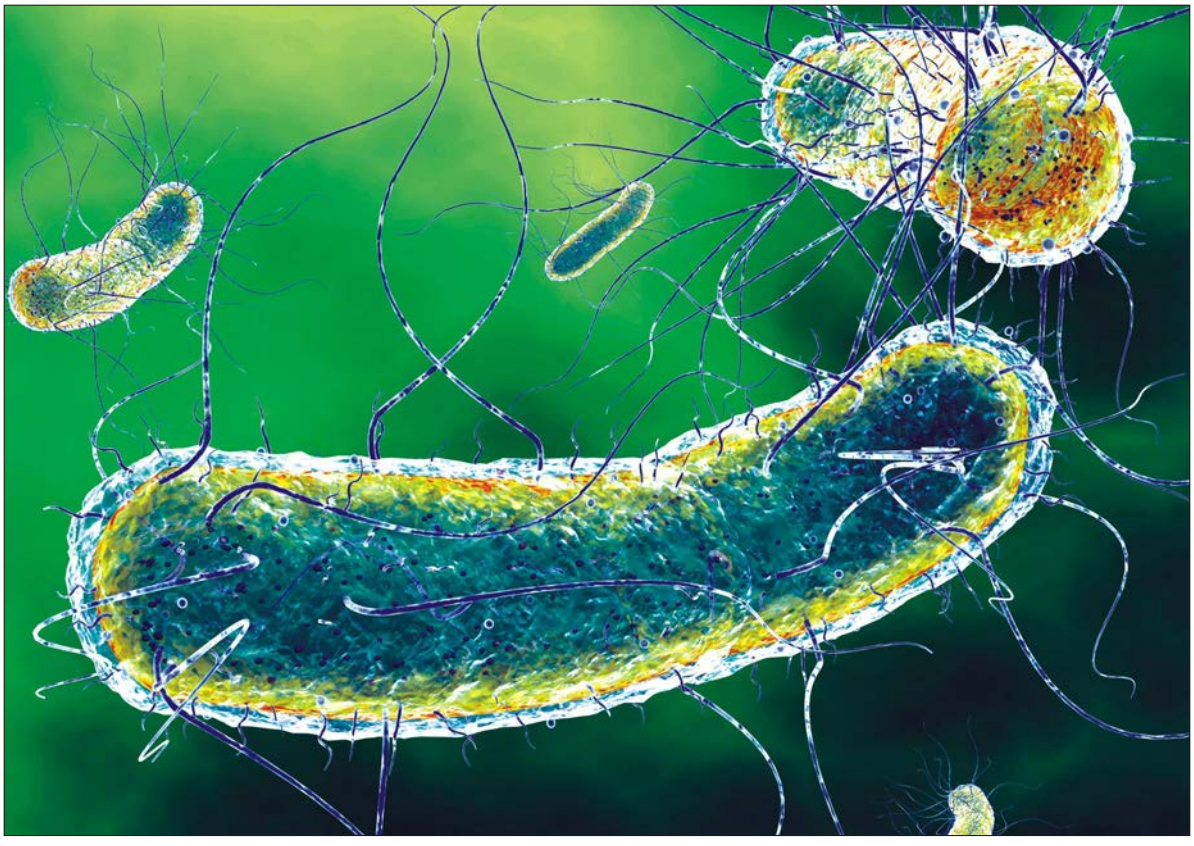

Current Research in Bacteriology

ISSN 1994-5426

science alert

ANSInet
an open access publisher
http://ansinet.com

Current Research in Bacteriology 1 (1): 1-6, 2008
ISSN 1994-5426

Lipid Content and *in vitro* Antimicrobial Activity of Oil Seeds of Some Indian Medicinal Plants

[1]Ujjwal Neogi, [1]Ruchi Saumya, [2]Rashmi K. Mishra and [1]K.C. Raju
[1]Department of Microbiology,
Administrative Management College, Bangalore, India
[2]Department of Botany, Dr. Babasaheb Bhimrao Ambedkar Bihar University, Bihar, India

