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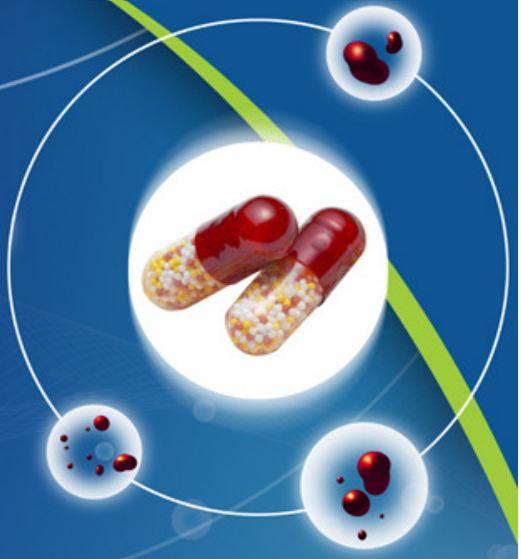

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Research Article

ESSENTIAL OIL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *COLUBRINA ASIATICA* (L.) BRONG

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ABSTRACT

The essential oil obtained from the fresh leaves of *Colubrina asiatica* (L.) Brong. by hydrodistillation was analysed by gas chromatography and gas chromatography-mass spectro metry. The essential oil content quantified showed presence of 10 compounds in which, dodecamethylcyclohexasiloxane has showed the highest (17%) and Dehydro-N-[4,5-methylenedioxy-2-nitrobenzylidene]-tyramine showed the lowest percentage (1.9%).α-cubebene, comprised of 14% .The in vitro antimicrobial activity of the leaf oil was studied by MIC technique. The leaf essential oil was tested against both Gram-positive and Gram-negative bacterial strains. Antibacterial activity was significant against Gram positive bacterium, Staphylococcus aureus.

Key words: *Colubrina asiatica*, essential oil composition, α-cubebene and antibacterial activity.

INTRODUCTION :

Colubrina asiatica Brong. is a glabrous, scandent or sprawling shrubs of Rhamhanaceae family [1]. This species is reported to occur in Tropical America, Southeastern Asia, Malesia, Tropical Australia and Polynesia (to Hawaii), Coastal east Africa, extreme Southeastern India, southern Burma, Andaman and Nicobar Islands, Sumatra, Australia and Hawaii [2-3]. In India, the species is wide spread in littoral scrub forests, tidal forests of Orissa and Ghats of Konkan [4-5]. The plant has some economic value, leaf contains saponin like principle which is used as soap substitute, can be used to prepare bowels, used to wash and whiten textile kilts and garments made from *Cyphlophus heterophvllus* in Samoa [6].

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It can be used for food, medicine, as a fish poison, used as chewstick, tooth cleaners. The fruit is used to produce a soft drink [7]. Perusal of previous literature available revealed that this promising medicinal plant is underutilized hence it was thought worthwhile to evaluating certain bioactivities of the essential oils hydro-distilled from leaves of *C. asiatica* grown in Coastal areas of India, in order to support the possibility of their uses as a natural resource in therapeutics and to demonstrate the correlation between chemical composition and bioactivity. In this respect antimicrobial activities of the oil was evaluated.

Material and Methods

Plant material

Leaves, stem and seeds of *C. asiatica* were collected in December 2011 from coastal area of Goa, India. The debris were later confirmed with taxonomist. The leaves were washed with

water to remove all unwanted plant materials and sand.

Extraction of essential oil

Hydrodistillation of the plant material was performed in a Clevenger-type apparatus for 3h and half. The oil obtained was light yellow, liquid at room temperature and its odor was agreeable. After its isolation, the essential oil was collected and stored in steeled glass vials in refrigerator at 4-5°.

Analysis of the oil:

The samples were analysed by GC-MS (Schimadzu) using capillary column. The GC-MS conditions were as follows; injection volume (1 mL), temperature programme 80°C to 160°C for 5 min at 10°C/min; 160°C to 235°C for 5 min at 5°C/min and 235°C to 290°C for 5 min at 50°C/min.; injector temperature (280°C), MS transfer line (290°C), ion source (200°C) split ratio (1:10) and mass range at 50-450. Data was analysed by compared to a SI (standard index) from the NIST library available.

