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RESEARCH ARTICLE

Chemical Composition of Essential Oil from *Cinnamomum Riparium* Gamble and its Antibacterial and Antioxidant Screening

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ABSTRACT

Cinnamomum riparium Gamble (Lauraceae) is a tree growing along the streams in evergreen forests and traditionally used for treating conditions like wounds, fever, intestinal worms, headache, inflammations, menstrual problems etc. and the activity, according to tribals, is mainly due to the volatile oil present in it. But as per published reports much works have not been carried out so far using its volatile oil. Hence it was thought worth to do the research work on this plant so that the claimed properties could be scientifically proved and if possible novel compounds or even effective formulations could be released finally for the use of the public at a low cost. For that the leaves and bark were collected from Cherupuzha, Kannur District and subjected to successive solvent extraction using pet ether, chloroform and ethanol (70%). Maximum yield was obtained for ethanol extract. Then the leaf and bark oils were also extracted using a clavenger apparatus. As the next step, the leaf and bark extracts as well as leaf and bark oils were tried for antioxidant activity using DPPH and H_2O_2 radical scavenging assays. Both of them gave significant results. Then the oils were subjected to GC-MS analysis. The main constituents present in both the leaf and bark oil were methyl eugenol, safrole, linalool, alpha-caryophyllene, elemicin etc. Both the leaf and bark oils were then used for antimicrobial studies against 2 Gram +ve and 2 Gram –ve organisms using agar well diffusion method. Bark oil was found to be more active than leaf oil. Hence it could be concluded that the volatile oil of *Cinnamomum riparium*, a wild growing species of the genus *Cinnamomum*, very widely used by the tribals of Kannur District, is a potent antimicrobial drug. The phytochemical studies (GC-MS) give the possible compounds responsible for the results as it is evident in other reported works too. The antioxidant studies give supportive results to confirm the above said activities.

KEYWORDS

Cinnamomum riparium; antibacterial; antioxidant; GC-MS

INTRODUCTION

Cinnamomum is a large genus consisting of evergreen aromatic trees and shrubs belonging to the Laurel family, Lauraceae. The genus contains over 300 species, distributed in tropical and subtropical regions of America and Asia.[1]

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Cinnamomum riparium Gamble growing along the streams in the Travancore hills, Coorg and in Western Ghats has been traditionally used for the treatment of wounds, fever, intestinal worms, headache, menstrual problems etc.[2] The present study highlights the GC-MS analysis of the volatile oil extracted from the leaves and bark of *Cinnamomum riparium* Gamble and their antibacterial and antioxidant screening.

MATERIALS AND METHODS

Plant Material

The leaves and bark of *Cinnamomum riparium* Gamble (Lauraceae) were collected from the river banks of Cherupuzha, Kannur District, Kerala and was identified by Mr.Biju K., Asst.Professor, (taxonomist), Govt. College, Kasaragod. A voucher specimen was kept (MDCP/CR 012) in the herbarium of the Pharmacognosy Laboratory of Malik Deenar College of Pharmacy, where this work was carried out.

Volatile Oil Extraction

Around 50 g each of fresh leaves and bark were cut into small pieces and taken separately in 1L distillation flask, and 250 ml of water was added to it. The oil was extracted with the help of a Clevenger apparatus.[3]

GC-MS Analysis

GC-MS analysis of the essential oil was performed using Agilent model GC6890 N coupled with a HP 5975 B mass selective detector. HP 5 30m x 0.32mm x 0.25µ capillary column was used with helium as a carrier gas and an injection volume of 1 µl was employed (split ratio of 10:1). During the run the temperature programming was as follows 40^{o}C for 3 min and rise at 5^{o} C / min to 280^{o} C, isotherm for 10 min; Post run 10 min at 300^{o} C. GC-MS operation condition: injector temperature- 220^{o}C; transfer line-240^{o}C; oven temperature programme-: 40^{o}C- 300^{o} C (3^{o} C min-1); carrier gas –He at 1.4mL min-1 Mass spectra: Electron impact (EI +) mode 70ev, ion source temperature 230^{o}C. Retention time and mass spectra were compared with the libraries (MP and NIST) and co injecting with standards. The results are shown in Table No 1 and 2.

Antibacterial Activity

Antibacterial activity of essential oils of leaf and bark was evaluated by agar well diffusion method. Mueller Hinton agar plates were prepared aseptically. The test organisms were spread on the plates and the wells were then punched into agar medium and filled with samples and standard drug (Ciprofloxacin).The plates were incubated for 24 hours at 37^{o}C.[4,5] The results are shown in Table No 3 and Fig No 3 to 6.