Abstract: The objectives of this study were to analyze the lipid content of 10 oil seeds of different plant families and the antimicrobial activities of these plant seeds that can add a new dimension in the alternative medicinal field of Indian origin. Chemical analysis reveals that the major components of all the seeds were myristic acid, ricinoleic acid, linoleic acid, palmitic acid, lauric acid and oleic acid. The other 4 components i.e., linolenic acid, palmitoleic acid, steric acid and arachidic acid present less than 30% of plant seeds. The primary screening tests for antibacterial and antifungal activities were shown positive for all the compounds. *Escherichia coli* and *Candida albicans* has shown a zone of clearance ranging 11-15 mm whereas *Pseudomonas aeroginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Aspergillus niger* and *Penicillium notatum* showed the range between 16-20 mm. It is assumed that oxidative effect could plausibly play an important role in the antimicrobial function of fatty acids. The higher oil content were found to be on *Aegle marmelos* (49%), *Prunus amygdalus* (48%), *Cardiospermum halicacabum* (47%), *Brassica alba* (38%) and can suggest as an agent of conservation in the cosmetic and/or food industries, as an active compound in medical preparations and as a disinfectants.

Key words: Oil seeds, lipid analysis, antimicrobial activity, agar well diffusion method

INTRODUCTION

The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s (Dorman and Deans, 2000).

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Parekh and Chanda, 2007). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996).

Corresponding Author: Ujjwal Neogi, Virology Laboratory-II, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110067, India Tel: +919953077149

India is a varietals emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition (Parekh and Chanda, 2007).

Hydroxy fatty acids shows wide range of antifungal (Sjogren *et al*., 2003) and antibacterial (Mundt *et al*., 2003) activities. Considering the extensive applications of hydroxy fatty acids in medicinal importance, an attempt has been made to evaluate the antibacterial and antifungal activity of crude hydroxy fatty acids containing oil seeds of different plant seeds. Previous investigations showed the antimicrobial activities of different plant part of *Mimusops hexandra*, *Prunus amygdalus* (Yosef *et al*., 2007) *Brassica alba* (Ceylan and Fung, 2004), *Cardiospermum halicacabum* (Parekh and Chanda, 2007), *Aegle marmelos*, *Anacardium occidentale* (Ojewole, 2004), *Jatropha gossypifolia* (Ogundare, 2007), *Hevea brasiliensis*, *Psoralea corylifolia* (Prasad *et al*., 2004) and *Bauhinia retusa*.

In this study we describe in details the lipid contents of 10 oil seeds of different plant families and the antimicrobial activities of these plant seeds on common human pathogens including food borne, intestinal, nosocomial pathogens and dermatophytes.

MATERIALS AND METHODS

Plant Materials

The seed samples belonging to different plant families were collected from Bidhan Chandra Krishi Viswavidalaya, Mohanpur, West Bengal and Pusa Agricultural Research Institute, Bihar, from the full grown vegetative plants during rainy season and tabulated in (Table 1) with the sample Number.

Extraction of Oils (Soxhlet Method)

Lipid extraction was carried out by Soxhlet extraction method using petroleum ether (40-60°C). A known weight of seeds were kept in the Soxhlet's apparatus and 250 mL of petroleum ether (40-60°C) was added and extracted for 10 h the solvent was recovered by distillation and the fat recovered in a minimum volume of ether was transferred into a pre-weighed clean beaker. The solvent was evaporated at 55°C in incubator. Finally oil was weighed. The direct thin layer chromatography of seed oils revealed the presence of hydroxy fatty acids (Prost and Wrebiakowski, 1972).

Fatty acid Analysis by Gas Chromatography

Preparation of Sample for Gas Chromatography

One milliliter of 0.5 M KOH was added to 0.5 mL of sample and kept it at 86°C in water bath for 10 min. Cooled and added 2 mL of hexane, vortex for 15 min and centrifuged to take out the hexane phase. To aqueous layer 1 mL of 0.7 NH_4Cl and 2 mL of hexane centrifuge at 5000 rpm for 15 min. The hexane layer was collected. After that the hexane layers were evaporated. 0.2 mL of benzene and 0.5 mL of BF_3 was added and kept at 86°C in water bath for 10 min. After that cool and add 1 mL of

