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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *KANDELIA CANDEL*

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ABSTRACT

Plant essential oils are well known for their numerous applications. An attempt was made to extract the essential oil from the mangrove plant *Kandelia candel* collected from the local costal area of Sidhudurg district, Maharashtra. The antimicrobial potential of the extracted oil was investigated using agar cup method against clinical isolates and results were found to be very encouraging. Chemical characterization of this oil was carried out using GC-MS.

KEYWORDS: Mangroves, *K. candel*, bioassay, Gas chromatography, Mass Spectrometry.

INTRODUCTION

Mangroves represent an intertidal wetland ecosystem formed by a unique association of animals and plants in the coastal areas and river estuaries throughout the low lying tropical and sub-tropical latitudes.[1] The ecological, economical and medicinal importance of flora and fauna from mangroves is well recognised and continue to being explored.

India has a rich diversity of mangroves with thirty nine species of mangrove plants.[2] *Kandelia candel* is an important member of Indian mangroves and is distributed along both the east and west coast of India. However, work on this plant has been mainly carried out by the researchers of China and Hong Kong who have reported antimicrobial and antifungal activities of its extracts.[3,4] A perusal of literature indicates that there is dearth of information

on the antimicrobial potential of the essential oils extracted from this plant. In view of this, the present investigation was undertaken.

MATERIALS AND METHODS

Collection of the sample

Mangrove plant *K. candel* was collected from local coastal area of Devgad, Konkan, Maharashtra by hand picking. The collected plant was identified by an expert taxonomic and then separated into individual parts such as stem and leaves, followed by a thorough washing under the running tap water in the laboratory order to remove dirt, germs and other contaminants from the sample.

Extraction of essential oil

Air-dried aerial parts of *K. candel* were subjected to hydro-distillation using a Clevenger – type glass apparatus for about seven hours to obtain a colourless volatile oil. The oil was dried over anhydrous sodium sulphate to remove traces of moisture followed by exhaustive extraction of the distillate with diethyl ether to obtain a yellow coloured mixture. After removal of the solvent, the oil yields were calculated on a dry weight basis of the plant material. The oil was kept in sterile sample tubes in refrigerator at a temperature of 4°C covered with aluminum foil to protect it from light until further used.

Antimicrobial activity of the essential oil

Antimicrobial activity of the extracted essential oil was evaluated using standard agar cup method against ten test organisms. Antimicrobial activity was recorded as the width of the clear zone of inhibition (in mm) surrounding the agar well. The experiments were repeated three times and the mean was used in the analysis.

Chemical characterization of the extracted oil

The analysis of the chemical composition of the essential oils was made using gas chromatography - mass spectrometry (GC-MS) with capillary column of fused silica Column and Helium as a carrier gas.

The retention index (RI) of the substances was obtained by co-injection of the essential oil with a standard mixture of hydrocarbons. The identification of the chemical constituents was effected by means of a comparative analysis of the mass spectrum of the substances with

those of the database of the system GC-MS (Nist 62.lib) and the literature of retention indices.

RESULTS

Clevenger type hydro-distillation apparatus successfully extracted essential oil from the aerial parts of *K. candel*. The oil after purification with anhydrous sodium sulphate and diethyl ether produced a yield of 0.29% crude oil which was yellowish in colour and with a characteristic odour.

Antimicrobial activity of the extracted oil

The essential oil of *K. candel* showed promising antimicrobial activity against all the test pathogens. Highest activity was observed against *Candida albicans* followed by that against *Salmonella typhii*. Least inhibition was observed of *Bacillus subtilis* (Table 1).

Table 2: Antimicrobial activities of crude essential oil of *K. candel* against pathogens.

Test pathogens	Zone of inhibition by the essential oil of *K. candel*
Escherichia coli	11 mm
Pseudomonas aeruginosa	9 mm
Staphylococcus aureus	15 mm
Shigella flexineri	10 mm
Aspergillus fischerii	13 mm
Salmonella typhii	18 mm
Vibrio cholerae	13 mm
Klebsiella pneumoniae	9 mm
Candida albicans	19 mm
Bacillus subtilis	8 mm

Chemical characterization of the extracted oil

The extracted oil when analyzed using GC-MS showed presence of many major and minor peaks (Fig. 1) Some major compound present in the oil of *K. candel* were identified by comparing their retention indices with the library references and are shown in the Table 2.

