CHAPTER 9

PUBLICATION & PRESENTATIONS

2. Poster paper presentation on “Phytochemical Screening and Antimicrobial Activity of Leaves Extracts from *Pandanus tectorius Soland ex Parkinson*” at 64th Indian Pharmaceutical congress held at Chennai during 7-9 December 2012.


Studies on Antimicrobial Activity of the Essential Oil Extracted from *Melaleuca leucadendron* (Linn.) Leaves

Dehghan M H G, Abubakar Salam Bawazir

**Abstract:** *Melaleuca leucadendron* (Linn.), (Myrtaceae) endemic to western part of Maharashtra, were collected from Mumbai. The essential oil (cajeput oil) was hydrodistilled by using Clevenger apparatus. Cajeput oil has been historically reported for its antiseptic and anti-inflammatory actions. The antimicrobial activity of the essential oil was evaluated using Well-agar plate diffusion method and the minimum inhibitory concentration (MIC) was determined. The essential oil was observed to be effective against both gram-positive and gram-negative bacteria; the essential oil is most effective against *Bacillus subtilis* and *Staphylococcus aureus* whereas the MIC for *Pseudomonas aeruginosa* and *Escherichia coli* was observed to be 2% and 10% respectively. The result suggests that the essential oil of this variety of *Melaleuca leucadendron* possesses constituents with antibacterial properties and thus has a potential for use as an antibiotic agent.

**Key Words:** *Melaleuca leucadendron*, Essential oil, Antimicrobial activity.

**INTRODUCTION**

The family Myrtaceae comprises of about 140 genera and more than 3000 species, mostly shrubs or trees with schizogenous secretory cavities containing aromatic volatile oils, some of which are used for their therapeutic action such as oil of eucalyptus. *Melaleuca leucadendron* (Linn.) locally named as kayaput also belongs to this family. The flowers of the plant are creamy white in colour. Leaves are oblong-elliptic, acuminate, subacute, nerves vertical, 5-7 glabrous (except when young) 3.8-12.5 cm long and 5-25mm wide (largest on young shoots). The oil obtained from *melaleuca* species was traditionally applied topically as an insect repellant, scabicide & for traumatic pain. The oil was prescribed in chronic pityriasis, psoriasis, eczema & acne. Various types of oils from *melaleuca* species have industrial applications as antiseptic to be used as antimicrobial agents & use as sources of therapeutic agent. The antimicrobial properties and essential oil composition of *H. alternifolia* have been investigated extensively. The chemical composition of *H. quinquervia* has also been studied before. However, the antimicrobial properties of the essential oil of *H. leucadendron* have been relatively unexplored until recently. The essential oil is light yellowish in color and clear liquid having characteristic odor. The specific gravity of oil is 0.915-0.928 at room temperature with percentage yield of 1% v/w, soluble in methanol and ether, the essential oil is light sensitive.

The taxonomical hierarchy as shown in Table 1, shows the point of deviation of plants belonging to the same family Myrtaceae at species level.

**MATERIALS AND METHODS**

Fresh leaves of *Melaleuca leucadendron* were collected and authenticated from Botany Department of Dr Babasaheb Ambedkar Marathwada University, Aurangabad with accession number 7715 in herbarium of the University and then cut into small pieces. The prepared plant material was immediately hydro-distilled by Clevenger apparatus for 3 hours in successive batches. After cooling, the oil yield was recorded for every batch and the essential oil was collected and purified. The yield of essential oil obtained from hydrodistillation of fresh leaves was 1.0% (v/w).

