Advances in Biological Research 4 (6): 301-304, 2010
ISSN 1992-0067

Chemical Composition and Antimicrobial Activity of the Essential Oil from Bark of *Pittosporum dasycaulon* Miq.

[1]C.T. Sadashiva, [2]P. Sharanappa, [1]A.B. Remashree, [1]A.V. Raghu
P.S. Udayan and [1]Indira Balachandran

[1]Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Malappurum, Kerala, India
[2]Department of Bioscience, University of Mysore, P.G. Centre, Hassan, Karnataka, India, 573201

Abstract: Essential oil from bark of medicinal tree *Pittosporum dasycaulon* obtained by hydrodistillation was analyzed through Gas Chromatography and Gas Chromatography/Mass Spectrometry. The essential oil was tested for antimicrobial activity by the disc-diffusion method and minimal inhibitory concentration (MIC) was also determined. The main compounds found were dodecanal (53.43%) undecane (20.84%) hexadecanal (9.95%), dodecanoic acid (3.6) and 1-tridecanol (2.15%) The oil sample was active against *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli* not active against *Bacillus subtilis*. The MIC values ranged from 25 to 100 µl/ml.

Key words: *Pittosporum dasycaulon* · Antimicrobial activity · Essential oil

INTRODUCTION

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition [1]. Essential oils are important constitutents of some higher plants comprising monoterpenes, sesquiterpenes, arylpropanoids and other derivatives. The antimicrobial properties of essential oils have been recognized long ago and they have been scientifically established [2].

The plant family Pittosporaceae consists of 9 genera represented by 250 species, 160 of which occur in the genus *Pittosporum* Banka ex. Soland and largely distributed in tropical and subtropical regions of old world, Africa, Asia, Australia and New Zealand [3] and in India Pittosporum the genus represented by 11 species.

The species of *Pittosporum* is widely used as medicinal plants. The flowers, root, bark and leaves are used as anti-inflammatory, antiseptic and in rheumatic disorders. The main components being essential oils, oleoresin, triterpenoid saponins, stigmasterol [4-6]. In India bark of Pittosporum genus used as anti-inflammatory, antispasmodic and for narcotic effects [7,8]. Bark also useful in chronic bronchitis and also administered in leprous affections and rheumatic swellings [7]. The oil is applied topically in sprains, brusies, sciatica, rheumatism, chest affection and in certain skin diseases [8]. Paste of root applied to dropsical and rheumatic swelling [7]. The native Maori people of New Zealand are known to have add the gum, leaves and flowers of *P. eugenoids* to oils, which were used to anoint their bodies [9]. Antimicrobial activity of essential oil from several Pittosporum species has been reported [10].

Pittosporum dasycaulon Miq. is an small tree, the leaves are alternate, exstipulate, elliptic, acuminate, cuneate 8-15 x 2-3.5 cm, glabrous with age and inflorescnece is umbels of short racemes. [11]. *P. dasycaulon* is endemic to South West India and distributed in moist deciduous and evergreen forests of Western Ghats above 800 m in the states of Maharastra, Karnataka, Tamil Nadu and Kerala.[3-8]. The bark and stem of *P.dasycaulon* have been used in folk medicine as an antibacterial and antifungal to treat infection [8]. This is the first attempt to analyze the chemical composition and antimicrobial activity of essential oil of *P. dasycaulon*..

METERIALS AND METHODS

Plant Material: Plant material from *P. dasycaulon* was collected in Nellyampathy of Palakkad district, Kerala State, India in the month of May 2008. The species was identified by Dr. P.S. Udayan, Taxonomy Division,

Corresponding Author: Dr. C.T. Sadashiva, Department of Pharmacology,
Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala 676705, India.

Arya Vaidya Sala and voucher specimen (No.05074 dt.29.5.2008) has been deposited in the Herbarium of the Arya Vaidya Sala, Kottakkal, Kerala, India.

Oil Isolation: Fresh bark of *P. dasycaulon* hydrodistilled in Clevenger-type apparatus. After 2 hour of distillation, the essential oil was removed from the surface of the water. The oils were dried over anhydrous sodium sulphate. The samples were sealed and kept in dark glass vials in the refrigerator for further analysis.

GC/FID Analysis: Capillary gas chromatography was performed using a Hewlett-Packard 6980 gas chromatography with fused silica capillary column HP-5 (5% diphenyl and 95% dimethylpolysyloxane, 60 m x 0.25 mm, 0.25 μm film thickness). Helium gas used as carrier gas (1ml/min) and temperature programming from 70 to 290° C (2° C/min), injector temperature 270° C and detector temperature 300° C.

