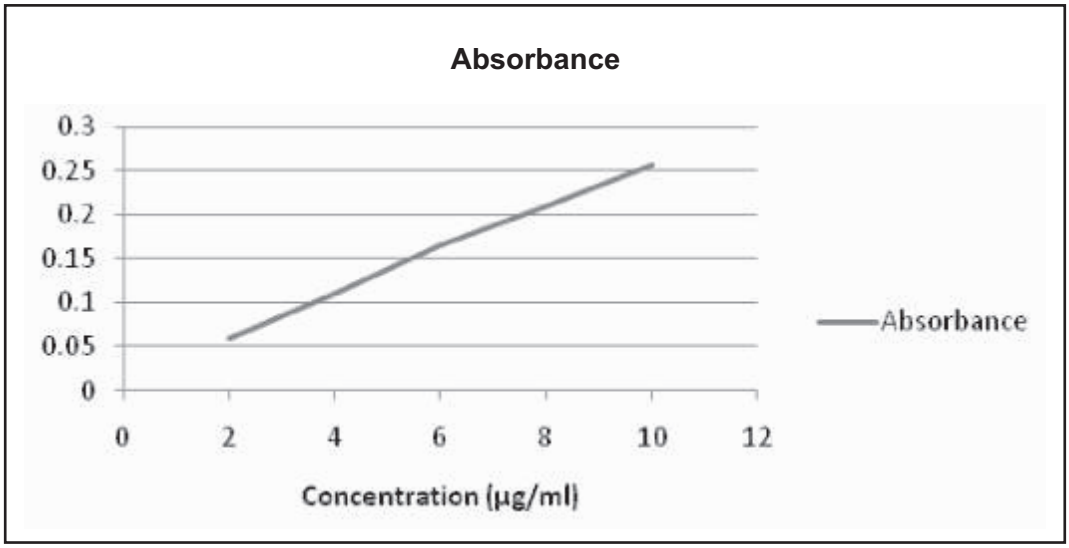

Figure-1 Calibration Curve

Results are summarised in Table-2. Results showed increase in concentration of drug with time. However, it was observed that the permeability improved when formulation containing essential oil was evaluated. The results recommend that the diffusibility of drug has improved when lemon grass essential oil was added to the formulation. Hence, the lemon grass oil can be exploited as a permeability enhancer in all tropical delivery systems (Table-3).

Table-3 Percentage drug release in various gel formulations with and without addition of eucalyptus oil.

Time	Diclo. Gel		Diclo. + Essential Oil	
	Abs	% Release	Abs	% Release
30 min	0.081	13.9	0.090	14.0
60 min	0.120	18.3	0.180	24.6
90 min	0.186	26.3	0.204	28.6
120 min	0.210	33.9	0.240	41.5
150 min	0.255	45.2	0.300	49.9

References

1. Surver, C. and Davis, F. A. Bioavailability and Bioequivalence, In Walter, K A. Dermatological and Tran dermal, Marcal Dekker, INC, Newyork, 2002, Pp. 119.
2. Ansel, H. C. and Allen, L. V. Pharmaceutical Dosage forms and Drug Delivery System, 7th edition, Lippincott William and Wilkens, Baltimore, 2000: 244-246.
3. Maciel, M. V.; Morais, S. M.; Bevilaqua, C. M. L.; Silva, R. A.; Barros, R. S.; Sousa, R. N. and Souza-Neto, M. A. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyialongipalpis. *Veterinary parasitology*, 2010, 167 (1), 1-7.
4. Reichling, J.; Schnitzler, P.; Suschke, U. and Saller, R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and

cytotoxic properties-an overview. *Forschende Komplementrmedizin,* 2006, 16 (2), 79.

5. Mainya, N. O.; Tum, P. and Douglas, R. M. Optimization of Essential Oil Extraction from Eucalyptus grandis Leaves by Clevenger Distillation. *International Journal of Science and Research*, 2016, 5(7), 493-496.
6. Milo, S.; Nikoli, C. A. and Jasmina, Glamo. Industrial Crops and Products 52 (2014) 183– 190.
7. Franz, T.J. Percutaneous absorption. On the relevance of In vitro data. *J. Invest. Dermatol.*, 1975, 64: 190–195. 2.
8. Franz, T. J. The finite dose technique as a valid in vitro model for the study of percutaneous absorption. *Curr. Probl. Dermatol.*, 1978, 7: 58–68.
9. Siewert, M.; Dressman, J.; Brown, C. K. and Shah, V. P. FIP/AAPS guidelines to dissolution/In vitro release testing of novel/special dosage forms. *AAPS Pharm. Sci. Tech.*, 2003, 4, Article 7.