Culture Used

Staphylococcus aureus NCIM 5021, *Bacillus subtilis* NCIM 2010, *Escherichia coli* NCIM 2027 and *Pseudomonas aeruginosa* NCIM 5029 were collected from the National Chemical Laboratory, Pune and stored in our Pharmaceutical Biotechnology Laboratory.

Antioxidant Studies

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH)[6]

The free radical scavenging activity of both leaves and bark oil of *Cinnamomum riparium Gamble* was studied by its ability to reduce the DPPH, a stable free radical that can donate an electron or hydrogen to DPPH, which can react with it and thereby bleach the DPPH absorption. DPPH is a purple coloured dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the formula as indicated below:

Percentage of inhibition = [(Ao – A1) / Ao] x 100

Where Ao = Absorbance of the control; A1 = Absorbance of the sample/ standard

The results are given in the Table No 4 and Figure No 7.

Hydrogen peroxide Scavenging Assay[7]

Hydrogen peroxide scavenging assay is based on the principle that the scavengers of hydrogen peroxide can donate electron to H_2O_2, thus neutralizing the H_2O_2 to water. The ability of the sample drug to effectively scavenge hydrogen peroxide was compared with that of

ascorbic acid, the standard. The results are given in Table No.5 and Figure No.8.

Percentage inhibition = [(A control– A test) / A control] x 100

Statistical Analysis

Data obtained were described as mean ± SEM. The probability (p) values were determined by paired t- test using Graph pad software and p > 0.05 were considered as significant.

RESULTS

Yield of the Oils

Based on the material used, the percentage yield of leaf oil and bark oil obtained was 1.3% v/w and 1.5 % v/w respectively.

GC-MS Analysis

GC-MS analysis of both the leaf and bark oil of *C. riparium* was carried out. When the leaf oil showed the presence of twelve major compounds, the bark oil showed eighteen major compounds.

The relative percentage of individual components was obtained from the % peak area from GC-MS data. The mass spectrum obtained after the spectral matching in the stored search libraries are depicted below.

Antibacterial Activity

Antimicrobial screening was carried out with the bark and leaf oils using two gram positive organisms namely *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative organisms namely *Escherichia coli* and *Pseudomonas aeruginosa*. The results showed remarkable inhibition of bacterial growth against the tested organisms. While comparing the activity of the oils, bark oil showed superior activity than the leaf oil.

If the diameter of zone of inhibition is,

17 mm and above; the drug is sensitive to the organisms.

13 – 16 mm; the drug is moderately sensitive.

< 12 mm; the drug is resistant.

Table 1: Compounds identified from the leaf oil by GC MS analysis.

Sl.No.	Compounds	Retention time	% of Total
1	Beta-phellandrene	7.145	0.21
2	Linalool	7.740	0.17
3	Cis 4 decanal	8.600	0.055
4	Methyl chavicol	8.704	0.107
5	Safrole	9.594	34.060
6	Copaene	10.286	0.042
7	Methyl eugenol	10.449	62.737
8	Caryophyllene	10.698	0.803
9	Alpha-humulene	10.956	0.150
10	Myristicin	11.323	0.050
11	Cadinene	11.367	0.070
12	Elemicin	11.454	0.209

Table 2: Compounds identified from the bark oil by GC MS analysis

Sl.No.	Compounds	Retention time	% of Total
1	1-R Alpha pinene	6.163	0.087
2	Beta-pinene	6.636	0.039
3	Cymol	7.081	0.075
4	Cineol/Eucalyptol	7.185	0.174
5	Linalool	7.782	2.791
6	Ocimene	8.025	0.018
7	Terpeneol	8.574	0.079
8	Methyl chavicol	8.714	0.221
9	Safrole	9.714	26.579
10	Copaene	10.309	0.479
11	Methyl eugenol	10.523	52.710
12	Alpha-caryophyllene	11.025	0.381
13	Cadinene	11.417	0.226
14	Elemicin	11.530	0.397
15	Trans nerolidol	11.606	0.116
16	Tetra decanal	11.886	0.228
17	Selinenol	12.230	0.205
18	Beta-Guaiene	12.307	0.304

Table 3: Results showing antimicrobial activity of leaf and bark oils using agar well diffusion method

Sl No.	Organism	Diameter of the zone of inhibition (mm)						
		Standard (µl/ml)	Leaf Oil (µl/ml)			Bark Oil (µl/ml)		
		25	25	50	100	25	50	100
1	*Bacillus subtilis*	23	16	19	21	17	18	20
2	*Staphylococcus aureus*	22	13	15	16	17	18	19
3	*Escherichia coli*	24	15	17	17	16	18	19
4	*Pseudomonas aeruginosa*	22	14	15	18	11	16	19

The results were interpreted as per Kirby Bauer method. Accordingly, the diameter of zone of inhibition and antimicrobial activity of the oils were correlated as follows.