**Screening of Antimicrobial Potential**

The essential oil was screened for its antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus* and *Candida albicans*. The antimicrobial activity of the essential oil of the plant was determined by the Well-agar plate diffusion method. 20ml of sterile nutrient agar (NA), potato dextrose agar (PDA) and Malt Glucose Yeast and Peptone agar (MGYP) were aseptically transferred to sterilized Petri dishes 9cm in diameter each at 45°C 82°C. Followed by inoculation of bacteria and fungal strains (0.5ml). The plates were held for 15-30 minutes at room temperature 25°C 42°C. The bore where prepared in the petri dishes using boron and sample were added in the bore using micropette. Then the bacterial culture plates were incubated at 37°C for 24 hours and the fungal culture plates were incubated at 22°C for 48 hours and the diameters of the inhibition zones formed on the NA, PDA and MGYP were evaluated in millimeters.

**Estimation of Minimum Inhibitory Concentration (MIC)**

The standardized 0.5% polysorbate-80 (sterile) in distilled water was used for the estimation of MIC. The different concentrations of the essential oil were prepared by using two fold serial dilution techniques. The pure essential oil was added to the polysorbate-80 to form the solutions of different concentrations. The bacterial and fungal cultures were prepared in triplicate and all were incubated. After incubation the MIC was estimated, the mean MIC and standard deviation calculated.

**RESULT & DISCUSSION**

The essential oil from *Melaleuca leucadendron* was observed to be effective against both gram-positive and gram-negative bacteria. As evident from Table 2, the essential oil possessed a good antibacterial activity against *Staphylococcus aureus*, *Salmomella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli* but was not effective as an anti-fungal agent against *Candida albicans*. These results are in variance with the findings of Puangnoi Loihakhongsom and Watcharin Rangjaitrorn, who reported good antifungal activity for the essential oil obtained from *Melaleuca leucadendron* var minor Duthie.

The essential oils from the species of *Melaleuca* are an important source of phytomedicinal products, such as cineole, terpinol and its acetate and sesquiterpenes. From the MIC (Table3) it is evident that the essential oil is most effective against *Bacillus subtilis* and *Staphylococcus aureus* whereas the MIC for *Pseudomonas aeruginosa* and *Escherichia coli* is 5% and 10% respectively.

**CONCLUSION**

The result suggests that the essential oil of this variety of *Melaleuca leucadendron* possesses constituents with antibacterial properties and thus has a potential for use as an antibiotic agent.

**REFERENCES AND NOTES**

1. Trease and Evans Pharmacognosy; Editor W.C Evans, 13th Edition, Published by New Elsevier Ltd, New Delhi, 2006; 274.
8. V. Satish, V. D Ravichandran, Usha Gavani and Padma Parihar; Antimicrobial studies on the extracts of *Cocos nucifera* Linn and *Hyptis suaveolens* Poit, Indian Journal of Natural Products & Resources 2010; 1: 49-52.

### Chapter 9: Publication & Presentation

#### Table 1: Taxonomical Hierarchy of *Melaleuca Leucadendron*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Myrtales</td>
</tr>
<tr>
<td>Family</td>
<td>Myrtaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Melaleuca</td>
</tr>
<tr>
<td>Species</td>
<td><em>Leucadendron Leucadendron</em></td>
</tr>
</tbody>
</table>

#### Table 2: Antimicrobial activities of essential oil from *Melaleuca leucadendron*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> NCIM-2079</td>
<td>Nutrient Agar</td>
<td>++</td>
</tr>
<tr>
<td><em>Escherichia coli</em> NCIM-2065</td>
<td>Nutrient Agar</td>
<td>++</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> NCIM-2200</td>
<td>Nutrient Agar</td>
<td>++</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC6633</td>
<td>Nutrient Agar</td>
<td>++</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> NCIM-304</td>
<td>Potato dextrose agar</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida albicans</em> NCIM-3471</td>
<td>MGYP Agar</td>
<td>-</td>
</tr>
</tbody>
</table>

**Remark:**
- No activity
- Diameter of clear zone = 05-10 mm (less activity)
- ++ Diameter of clear zone = 11-20 mm
- +++ Diameter of clear zone = 21-30 mm