Anti-Spermatogenic Effect of *Solanum xanthocarpum* in Guinea Pigs

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Abstract- The alcoholic extract of *Solanum xanthocarpum* (seeds) was studied in male guinea pigs. The doses 100, 300 and 500 mg/kg b.w. per day were fed to 3 groups (dose wise, 2nd, 3rd and 4th) with 1st control group as experimental for 60 days. The body weight were recorded after 60 days. Slight weight reduction was noted at higher doses. The rats of 60 days treated with different doses of first set were mated with normal female Guinea pigs. The treated Guinea pigs show normal mating. After mating test done, 05 Guinea pigs were killed and their genital organs (Testes Epididymes and Vasa deferentia) were taken out and weighed. The organ weights were slightly reduced. These organs were processed for histological examination. In the seminiferous tubules, the spermatogenesis was arrested. The tubules became reduced in size. The Leydig's cells became atrophied. The lumen was filled with cellular debris. The epididymes and Vasa deferentia were also devoid of spermatozoa. The 05 treated Guinea pigs for 60 days were left without treatment for 30 days. After 30 days of withdrawal period, these Guinea pigs were allowed to mate with normal female Guinea pigs. These male Guinea pigs showed return of fertility state. The mated female Guinea pigs became pregnant as they showed implants on day 10th of pregnancy on laparotomy. The effect was reversible. These changes were not found in control set of female Guinea pigs.

Keywords: Male Reproduction, Contraception, Reproductive Biology, Spermatogenesis, Antifertility effect, *Solanum xanthocarpum* (seeds).

Introduction

Worldwide search on fertility regulating agents is going on to check the problem of ever growing population. Hormonal drugs are available for the purpose but they are not free from side effects. Hence, the search for easily available drug from indigenous medicinal plants is proposed, which could be effectively used in place of the 'Pill'.

Indian medicinal plants associated with antifertility property are numerous. They are enlisted under the possible antifertility plants (Chaudhury, 1966 and Farnsworth *et al.,* 1975). *Solanum xanthocarpum* Linn. (Family – Solanaceae) is one of them. The plant is known as Kateli or Kantkari. The seeds of this plant are considered as a local contraceptive by tribals of India. The powdered seeds and steroidal fraction, Solasodine (Dixit and Gupta, 1982) of this plant was reported to interfere with spermatogenesis of Indian dog (*C. familiaris)*. Rao (1986, 88) reported antifertility effects in male albino rats. Antifertility study of this plant in male Guinea pigs is lacking. On the basis of above information, the experimental work was done on male Guinea pigs (*Cavia porcellus*).

Material and Methods

The alcoholic extract of *Solanum xanthocarpum* Linn seed powder was obtained using soxhlet apparatus. It was dried under reduced pressure and low temperature. The three doses of dried extract (100, 300 and 500 mg/kg) were prepared with 5% of Gum acacia powder as vehicle. All the doses were dissolved in distilled water separately and fed to male Guinea pigs orally with the help of specially designed feeding needle fitted into a syringe. The controlled group (1st group) was fed with vehicle only. The male Guinea pigs (*Cavia porcellus*) weighing between 450-500 gms were used. They were procured from local dealer and maintained as per guidelines of Institutional Animal Ethical Committee appointed by

Principal. Besides, pelleted food and water, the leafy vegetables like spinach, cabbage, bottle guard, cucumber and soaked bengal gram were provided because Guinea pigs are herbivorous like rabbit. The three doses 100, 300 and 500 mg/kg b.w. per day fed for 60 days to different groups (dose wise) of male guinea pigs. The 10 Guinea pigs were used for each dose. After 60 days of feeding of each dose, the Guinea pigs were mated with normal female Guinea pigs than 05 Guinea pigs from 2nd, 3rd and 4th (control also) were killed. First group was served as control. Their testes, epididymes and Vasa deferentia was taken out and processed for histological study. Rest 05 Guinea pigs from each dose groups were left for study of fertility performance and reversibility, if any.