Table 1: Medicinal plant of different plant families

Family	Genus and species	Sample No.
Sapotaceae	*Mimusops hexandra*	S1
Rosaceae	*Prunus amygdalus*	R2
Cruciferae	*Brassica alba*	C3
Sapindaceae	*Cardiospermum halicacabum*	S4
Rutaceae	*Aegle marmelos*	R5
Anacardiaceae	*Anacardium occidentale*	A6
Euphorrbiaceae	*Jatropha gossypifolia*	E7
	Hevea brasiliensis	E8
Leguminosae	*Psoralea corylifolia*	L9
	Bauhinia retusa	L10

water. The aqueous phase was extracted 3 times with 2 mL of hexane. The hexane layer was pooled and 5 mL of water was added. The hexane layer was separated after centrifugation. The hexane was evaporated and benzene was added to ready the sample for gas chromatography (Goud *et al*., 2007).

Gas Chromatography

After the gas chromatography the fatty acids were identified by using authentic fatty acid standards obtained from Sigma, USA. The relative percentages of the fatty acids were determined using Shimadzu C-R 3A integrator connected to gas chromatography.

Preparation of Drugs Solution

Each test compound (10 mg) was dissolved in dimethyl formamide (10 mL) to give solution of 1000 μg mL^{-1} and 0.1 mL of this solution was used.

Microorganisms

The organisms *Escherichia coli*, *Pseudomonas aeroginosa*, *Salmonella typhi*, *Staphylococcus aureus* were obtained from Vaidehi Medical College, Bangalore. Three fungi culture *Aspergillus niger*, *Candida albicans* and *Penicillium notatum* were collected from different colleges in Bangalore.

Antimicrobial Assay

The antimicrobial properties were evaluated by agar well diffusion method (Perez *et al*., 1990) using Mueller Hinton agar (Hi-media) for bacteria and Sabouraud's dextrose agar for fungi. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (25 mL) and Sabouraud's dextrose broth (25 mL). The culture flasks were incubated at 37°C for 24 h (bacteria) and 25°C for 7 days (fungi), respectively.

One milli liter of inoculums was inoculated into the 45-50°C cooled agar and plated. Using the cork borer wells was made and different extracts having 100 μg mL^{-1} concentration were transferred using a micropipette. Then the plates were kept in the refrigerator for 5 min for diffusion and incubated at 37°C for 24 h and 25°C for 7 days, respectively. The control experiment was carried out with tetracycline. Zone of inhibition was measured in millimeter. The experiments were carried out for ten successive trials and the average values are presented.

RESULTS AND DISCUSSION

The oil contents of different oil seeds containing hydroxy fatty acids are tabulated in (Table 2) after extraction of oil by Soxhlet Method.

The highest oil contains of the oil was found in *Aegle marmelos* (49%) and the lowest in *Hevea brasiliensis* and *Psoralea corylifolia* (14%). Direct thin layer chromatography revealed the presence of hydroxy fatty acids in all the plant species.

Table 2: The oil contents of different oil seeds containing hydroxy fatty acids

Oil seeds	Percentage of oil
Mimusops hexandra	17.0
Prunus amygdalus	48.0
Brassica alba	38.0
Cardiospermum halicacabum	47.0
Aegle marmelos	49.0
Anacardium occidentale	32.5
Jatropha gossypifolia	33.0
Hevea brasiliensis	14.0
Psoralea corylifolia	14.0
Bauhinia retusa	20.0

Table 3: Fatty acid composition of different oil seeds represented in (%)

Samples	Lauric	Myristic	Palmitic	Stearic	Oleic	Ricinoleic	Linolenic	Linoleic	Arachidic	Palmitoleic
S1	13.6	10.5	25.4	8.2	27.8	14.5	-	-	-	-
R2	-	9.5	-	15.3	20.5	12.5	-	18.4	23.8	-
C3	6.9	12.8	45.4	-	10.5	12.4	-	12.0	-	-
S4	4.7	5.6	12.7	9.7	32.9	8.6	-	13.3	12.8	-
R5	-	4.3	38.8	-	12.6	12.5	18.4	13.4	-	-
A6	8.0	13.8	38.0	-	-	13.2	-	15.2	-	11.8
E7	8.1	16.4	-	-	11.3	14.7	-	22.6	27.1	-
E8	6.6	12.7	18.2	-	-	11.7	-	38.0	-	12.8
L9	6.4	7.3	12.7	-	-	7.6	22.0	44.0	-	-
L10	-	6.6	18.3	-	24.4	16.7	-	34.4	-	-