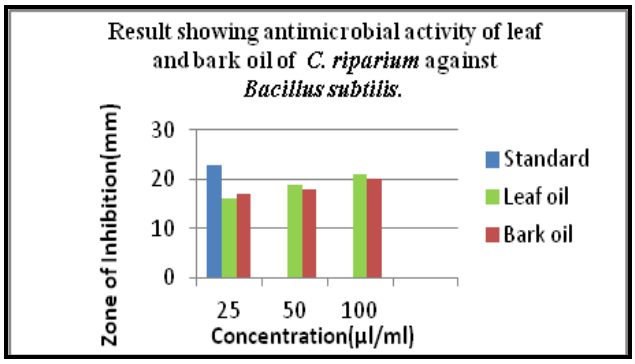

Figure 1: Result showing antimicrobial activity of leaf and bark oil of *C. riparium* against *Bacillus subtilis*

Both the leaf and bark oils were highly sensitive at 25, 50 and 100µl/ml. Also leaf oil (25µl/ml) was found to be moderately sensitive.

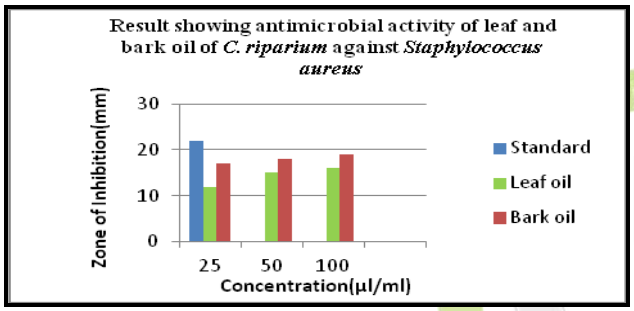

Figure 2: Result showing antimicrobial activity of leaf and bark oil of *C. riparium* against *Staphylococcus aureus*

At all concentrations (25, 50 and 100µl/ml) bark oil was found to be sensitive. Leaf oil at all concentrations (25, 50 and 100µl/ml) were moderately sensitive.

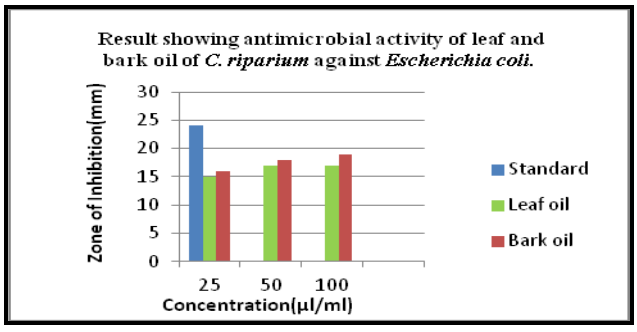

Figure 3: Result showing antimicrobial activity of leaf and bark oil of *C. riparium* against *Escherichia coli*

Both the leaf and bark oil were found sensitive at 50 and 100 µl/ml concentrations. Also the oils at 25µl/ml was moderately sensitive.

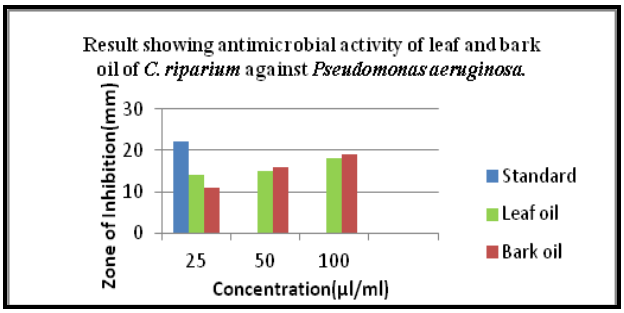

Figure 4: Result showing antimicrobial activity of leaf and bark oil of *C. riparium* against *Pseudomonas auruginosa*

Both the leaf and bark oil at a concentration of 100 µl/ml were found to be sensitive. The leaf oil at a concentration of 25, 50 µl/ml and bark oil at a concentration of 50µl/ml showed moderately sensitive activity. The bark oil at a concentration of 25µl/ml showed resistance to this organism.