The parameters taken for study were (i) Body weight and genital organ weight (ii) Histology of testes, epidiymes and Vasa deferentia (iii) Fertility Performance Test: Treated males were mated with fertile female Guinea pigs (iv) Reversibility study was made after 30 days of withdrawal of treatment.

Results and Discussion

The treatment did not reduce the body weight at any dose, but at higher doses, it reduced the genital organ weight significantly (Table-1). After 60 days of treatment, with higher doses (300 and 500 mg/kg), caused arrest of spermatogenesis at spermatid stage in seminiferous tubules, distorted the germinal epithelium, atrophied the primodial germ cells including atrophy of Leydig's cells in interstitium. The seminiferous tubules were also filled with oedematus fluid (Figure-1 and 2). The lumens of epidiymes were devoid of spermatozoa with changes in epithelium and stereocilia. Loss of connective tissue and vascularity were seen (Figure-3 and 4). The vasa differentia was also devoid of spermatozoa with other changes like too much folded epithelium without stereocilia (Figure-5 and 6). Higher doses caused 100% infertility in the treated male Guinea pigs. The effect appeared to be reversible (Table-2). The dose 100 mg/kg was not very much effective to control spermatogenesis.

No untoward effect on body weight, but significant reduction in Genital organ weight was noted at higher doses (Table-I). After 60 days of treatment, the arrest of spermatogenesis and other histopathological changes were found in testes and epididymes. No spermatozoa could be seen in Vasa deferentia. Fertility was greatly affected at higher doses (Table-2). Reversible antifertility effect was noted (Table-2).

According to Sinha and Mathur (1990), the feeding of plant extract (neem oil) may cause androgen deficiency which in turn responsible for decrease of testicular weight. The genital organ weight reduction was also reported by Rao (1986) in male albino rats with application of *S. xanthocarpum* seed alcoholic extract. This indicates an anti-androgenic effect. The weight of testes is known to be a good index of FSH secretion. It is confirmed that both steroidal and non-steroidal agents inhibit pituitary gonadotropins either acting directly on pituitary or through the hypothalamo-hypophyseal axis. Bustos-Obregon and Lopez (1973) observed the effect of plant alkaloids on spermatogonium of testes of albino rat. The change in the testicular weight corresponds to the presence or absence of postmeiotic cells (Nelson and Patanelli, 1965). Paul *et al.* (1953) have also demonstrated the reduction in weight of testes and accessory organs in the absence of spermatids and spermatozoa.

Rao (1988) also reported severe histopathological changes in the genital organs (testes, epididymes and Vasa deferentia) of male albino rats after 60 days of treatment with alcoholic extract at 100 mg/kg dose of *S. xanthocarpum*. Shishodia *et al.* (2010) reported effect of alcoholic extract of *Malva viscus conzattii* (Greenum) 25 mg dose on germinal components and Sertoli cells of testes of male albino rats. Our results confirm this effect in Guinea pigs also. The higher dose (500 mg/kg) caused more potent effect. Setty *et al.* (1977) reported spermicidal effect of *S. xanthocarpum* seed extract. Dixit *et al.* (1982) reported the

histopathological changes in testes of male dogs (*C. familiaris*) with pure compound, Solasodine obtained from seeds of *S. xanthocarpum*.

The above study confirms the anti-spermatogenic effect of *S. xanthocarpum* seeds in male Guinea pigs.

Table-1 Effect of *S. xanthocarpum* alcoholic extract on body weight (gm) and genital organ weight (mg) of male guinea pigs administered at different doses for 60 days. Five animals used in each group. Values are mean S.E.

Dose (mg/kg)	Body weight (gm)		Genital Organ Weight (mg)	
	Initial	Final	Testes	Epididymes
Control	482.16 ? 3.12	485.36 ? 1.98	965.13 ? 3.22	325.20 ? 6.12
100 mg	480.81 ? 1.52	483.21 ? 1.45	963.25 ? 7.52	316.71 ? 1.93
300 mg	471.13 ? 1.51	470.22 ? 1.36	928.18 ? 1.31*	301.12 ? 2.33*
500 mg	483.15 ? 2.36	471.31 ? 2.78	866.56 ? 2.91*	265.67 ? 3.89*

* P values < 0.05

Table-2 Effect of *S. xanthocarpum* alcoholic extract on fertility of male Guinea Pigs at different doses (Table also shows reversibility, if any)