- : Absent

Table 4: Screening results for antimicrobial activity of the oil seeds by the agar well diffusion method

	Zone of inhibition (mm)										
Organisms	Control	S1	R2	C3	S4	R5	A6	E7	E8	L9	L10
Escherichia coli	26	12±0.5	14±0.5	13±0.5	15±1.1	11±1.1	14±0.7	13±0.4	12±0.6	12±0.8	13±0.8
Pseudomonas aeroginosa	24	16±1.2	17±0.6	16±0.6	15±1.2	20±1.2	18±0.6	15±0.6	16±0.8	17±0.9	19±1.2
Salmonella typhi	21	19±1.3	20±0.9	17±1.5	18±0.6	16±1.6	14±0.8	16±0.8	19±1.2	20±0.2	20±1.6
Staphylococcus aureus	20	16±0.7	17±1.3	18±1.1	19±0.8	18±0.8	19±0.5	20±0.2	17±1.5	17±1.2	18±0.6
Candida albicans	24	11±1.4	15±1.2	12±0.5	15±0.0	13±0.5	14±1.5	15±1.0	13±0.8	14±1.5	13±0.8
Aspergillus niger	21	14±0.6	18±0.7	17±0.0	16±1.2	19±1.0	18±0.5	15±1.5	15±2.0	15±1.8	18±1.0
Penicillium notatum	20	16±0.8	20±0.8	16±0.6	18±1.6	17±0.5	15±1.5	14±0.5	19±1.5	16±0.8	20

N.B. diameter of the well: 10 mm

After the gas chromatography the composition and percentage of fatty acid was tabulated in the Table 3.

In all tested oils 10 components were identified. The major components of all the seeds were myristic acid and ricinoleic acid, linoleic acid, palmitic acid, lauric acid and oleic acid. The other 4 components i.e., linolenic acid, palmitoleic acid, steric acid and arachidic acid present less than 30% of plant seeds. Antibacterial activity of the different plant oil seeds containing are presented in (Table 4).

All the bacterial strains demonstrated some degree of sensitivity to the oil seeds tested. *Escherichia coli* and *Candida albicans* showed a zone of clearance ranging 11-15 mm whereas *Pseudomonas aeroginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Aspergillus niger* and *Penicillium notatum* showed the range between 16-20 mm. The control showed the zone diameter ranging 20-26 mm.

Natural hydroxy fatty acids isolated from different plant seeds showed a moderate antibacterial and antifungal activity. The antifungal property of fatty acids is due to its detergent-like activity which affects the structure of cell membranes of the target organisms. Indeed, cis-9-heptadecenoic acid, a compound similar to the 3-hydroxy fatty acids identified that readily partitions into the lipid bilayers of fungal membranes (Avis and Belanger, 2001). This increases membrane permeability and the release of intracellular electrolytes and proteins and, eventually, leads to cytoplasmic disintegration of fungal cells. It is assumed that this oxidative effect could plausibly play an important role in the antimicrobial function of fatty acids (Kim Young *et al*., 2006).

Gram-positive bacteria are more resistant then Gram negative bacteria to the antibacterial properties of plant volatile oils (Fredj *et al*., 2007). However, *Brassica alba*, *Cardiospermum halicacabum*, *Jatropha gossypifolia* and *Bauhinia retusa* oils appeared preferentially more active with

respect to Gram reaction, exerting greater inhibitory activity against Gram-positive organisms *Staph. aureus*. In our study *E. coli* showed the more resistant. It may be due to the presence of plasmid conferring resistance. Among the fungi *Candida albicans* was more resistant compared to the other two.

Chemotherapeutic agents, used orally or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious disease (Dorman and Deans, 2000). The tested plant products appear to be effective against a wide spectrum of microorganisms, both pathogenic and nonpathogenic bacteria and fungi. Administered orally, these compounds may be able to control a wide range of microbes but there is also the possibility that they may cause an imbalance in the gut microflora, allowing opportunistic pathogenic coliforms to become established in the gastrointestinal tract with resultant deleterious effects. Further studies on therapeutic applications of volatile oils should be undertaken to investigate these issues, especially when considering the substantial number of analytical studies carried out on these natural products.