#### Table 3: Minimum Inhibitory concentration (MIC) of the essential oil of *Melaleuca leucadendron*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> NCIM-2065</td>
<td>10 %w/v + 5.52</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> NCIM-2200</td>
<td>5 %w/v ± 3.33</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> NCIM-2079</td>
<td>125 ppm ± 8.74</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC6633</td>
<td>100 ppm ± 5.42</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> NCIM-304</td>
<td>No activity</td>
</tr>
<tr>
<td><em>Candida albicans</em> NCIM-3471</td>
<td>No activity</td>
</tr>
<tr>
<td>Control diluent</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Scientific Abstracts
Pharmacognosy, Indigenous drugs, Herbal formulations and Phytochemistry

Phytochemical screening and antimicrobial activity of leaves extracts from Pandanus tectorius Soland ex Parkinson
M. H. G. Dehghani; Bawzzir Abubakar Salam
Maulana Azad Educational Trust’s Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Dr. Rafiq Zakaria Marg, Rauza Baugh, P O Box – 33, Aurangabad-431001, Maharashtra, India.

The study on phytochemicals and antimicrobial activity of the extracts of Pandanus tectorius found in the region of Marathwada is reported. The dried powdered leaves were extracted successively with petroleum ether (60-80%), ethyl acetate and methanol. Also, the dried powdered leaves were directly extracted with methanol to get total methanol extract.

Materials and methods: The extracts were screened against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus flavus & Candida albicans. The antimicrobial activities of the extracts of the plant were assayed by diffusion method and minimum inhibitory concentration (MIC) was determined. Phytochemical screening was done using Thin Layer Chromatography (TLC), Column Chromatography (CC), High Pressure Thin Layer Chromatography (HPTLC) and Gas Chromatography with High Resolution Mass spectrometer (GC-HRMS).

The extracts, especially petroleum ether and total methanol, were very effective against Gram-positive, Gram-negative bacteria such as Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, and fungi like Aspergillus flavus but not much effective against Candida albicans.

The Phytochemical screening confirmed the presence of squalene, stigmastanol, β-sitosterol, tirculan triterpene and lupenone which are compared with available reference standards (Sigma-Aldrich). These soft extracts may further be studied for their use as potential ingredients for topical herbal antimicrobial formulations.
Chapter 9 Publication & Presentation

64th INDIAN PHARMACEUTICAL CONGRESS

Certificate

This is to certify that Prof./Dr./Mr./Ms. Bawazir Abubakar Salam has presented a paper entitled Phytochemical Screening and Antimicrobial Activity of Leaves Extracts from Pandanus Tectorius Soland ex Parkinson in the Scientific Poster Session of 64th IPC held at Chennai, SRM University, Kattankulathur during 7th – 9th December 2012.

Dr. N. Udupa
Convenor, Scientific Services, IPA

Prof. K. Chinnasamy
President, IPA

Shri. S.V. Veeramani
Chairman, LOC

Dr. T. K. Ravi
Chairman, Scientific Services - LOC

ORGANIZED BY:
Indian Pharmaceutical Congress Association

HOST:
Association of Pharmaceutical Teachers of India (APTI)
Acceptance of Abstract for presentation in the ISE 2012, Kolkata

Pulok Mukherjee
To Me
Nov 24, 2011

This message contains blocked images.

Presenting Author: Abubakar Salam Bawazir

Dear Sir/Madam

Greetings from Kolkata, India!

I am happy to let you know that the organizing committee has accepted the following paper for presentation in the 12th congress of International Society for Ethnopharmacology [ISE] to be held at Science City, Kolkata, India during February 17-19, 2012.

Abstract number: SNPSJU0105

Title of the Paper: ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF EXTRACTS FROM PANDANUS TECTORIUS PARKINSON

Presentation: Oral

Please complete your registration and accommodation [if required] by November 30, 2011 positively with due payment if you have not done it, failing which your presentation will be cancelled and it will not be printed in the conference document.

It will be our pleasure to get you here at Kolkata during this event and exchange your views on globalization of traditional medicine.