Doses (mg/kg)	No. of treated males	No. of normal females mated	Fertile matings*	Infertile matings**
After 60 days of continuous treatment				
Control	05	05	5 (100%)	0
100	05	05	2 (40%)	3 (60%)
300	05	05	0	5 (100%)
500	05	05	0	5 (100%)
After 30 days of withdrawal of treatment (Reversibility)				
Control	05	05	5 (100%)	0
100	05	05	5 (100%)	0
300	05	05	5 (100%)	0
500	05	05	5 (100%)	0

* Those which resulted in pregnancy

**Those which did not result in pregnancy.

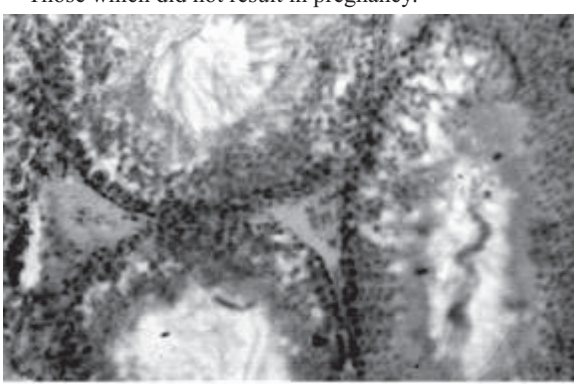

Figure-1 T.S. of testis of Guinea pig of control group for 60 days. Note full spermatogenic activity with spermatogonia, spermatid and spermatozoa in the seminiferous tubules and normal Leydig's cells in the interstitium X 450.

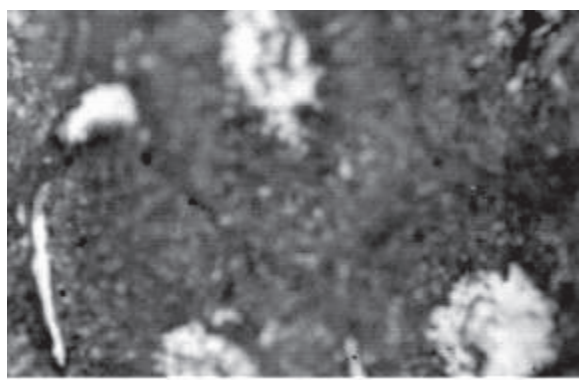

Figure-2 T.S. of testis of Guinea pig of treated group with S. xanthocarpum at 500 mg/kg dose for 60 days. Note the highly degenerated germ cells, oedematous fluid, Leydig's cell atrophy and arrest of spermatogenesis X 450.

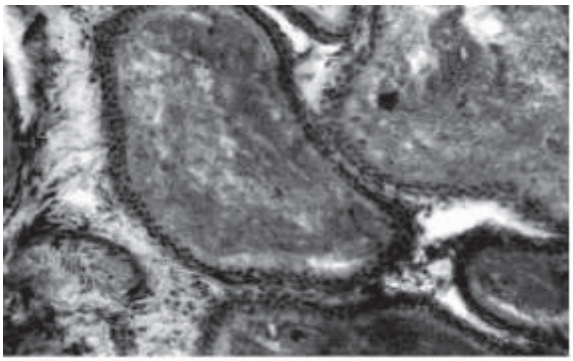

Figure-3 T.S. of epididymis of Guinea pig of control group for 60 days. Note the normal histological features, spermatozoa and stereocilia clearly visible in the lumen of ductules X 450.

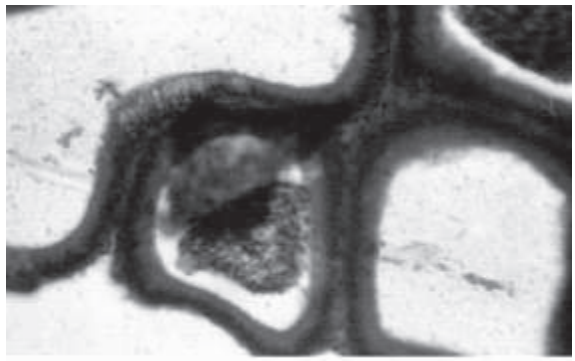

Figure-4 T.S. of epididymis of Guinea pig of treated group with S. xanthocarpum at dose 500 mg/kg for 60 days. Note reduced epithelial cell height, no spermatozoa, stereocilia and vacoules around the epithelial cell nuclei X 450.