As a conclusion, among the all species the higher oil content were found to be on *Aegle marmelos* (49%), *Prunus amygdalus* (48%), *Cardiospermum halicacabum* (47%), *Brassica alba* (38%) and can be suggested as an agent of conservation in the cosmetic and/or food industries, as an active compound in medical preparations and as a disinfectants.

ACKNOWLEDGMENTS

We are very much thankful to Dr. Anjan Bhattacharjee, Officer in Charge, MAP Project, Dept. of Agriculture Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur for providing the plant materials. Dr. Seemanthini Desai, Apollo Hospitals, Bangalore, India and Miss Hithyashi C B, Lecturer, Dayanand Sagar Institution, Bangalore for their valuable guidance and support.

REFERENCES

Avis, T.J. and R.R. Be´langer, 2001. Specificity and mode of action of the antifungal fatty acid cis-9-heptadecenoic acid produced by *Pseudozyma flocculosa*. Applied Environ. Microbiol., 67: 56-96.

Benkeblia, N., 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensm-Wiss u-Technol., 37: 263-268.

Ceylan, E. and Y.C. Fung, 2004. Antimicrobial activity of spices J. Rapid Methods Automation in Microbiol., 12: 1-55.

Colombo, M.L. and E. Bosisio, 1996. Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). Pharmacol. Res., 33: 127-134.

Dorman, H.J.D. and S.G. Deans, 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol., 88: 308-316.

Fredj, M.B.H., B. Marzouk, I. Chraief, K. Boukef and Z. Marzouk, 2007. Analysis of Tunisian *Ruta graveolens* L. oils from Jemmel. J. Food Agric. Environ., 5: 52-55.

Goud, N.R., U. Neogi and R. Saumya, 2007. Fungal production of omega-6 fatty acid: Gamma Linolenic Acid. Int. J. Biol. Chem., 1: 127-130.

Kim Young-Mi, L. Geon-Ho, Y. Young-Geun, K. In-Hwan, M. Kazuo, T. Hou Ching, K. Sun-Chul and K. Hak-Rvul, 2006. The effect of bio-converted polyunsaturated fatty acids on the oxidation of TAG containing highly unsaturated fatty acids. J. Ind. Microbiol. Biotechnol., 33: 17-21.

Mundt, S. S. Kreitlow and R. Jansen, 2003. Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. J. Applied Phycol., 15: 263-267.

Ogundare, A.O., 2007. Antimicrobial effect of *Tithonia diversifolia* and *Jatropha gossypifolia* leaf extracts. Trends Applied Sci. Res., 2: 145-150.

Ojewole, J.A., 2004. Potentiation of the antiinflammatory effect of *Anacardium occidentale* (Linn.) stem-bark aqueous extract by grapefruit juice. Methods Find Exp. Clin. Pharmacol., 26: 183.

Parekh, J. and S.V. Chanda, 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants, Turk. J. Biol., 31: 53-58.

Perez, C., M. Paul and P. Bazerque, 1990. An antibiotic assay by the agar well diffusion method. Acta Bio. Med. Exp., 15: 113-115.

Prasad, R.N., C. Anandi, S. Balasubramanian and K.V. Pugalendi, 2004. Antidermatophytic activity of extracts from *Psoralea corylifolia* (Fabaceae) correlated with the presence of a flavonoid compound. J. Ethnopharmacol., 91: 21-24.

Prost, E. and H. Wrebiakowski, 1972. Evaluation of soxhlet's and bligh and dyer's methods in the determination of fat in meat. Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 149: 193-196.

Sjogren, J., J. Magnusson, A. Broberg, J. Schnurer and L. Kenne, 2003. Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* milab 14. Applied Environ. Microbiol., 69: 7554-7557.

Yosef, H.A.A., N.M. Morsy, M.R.H. Mahran and H.Y. Aboul-Enein, 2007. Preparation and reactions of optically active cyanohydrins using the (R)- hydroxynitrile lyase from *Prunus amygdalus*. J. Iran Chem. Soc., 4: 46-58.