Determination of minimum inhibitory concentration (MIC)

For the determination of MIC, which represents the concentration that completely inhibits the growth of microorganisms, a micro-dilution broth susceptibility assay was used the emulsification was carried out with a solution of 0.2% agar [8]. The 40, 80, 100, 150, 200, 250 and 300 µl of EO were added to 1960, 1920, 1900 1850, 1800, 1750 and 1700µl of 0.2% agar respectively. Total volume (2ul) of each dilution was added aseptically to 18ml of culture medium. The tubes were sterilized in an autoclave for 20 min at 120 ° C and were stirred by a vortex tube to disperse the EO. Finally seeding is done by the filing of the inoculums containing 10µl of 10^8 germs/ml. The results are seen after 24 hour of incubation at 37°C. Final concentrations obtained of 15, 12.5, 10, 7.5, 5, 4 and 2µl / ml.

Results and Discussion

The hydrodistillation of the dried aerial parts of C. asiatica gave light yellowish oil with yield of 0.6 % (w/w). As shown in Table 1, 10 components were identified in this oil, which presented about 96.9 % of the total composition of the oil. The major constituents of the essential oil were dodecamethylcyclohexasiloxane (17 %) and decamethylcyclopentasiloxane showed the lowest percentage (1.6%).α-cubebene, comprised of 10%. Tables 2 give a summary of the results of the antimicrobial screening of *C. asiatica* oil. Measurements of minimal inhibitory concentration (MIC), indicate that the lowest MIC value (5µl/m) *Staphylococcus aureus* was the second vulnerable microorganism to the EO with MIC (7.5 µl/ml) and zone inhibition (20 mm), *Escherichia coli* was the third vulnerable with MIC (10µl/ml) and zone inhibition (18mm). The oil had considerable activity against Gram-positive bacteria. The oil was more active against Gram-positive bacteria than Gram-negative bacteria; the least activity was against *Escherichia coli.*

These compounds were reported to be in many personal care products such as toiletries. volatile compounds is essential to determine the predominant components and their composition in order to investigate their bioactivity including antioxidant and antibacterial activities. A number of reports have shown that plant volatile compounds exhibited potent antioxidant and antibacterial activities [9]. α-cubebene was reported to show potent antibacterial properties[10] and antioxidant properties[11]. Gram-positive bacteria are more sensitive to plant oils and extracts than Gram-negative bacteria [12]. Our findings suggests feasibility of application of *C. asiatica* oil in treatments of the infections caused by those microorganisms.

Table 1. Composition of essential oil in *C. asiatica* leaves

Peak	Rt	Compound name	%
1.	13.4	Dodecamethylcyclohexasiloxane	**19**
2.	20.1	Tetradecamethyl-cycloheptasiloxane	14.3
3.	16.2	α-Cubebene	14
4.	2.5	2,4-Dimethylhexane	13
5.	5.3	6,6-Methylenebicyclo[3.1.1]heptanes	12
6.	21.9	Cadina-1(10),4-diene	6.2
7.	27.1	Hexadecamethyl-cyclooctasiloxane	4.9
8.	6.6	Octamethylcyclo-tetrasiloxane	4.1
9.	18.1	Isocaryophillene	3.2
10.	6.9	Dehydro-N-[4,5-methylenedioxy-2-nitrobenzylidene]-tyramine	2.3

Table 2: determination of MIC and disc diffusion of the *C. asiatica* essential oil

Organism	Inhibition zone(mm)	15µl/ml	12.5µl/ml	10µl/ml	7.5µl/ml	5µl/ml	4µl/ml	2µl/ml
E.coli	**18**	**-**	**-**	**-**	**±**	**±**	**+**	**+**
S.aureus	**20**	**-**	**-**	**-**	**-**	**±**	**±**	**+**
B.cereus	**54.5**	**-**	**-**	**-**	**-**	**-**	**+**	**+**

- : MIC (minimal inhibition concentration); ± : low to medium growth; + : medium to good growth

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