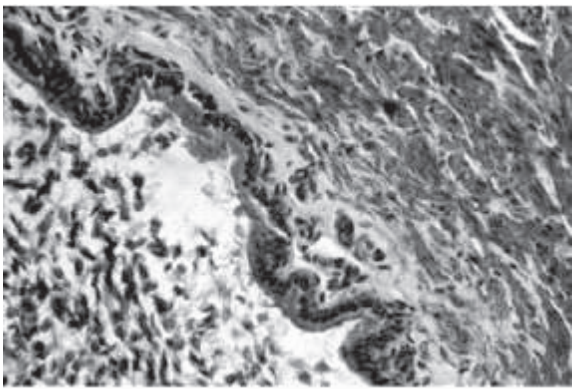

Figure-5 T.S. of vas deferens of Guinea pig of control group for 60 days. Note the normal structure, distended lumen with spermatozoaand less folded epithelium X 450.

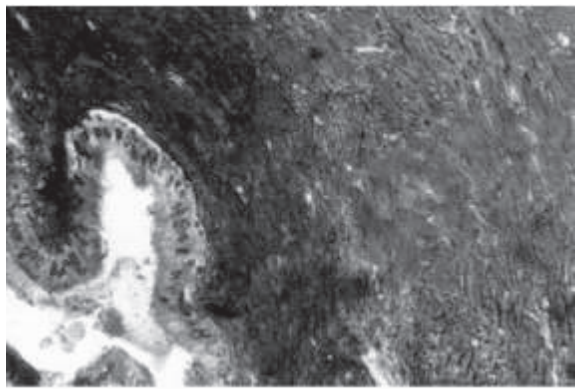

Figure-6 T.S. of vas deferens of Guinea pig of treated group with S. xanthocarpum at dose 500 mg/kg for 60 days. Note the lumen devoid of spermatozoa and folded epithelium X 450.

Acknowledgement

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References

- Bustos-Obregon, E. and Lopez, M.L. *Andrologia*, 1973, 5: 243-247.
- Chaudhury, R.R. Special report series, 1966, No. 55, I.C.M.R., New Delhi
- Farnsworth, N.R. *et al. J. Pharma. Sci. U.S.A.,* 1975, 64(4) : 535-558.
- Dixit, V.P. and Gupta, R.S. *Int. J. Androl.,* 1982, 4: 1-8.
- Nelson, W.O. and Patanella, D. J. In: Agents Affecting Fertility (Eds. Austin, C.R. and Perry, J.S.) Little Brown, Boston Mass, U.S.A., 1965.
- Paul *et al. Endocrinol*., 1953, 53 : 585-588.
- Rao, M. V. *Bio-Sci. Res. Bull.,* 1986, 2: 22-28.
- Rao, M. V. *Indian J. Exp. Biol.,* 1988, 26(2): 95-98.
- Setty *et al. Indian J. Exp. Biol.* 1977, 15: 231-232.
- Shishodia, A. *et al.*, *J. Env. Bio-Sci.,* 2010, 24(2): 247-251.
- Sinha, S. and Mathur, R. S. *Indian J. Exp. Biol.* 1990, 28: 752-756.

About Flowers

Holarrhena antidysenterica (Kutaja)

Aegle marmelos (Bael)

Punica granatum (Anaar)

Cyperus rotundus (Nagar Motha)

***Holarrhena Antidysenterica* (Kutaja)**

Kingdom	:	Plantae
Division	:	Tracheophyta (Tracheophytes Or Vascular Plants)
Sub Division	:	Spermatophytina (Spermatophytes Or Seed Plants)
Class	:	Magnoliopsida
Order	:	Gentianales
Family	:	Apocynaceae (Apocyns, Dogbane)
Genus	:	Holarrhena
Species	:	*Holarrhena antidysenterica* Wall ex G. Don

Holarrhena antidysenterica plant grows as a deciduous shrub up to 3ms high. The leaves are opposite, ovate and 10-cm long. The stem has several branches. The flowers are corymb-like cymes. The flowers have five white petals and grow up to 2-3cm high. The fruits are cylindrical and paired and the seeds are light brown. It is a medicinal plant in Ayurveda. The botanical name of the plant itself explains the main medicinal property of plant that is explained in Veda for the treatment of diarrhea.

Bark and seeds of the plant are used medicinally. The bark contains the alkaloids such as regholarrenine A, B, C, D, E, and F. It is pungent, bitter, and astringent in taste. It is used both internally and externally. It is used for the treatment of dysentery caused due to amoeba.The seeds are antibilious and promote conception. It is also used for toning up vaginal tissue after delivery in women.