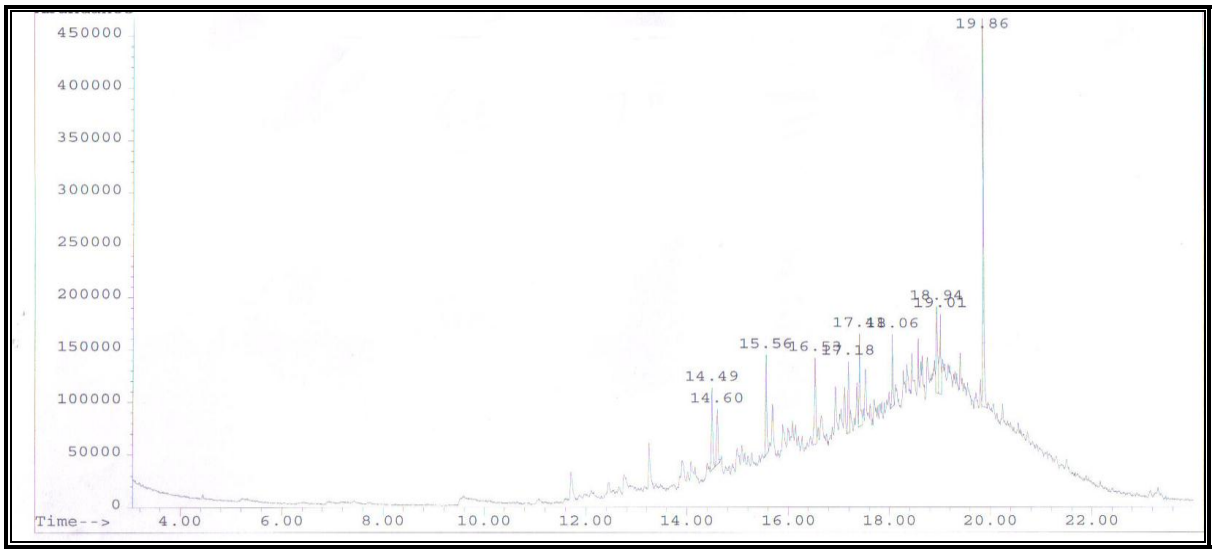

Figure 1: GC-MS spectrum of the extracted essential oil from *K. candel.*

Table 2: Major compounds identified in the essential oil of *K. candel* by GC-MS.

GCMS Retention time	Compounds	Total Area %
13.25	Hexadecane, Tridecane, Heptadecane	5.53
14.49	Heptadecane, Nonadecane, Heneicosane	7.97
14.59	Dodecane, 4,6-dimethyl-Octane, 2,6-dimethyl-Nonane	5.90
15.56	Octadecane, Hexatriacontane, Heneicosane	8.02
16.71	Dibutyl Phthalate, 1,2-Benzenedicarboxylic acid, buty	52.32
17.19	Naphthalene, Dibutyl-9H-Carbazole, 3-methoxy-1h-pyrazole, 3,4-bis(trimethylsily)	5.70
17.40	Eicosane, Heneicosane, Docosane	7.36
18.91	Mercaptoacetic acid, Bis(trimethyl Ethanedioic acid, Bis (trimethylsil 1,2-Benzenedicarboxylic acid	12.22
18.94	Phenol, 3-amino-2,3-dehydro-1,8-cineole Silane, tetra-2-prophenyl	9.19
19.00	Nonadecane, Heneicosane, Octadecane	7.28
19.85	Bis)2-ethylhexyl) phthalate, 1,2-Benzenedicarboxylic acid, diis 1,2-Benzenedicarboxylic acid,buty	25.53
19.88	Bis (2-ethylhexyl) phthalate, 1,2-Benzenedicarboxylic acid, diiso	11.69
21.10	Mercaptoacetic acid, Bis(trimethyl Ethanedioic acid, Bis (trimethylsil Tris (trimethylsilyl) borate	8.51

DISCUSSION

The present study indicates that Clevenger type hydro-distillation apparatus can be successfully employed for extracting essential oil from mangrove plant *K. candel*. In future, this apparatus can be used for extracting essential oils from other mangrove plants found along the local coastal area. An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. They are also known as volatile or ethereal oils. An

oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oils from plants have been used for thousands of years[5] in food preservation, pharmaceuticals, alternative medicine and natural therapies[6] Essential oils are reported to be potential sources of novel antimicrobial compounds especially against bacterial pathogens.[7]

The essential oil showed promising activity against gram positive as well as gram negative bacteria, against yeast like *C. albicans* and against fungi indicating its broad spectrum activity. An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable.[8] Extensive leakage from bacterial cells or the exit of critical molecules and ions lead to death.[9] So it is suggested that essential oils from other mangrove species should also be explored.

The antibacterial activity is attributed to the presence of some active constituents in the oils. GC-MS analysis of the isolated oil recorded many major as well as minor peaks confirming presence of many compounds. Among them few peaks were identified using available standards as Benzenedicarboxylic acid, Cyclohexanone, Dibutyl Phthalate, Heneicosane and Mercaptoacetic acid. Studies are ongoing to separate individual compounds in the purified form to know exact bioactive principle present in it and to obtain a potent antimicrobial drug.

There are very few reports on the isolation of essential oil from the mangrove species. Brophy *et al.*, (1993) extracted essential oil from *Osbornia octodonta* but antimicrobial activity of this oil was not studied by the researcher.[10] Shah (2005) isolated crude essential oil from *Aegiceras corniculatum* and carried out GC MS and antimicrobial analysis of this oil.[11] The results for antimicrobial activities in the present study are on the similar lines with the author (though the test organisms are different) but results for GC MS vary significantly indicating presence of different bioactive compounds in the essential oil isolated from different mangrove species.

The volatile oil from the leaves of mangrove plant *Kandelia candel* was extracted by steam distillation by Huang *et al.*, (2005). According to the results by gas chromatography-mass spectrometry, 49 peaks were separated and 35 compounds were identified.[12] The main compounds were 2,6-bis(1,1-dimethylethyl)-4-methyl-phenol, germacrene-D, heptadecane, caryophyllene, nonadecane, pentadecane, and kaur-16-ene. It is noteworthy to mention that a few compounds are exactly the same as found in the oil in the present study but many novel

compounds were also detected in the present study. However, researchers did not investigate the antimicrobial potential of the oil extracted by them. Therefore, the present study is of significant importance.

CONCLUSION

Essential oil extracted from the mangrove plant *Kandelia candel* has a promising antimicrobial activity against clinical isolates especially against *C. albicans* and *S. aureus* which cause skin infections. Since this isolated oil was not found to be skin irritant after its application on skin, it is suggested that it can be used as local antiseptic against skin infections, without any side effects. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral applications.

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