GC/MS Analysis: Gas chromatography-mass spectrometry (GC-MS) analysis were carried out in a Agilent gas chromatography N6980 fitted with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 30m x 0.25 mm, film thickness 0.25μm), interfaced with an Agilent mass selective detector. Oven temperature program: 50-240° C at 5°C min^{-1}. Injector temperature 280°C. Helium used as carrier gas adjusted to column velocity of flow 2.0ml/ min^{-1}. Split ratio was 1:10, whereas split flow was 30.7-ml/ min^{-1}. Mass scan range 50 to 500 amu. One μl of sample (dissolved in hexane 100% v/v) was injected into the system. Compound identification was based on the comparison of retention indices, mass spectra and the NIST spectrometer data bank as well as comparison with literature data.

Microorganism and Growth Conditions: The essential oils of *P. dasycaulon* were tested against a panel of microorganism including *Staphylococcus aureus* (MTCC 3160), *Bacillus cereus* (MTCC 1306), *Pseudomonas aeruginosa* (MTCC 1034) and *Escherichia coli*(MTCC 1089) and *Bacillus subtilis* (MTCC 1133). These microorganisms, belonging to the Microbial Type Culture Collection(MTCC), were supplied by the Institute of Microbial Technology, Chandigarh, India.

Antimicrobial Activity: Antibacterial activity assays were carried out by disc diffusion method [12] with a minor modification. Two concentration of essential oil prepared by dissolving 5 μl/ml and 10 μl/ml in DMSO. Overnight bacterial suspension (100μl) adjusted to contain $1x10^6$ CFU/ml of bacteria, spread by a sterile glass rod on Nutrient Agar (NA) medium. The filter paper (Whatman No.1) discs (5 mm in diameter) were impregnated with 4 μl of the prepared essential oil concentration and put in the inoculated plates. The inoculated plates were incubated at 27±2°C for 24 h and then the inhibition zones were measured in diameter (mm) around of the discs. Antibiotic discs containing 1 μg of Cifroflaxin CF^1 (Himedia, India) was used as positive control and DMSO used as negative control. The assays were performed with three replicates.

Minimal inhibitory concentration (MIC): The minimal inhibitory concentration (MIC) of the essential oil was determined by a broth dilution technique [13] with some modification. The essential oil diluted to give with five different concentrations (25, 50, 100, 150 and 200 μl/ml) in nutrient broth. Using a standard micropipette, 0.05 ml of the 18 h old bacterial broth (10^6 CFU/ml) culture was introduced into each of the test tube with different concentration of essential oil. A set of tubes containing only growth medium plus each of the test bacteria was set up separately to serve as control. All the tubes were incubated at 27±2°C for 30 h. The minimum inhibitory concentration was taken as the lowest concentration of essential oil that will prevent growth of the bacterial strains. The same test was repeated with the antibiotic, cifroflaxin as positive control.

RESULTS AND DISCUSSION

Essential oil yield from fresh bark sample was 0.4%. The oil was analyzed by GC and GC-MS and the qualitative and quantitative composition are presented in Table 1. Seventeen compounds were identified in the essential oil which represent 99% of the oil respectively. The major compounds identified as dodecanal (53.43%), undecane (20.84%) hexadecanal (9.95%), dodecanoic acid (3.62%) and 1-tridecanol (2.15%). The essential oil was evaluated for antimicrobial activity against Gram-positive and Gram-negative bacterial strains by disc-diffusion method and was found to be active against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli.* However, no activity was show active against *Bacillus subtilis* (Table 2). The inhibition zones varied between 6.3 to 10.6 mm. The MIC values confirmed the activity against the tested microorganisms. The MIC values of oil ranged from 50 to 150 μl/ml (Table 3). Antibacterial activity was reported earlier in essential oil in plants belongs to other species of Pittosporum [14]. The antimicrobial activity of dodecanoic acid against bacteria including *Pseudomonas aureginosa*, *Staphylococcus aureus* has been well established [15].

Table 1: Constituents of the essential oil from bark of *Pittosporum dasycaulon*

Compound	Retention Time	Retention Indices	Percent in sample (%)
Nonane	6.776	916	0.64
Undecane	13.190	1115	20.84
Decanal	16.824	1204	0.43
Decane 2,4,6-trimethyl-	19.965	1121	0.43
Cyclopentadecanol	20.263	1987	0.62
1-undecanol	22.454	1357	0.92
Dodecanal	23.725	1402	53.43
1-tridecanol	25.607	1556	2.15
Methoxyacetic acid,10-undecenyl ester	26.706	1646	0.38
Dodecanoic acid	28.617	1570	3.62
Hexadecanl	29.733	1800	9.95
Rhodopin	44.244	4025	0.28
Heptadecane,0-octyl-	46.378	2442	0.70
Pyrrolidine,1-(1,6-dioxo octadecyl)-tetracontane	50.406	4395	1.32
1,2-benzenedicarboxylic acid,mono(2ethylhexyl)ester	51.533	2162	1.73
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	52.61		1.064