The plant is used for the treatment of skin diseases such as scabies, ringworm, itching and other infections. The plant is used as the rejuvenating agent for the immune system in the body.

It also cures rheumatoid arthritis and osteoarthritis.

In treatment of bleeding piles, Kurai checks the secretion of mucus and blood.

***Aegle Marmelos* (Bael)**

Kingdom Plantae	:	Plants
Subkingdom Tracheobionta	:	Vascular plants
Superdivision Spermatophyta	:	Seed plants
Division Magnoliophyta	:	Flowering plants
Class Magnoliopsida	:	Dicotyledons
Subclass	:	Rosidae
Order	:	Sapindales
Family Rutaceae	:	Rue family
Genus *Aegle* Corr. Serr.	:	aegle P
Species *Aegle marmelos* (L.) Corr. Serr.	:	Indian bael P

Aegle marmelos, a plant indigenous to India has been used by the inhabitants of the Indian subcontinent for over 5000 years. The leaves, bark, roots, fruits and seeds are used extensively in the Indian traditional system of medicine, the Ayurveda and in various folk medicine to treat myriad ailments. Bael fruits are of dietary use and the fruit pulp is used to prepare delicacies like murabba, puddings and juice. Bael fruits are also used in the treatment of chronic diarrhea, dysentery, and peptic ulcers, as a laxative and to recuperate from respiratory affections in various folk medicines. Scientific studies have validated many of the ethnomedicinal uses and reports indicate that the fruit possesses broad range of therapeutic effects that includes free radical scavenging, antioxidant, inhibition of lipid peroxidation, antibacterial, antiviral, anti-diarrheal, gastroprotective, anti-ulcerative colitis, hepatoprotective, anti-diabetic, cardioprotective and radioprotective effects. For the first time, this review critically assesses the nutritional values, phytochemistry and preclinical pharmacological properties of the bael fruit.

***Punica Granatum* (Anaar)**

Kingdom Plantae	:	Plants
Division Magnoliophyta	:	Flowering plants
Class Magnoliopsida	:	Dicotyledons
Subclass	:	Rosidae
Order	:	Myrtales
Family Punicaceae	:	Pomegranate family
Genus *Punica* L.	:	pomegranate P
Species *Punica granatum* L.	:	pomegranate P

Punica granatum, commonly known as Pomegranate, is a species of fruit-bearing deciduous shrub or small tree, growing to 5-8 m height. The leaves are opposite or sub-opposite, glossy, narrow, oblong, entire, 3-7 cm long and 2 cm broad. The flowers are bright red, with five petals each. Native to the southwest zones of Asia, the plant species was originally cultivated in the Caucasus region in ancient times.

Pomegranate is a rich source of potassium and antioxidant polyphenols. These polyphenols mainly include hydrolysable tannins called punicalagins. Polyphenols catechins, gallocatechins, and anthocyanins such as prodelphinidins, delphinidin, cyanidin, and pelargonidin are other phytochemicals present in the plant species.Pomegranate is great for curing a number of ailments and diseases. The juice of its fruit helps to reduce the risk of strokes, heart diseases and heart attacks. It prevents the formation of Low Density Lipoprotein (LDL) or "bad" cholesterol, through its antioxidant properties and hence, helps in the clearance of blood clots in the arteries.

The fruit is a rich source of vitamins and is particularly high in vitamin A, C E and folic acid.

It also helps to strengthen the normal human defense mechanism and prevents the occurrence of certain types of cancer. This is mainly because of the fact that the fruit contains high amounts of antioxidants called flavenoids, which are believed to counteract the actions of cancer-causing radicals and thereby promote good health.

***Cyperus Rotundus* (Nagar Motha)**

Kingdom Plantae	:	Plants
Subkingdom Tracheobionta	:	Vascular plants
Superdivision Spermatophyta	:	Seed plants
Division Magnoliophyta	:	Flowering plants
Class Liliopsida	:	Monocotyledons
Subclass	:	Commelinidae
Order	:	Cyperales
Family Cyperaceae	:	Sedge family
Genus *Cyperus* L.	:	flatsedge P
Species *Cyperus rotundus* L.	:	nutgrass P

Cyperus rotundus is a perennial plant, that may reach a height of up to 140 cm (55 inches). The names "nut grass" and "nut sedge" shared with the related species Cyperus esculentus are derived from its tubers, that somewhat resemble nuts, although botanically they have nothing to do with nuts.