With my best personal regards

Pulok K. Mukherjee, PhD, FRSC

Organizing Secretary
ISE 2012

Abstract Submission and Registration Log on: www.ise-snpsju.org

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naturalproductm@gmail.com

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“Traditional Medicines and Globalization – The Future of Ancient Systems of Medicine”

12th International Congress of Ethnopharmacology
February 17-19, 2012

Organized by
School of Natural Product Studies
Jadavpur University, Kolkata, India

Certificate

It is our great pleasure in certifying that
Mr./Ms./Prof./Dr. Abubakar Salam Bawariz
presented a research paper in the Scientific Oral / Short Lecture session of the 12th International Congress of Ethnopharmacology (ISE 2012) held at Science City, Kolkata during February 17-19, 2012.

Prof. S. Datta
Chairman
Dr. Marco Leoniti
President, ISE
Dr. Pulok K. Mukherjee
Organizing Secretary
May 12, 2011

setcpd dharwad

To Me

May 14, 2011

Dear Sir/Madam

Greetings from Dharwad.

I have received your abstract. Abstract has been reviewed and accepted for the oral presentation. The presentation should be done in ppt containing all important aspects about the topic. Time given is 13 min followed by 2 min discussion. Kindly register for the seminar. (If you have already registered please ignore).

See you during seminar.

Thanking you with kind regards

Dr A R Kulkarni
Chairman Scientific Committee
National Seminar on “Recent Trends in Drug Development & Drug Delivery of Phytopharmaceuticals”
SET’s College of Pharmacy
Dharwad
Chapter 9 Publication & Presentation

AICTE & ICMR Sponsored National Seminar on
Recent Trends in Drug Development & Drug Delivery of Phytopharmaceuticals
3rd & 4th June 2011
Organized by
Soniya Education Trust’s College of Pharmacy
J. R. Nagar, Dharwad - 580 002, Karnataka, India.

CERTIFICATE

Dr./Prof./Mr./Mrs./Ms./ ABUBAKAR secured 2nd Prize
participated as a Delegate / Presented Paper (Oral / Poster) in the National Seminar on RTDDD, 3rd & 4th June 2011, held at Soniya Education Trust’s College of Pharmacy, Dharwad, Karnataka.

Dr. V. H. Kulkarni Dr. M. S. Srinath
Convener Chairman
Organizing Secretary
Dr. P. V. Habbu
Abubakar Bawazir ISP 2011

Srinivas Patnala

To Me'Secretary ISP BGM'harchavh@rediffmail.com and 1 More...

Dec 20, 2010

Dear Mr Bawazir

Your abstract has been accepted for "Poster presentation" at the Scientific sessions of the First International Convention and 15th Annual Conference of the Society of Pharmacognosy to be held at Belgaum from 18-20 Feb 2011. To attend and present this abstract at the conference, please register at the earliest or before the due date noted.

Looking forward to seeing you at the conference

Regards

Scientific Convener

Phytochemical screening using GC-MS and Antimicrobial activity of Essential oil obtain from Melaleuca leucadendron Linn

Dr. M. H. G. Dehghan., Bawazir Abubakar Salam

Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria campus, Rauza Bagh,

P. O. Box – 33, Aurangabad-431001, India.
SCIENTIFIC PROCEEDINGS CERTIFICATE

This is to certify that

Prof./Dr./Mr./Ms./Mrs. Abubakar Bawazir

has participated and presented his/her research findings at the Oral/Poster session of the scientific proceedings during the 15th Annual Conference & First International Convention of Society of Pharmacognosy held at KLE University’s College of Pharmacy, Belgaum, Karnataka, India, from 18th to 20th February 2011.

Theme: Global Challenges & Opportunities in Herbal Medicines

Prof. Srinivas Patnala
Scientific Convener

Prof. F. V. Manvi
Chairman LOC

Prof. S. S. Jalalpura
Organizing Secretary LOC

Jointly organized by: KLE University’s College of Pharmacy and Regional Medical Research Centre, ICMR Belgaum