Table 2: Antimicrobial activity of essential oil from bark of *Pittosporum dasycaulon*

Inhibition zones in mm[a]				
Test organisms	5µl/ml	10µl/ml	Negative control	Control Ciproflaxin (1µg/disc)
Staphylococcus aureus (MTCC 3160)	6.3	8.3	-[b]	25
Bacillus cereus (MTCC 1306)	8.6	8.6	-	28
Bacillus subtilis (MTCC 1133)	-[b]	-[b]	-	30
Escherichia coli (MTCC 1089)	7.3	10.6	-	20
Pseudomonas aeruginosa (MTCC 1034)	6.6	8.3	-	35

Experiments were carried out in triplicate

[b] No activity

Table 3: Minimal inhibitory concentration of essential oil of *Pittosporum dasycaulon*

Test organisms	MIC (µl/ml)	Control (µg/ml)
Staphylococcus aureus (MTCC 3160)	150	10
Bacillus cereus (MTCC 1306)	150	10
Bacillus subtilis (MTCC 1133)	NT[a]	NT[a]
Escherichia coli (MTCC 1089)	50	5
Pseudomonas aeruginosa (MTCC 1034)	100	10

Experiments were carried out in triplicate

[a]NT Not tested.

Also the interaction between other major and minor components could have contributed to the increased antimicrobial activity. Our results firmly support the popular use of bark of *P. dasycaulon* in the folk medicine as antimicrobial agent. Therefore, the essential oil from this species is potential candidate to be used as antimicrobial agent.

CONCLUSION

It was that the major components in the bark of P. dasycaulon were dodecanal (53.43%), undecane (20.84%) hexadecanal (9.95%), dodecanoic acid (3.62%) and 1-tridecanol (2.15%). Essential oil of *P. dasycaulon* bark demonstrated a strong activity against both gram positive and gram positive bacteria. This investigation can be used essential oil in medicine and sources of antibacterial substances for possible treatment of many disease including bacteria and fungal infections.

REFERENCES

1. Buchbauer, G., 2000. The detailed analysis of essential oils leads to the understanding of their properties. Perfumer and Flavorist., 25: 64-67.

2. Deans, S.G. and G. Ritchie, 1987. Antibacterial properties of plant essential oils. Intl. J. Food Microbiol., 5: 165-180.
3. Sharma, B.D., N.P. Balakrishnan, R.R. Rao and P.K. Hajra, 1993. Flora of India. Vol.1 Calcutta: Botanical Survey of India.
4. Gurib-Fakim, A. And F.E. Demarne, 1994. Constitutents of the essential of the leaves of *Pittosporum balfourii* growing in rodrigues. Planta Medica, 60(5): 584-585.
5. Weston, R.J., 2004a. Composition of essential oil from the leaves of seven New Zealand species of *Pittosporum* (Pittosporaceae) J. Essential Oil Res., 16(5): 453-458.
6. Weston, R.J., 2004b. Aromatic componenets of the leaves of the New Zealand lemonwood tree *Pittosporum eugeniodes*. Z. Natforsch., 59c: 32-34.
7. Yoganarasimhan, S.N., 1996. Medicinal plants of India, Vol. I-Karnataka. Interline Publishing Pvt., Ltd., Bangalore, India.
8. Khare, C.P., 2007. Indian Medicinal Plants, An illustrated Dictionary. Springer-Verlag, Berlin, pp: 496.
9. Colenso, W., 1869. On the botany, geographic and oeconomic of the North Island of the New Zealand group. Trans. New Zealand Inst., 1: 36-37.
10. Jorge, R.M., B.C. Lurdes, C.M. Susana, B.D. Laurence and G.L. Norman, 2003. Composition and antimicrobial activity of the essential oils from invasive species of the Azoes, *Hedychium gardenirium* and *Pittosporum undulatum*. Phytochemistry, 64(2): 561-565.
11. Ramamurthy, T., 1976. Pittosporaceae. In: Flora of Hassan District. Saldahna and Nicholson (Ed.,) Amerind Publishing Company, New Delhi, India.
12. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolke, 1995. Manual of Clinical Microbiology 6th Ed. ASM, Washington, DC.
13. Atta-ur-Rahman, Choudhary, M.I. and W.J. Thomsen, 2005. Bioassay Techniques for Drug Development. Harwood Academic Publishers, Australia.
14. Anil, J.J., G. Varghese, S.R. Nediyaparanbu and G.S. Mathur, 2008. Composition and antibacterial activity of the leaf and fruit oils in *Pittosporum neelgherrensis* Wight et Arn. J. Essent. Oil. Res., 20(4): 380-382.
15. Babic, C., Nguyen-the, M.J. Amiot and I.S. Aubert, 2008. Antimicrobial activity of shredded carrot extracts on food-borne bacteria and yeast. J. Appl. Microbiol., 76(2): 135-141.