Antioxidant Studies

DPPH radical Scavenging Assay

The scavenging activity of leaf and bark oil was performed according to the accepted method given earlier. Accordingly, leaf oil at a concentration of 200µl/ml showed 73.42% of inhibition whereas bark oil in the same concentration exhibited only 68.35 % of inhibition. Again when the IC50 value was calculated the leaf oil required a concentration of 67.5µl/ml where as for the bark oil required 90µl/ml for the same. The standard used in the experiment was ascorbic acid and it required only 52.5µg/ml to produce 50% inhibition. The results are shown in Table no. 4.

Hydrogen Peroxide Scavenging Assay

The absorbance of the reaction mixture for different concentrations of standard, samples and control were recorded in triplicate. At a concentration of 200µg/ml ascorbic acid (standard) showed 80.02% inhibition whereas leaf oil and bark oil at this concentration exhibited 76.45% and 76.45% inhibition respectively. The results are shown in Table No 5. The IC_{50} value of leaf and bark oil was also determined and they were found to be 57.5µl/ml (leaf oil) and 90µl/ml (bark oil).

Table 4: Result showing DPPH radical scavenging activity of leaf and bark oil of *C. riparium*

Sl No.	Sample	Concentration (µl/ml)	Absorbance (nm)	Percentage inhibition (%)
1	Control	-	0.79±0.15	-
2	Standard	50	0.40±0.09	49.37
		100	0.25±0.07	68.25
		150	0.23±0.07	70.94
		200	0.18±0.03	77.78
3	Leaf oil	50	0.47±0.79	40.57
		100	0.26±0.26	67.08
		150	0.25±1.40	68.35
		200	0.21±0.67	73.42
4	Bark oil	50	0.55±1.39	30.38
		100	0.36±1.27	54.43
		150	0.32±1.37	59.49
		200	0.25±1.00	68.35

Values are mean ± SEM; n=3

Table 5: Results Showing Percentage Inhibition of Leaf and Bark oil of *C. riparium* Using Hydrogen Peroxide Scavenging Assay

Sl No.	Sample	Concentration	Absorbance (nm)	Percentage inhibition (%)
1	Control	-	0.98±0.00	-
2	Standard	50 µg/ml	0.47±0.05	55.14
		100 µg/ml	0.29±0.12	69.83
		150 µg/ml	0.23±0.46	76.45
		200 µg/ml	0.19±0.00	80.02
3	Leaf oil	50 µl/ml	0.51±0.00	48.01
		100 µl/ml	0.29±0.00	68.60
		150 µl/ml	0.26±0.00	72.78
		200 (µl/ml)	0.23±0.00	76.45
4	Bark oil	50 µl/ml	0.61±0.00	37.10
		100 µl/ml	0.45±0.00	54.02
		150 µl/ml	0.38±0.00	61.06
		200 µl/ml	0.29±0.00	68.60

DISCUSSION AND CONCLUSION

Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens.[5] Antimicrobial screening was carried out with the leaf and bark oils using two gram positive organisms namely *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative organisms namely *Escherichia coli* and *Pseudomonas aeruginosa*. Both the oils were found to be good antimicrobial agents against the tested organisms and bark oil was more active when compared with the standard, Ciprofloxacin. Also the activity was concentration dependent. In the related species of *Cinnamomum* also antimicrobial activity has been reported.[8,9]

GC-MS analysis of both leaf and bark oil was done for the identification of major phyto constituents and the report disclosed the presence of twelve major constituents in the leaf oil and eighteen constituents in the bark oil. Methyl eugenol was found as the major component in both the oils followed by safrole, linalool, alpha-caryophyllene, elemicin, humulene, pinene etc. Also some of the compounds obtained after GC-MS analysis are reported to have antimicrobial as well as antioxidant properties which supports the present work. For example methyl eugenol, the major component present in both the leaf and bark oils are reported to have anticancer properties in other drugs.[10-13] Also safrole present in both the oils have reported antibacterial activity.[14] Linalool is a compound with reported antimicrobial and antioxidant properties.[15-17] Also, humulene is found to have cytotoxic potential[5] and pinene has antibacterial activity[14] as reported elsewhere.

From the results obtained in the current investigation, it may be concluded that the volatile oil of *Cinnamomum riparium*, one of the wild growing species of the genus *Cinnamomum*, very widely used by the tribes of Kannur District, is a potent antimicrobial drug. Also it is found to have antioxidant effects. Further studies on pharmacological properties of the plant using test animals and cell cultures is warrented for validation of the drug as a future pharmaceutical candidate. Such a study is ongoing.

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