Nagarmotha (*Cyperus rotundus*), a cosmopolitan weed, is found in all tropical, subtropical and temperate regions of the world. In India, it is commonly known as Nagarmotha and it belongs to the family Cyperacea. The major chemical components of this herb are essential oils, flavonoids, terpenoids, sesquiterpenes, cyprotene, cyperene, aselinene, rotundene, valencene, cyperol, gurjunene, trans-calamenene, cadalene, cyperotundone, mustakone, isocyperol, acyperone, etc. Research studies have shown that it possesses various pharmacological activities such as diuretic, carminative, emmenagogue, anthelminthic, analgesic, anti-inflammatory, anti-dysenteric, antirheumatic activities. An extensive review of the ancient traditional literature and modern research revealed that the drug has numerous therapeutic actions, several of which have been established scientifically, which may help the researchers to set their minds for approaching the utility, efficacy and potency of nagarmotha.

Forth Coming Events

1. 12th World Congress on Pharmaceutical Sciences and Innovations in Pharma Industry
   February 26-27, 2018
   London, UK
   https://industry.pharmaceuticalconfrences.com/

2. 16th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery System
   March 19-20, 2018
   Berlin, Germany
   http://novel-drugdelivery-systems.pharmaceuticalconferences.com/

3. International Conference on Nano Medicine and Nanoparticles
   April 18-19, 2018
   Las Vegas, USA
   http://nanomedicine.pharmaceuticalconferences.com/usa/

4. Annual Global Experts Meet on Green Chemistry and Engineering
   September 20-22, 2018
   Berlin, Germany
   http://www.meetingsint.com/chemistry-conferences/greenchemistry

5. Annual Pharmaceutical Biotechnology Congress
   May 17, 2018
   Singapore
   http://phytochemicalsociety.org/?post_type=tribe_events

6. 10th International Conference on Chemical Education
   May 21-22, 2018
   Oslo, Norway
   http://www.omicsonline.org/conferences-list/national-products-chemistry

7. 4th International Conference and Exhibition on Natural Products, Medical Plants & Marine Drugs
   June 11-12, 2018
   Rome, Italy
   http://naturalproducts.pharmaceuticalconferences.com/

8. Advances in Phytochemical Analysis (Trends in Natural Products Research)
   July 2, 2018
   Liverpool, United Kingdom
   http://phytochemicalsociety.org/?post_type=tribe_events

9. International Conference on Pharmaceutical Oncology
   July 18-19, 2018
   Atlanta, USA
   http://oncology.pharmaceuticalconferences.com/

10. 20th International Conference on Medicinal Chemistry and Pharmacology
    July 25-26, 2018
    Vancouver, Canada
    http://www.omicsonline.org/conferences-list/pharmacognosy-and-phytopharmaceuticals

11. 4th World Congress on Medical Plants and Natural Products Research
    August 20-21, 2018
    Tokyo, Japan
    http://medicinalplants.pharmaceuticalconferences.com/

12. 8th International Conference on Environmental Chemistry and Engineering
    September 20-22, 2018
    Berlin, Germany
    http://environmentalchemistry.conferenceseries.com

13. International Conference on Biomedicine & Pharmacotherapy
    October 26-27, 2018
    Osaka, Japan
    biomedicineconferenceseries.com

14. 3nd International Conference on Pharmaceutical Chemistry
    October 29-31, 2018
    Brussels, Belgium
    http://pharmaceuticalchemistry.conferenceseries.com/

15. 9th International Congress of Environmental Research
    February 8-10, 2018
    Gwalior, Madhya Pradesh, India
    https://conferencealerts.com/show-event?id=191888

16. International Congress on Plant Based Natural Products: Phytocosmetics, Phytotherapeutics and phytonutraceutics Conference
    February 10-21, 2018
    Udaipur, Rajisthan, India
    https://conferencealerts.com/show-event?id=190693

17. Wellness India 2018 Expo
    August 20-22, 2018
    Pragati Maidan, New Delhi, India
    https://www.aoaweb.in/single-post/2017/09/27/Wellness-India-2018-Expo

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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. T**he 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 Phytochemistry and Ayurvedic Height for their endeavor in Herbal research.

Dr. Rajendra Dobhal
Director General

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