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A STUDY ON PHYSICAL, CHEMICAL, AND ANTIOXIDANT PROPERTIES OF ESSENTIAL OIL SEPARATED FROM FRESH BERRIES OF *CINNAMOMUM RIPARIUM* BY STEAM DISTILLATION TECHNIQUE

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ABSTRACT: *Cinnamomum riparium* Gamble belongs to the family Lauraceae is a red listed species by the International Union for Conservation of Nature (IUCN). It is a tree growing in evergreen forests and traditionally used for treating conditions like wound healing, fever, intestinal worms, headache, inflammations, menstrual problems etc. According to tribal's, this activity 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. Berries were collected from Paniyeli poru, near Periyar river, Ernakulam District, latitude 10.1720° N and longitude 76.5959° E Kerala, India. Berry essential oil was separated by steam distillation technique by using a Clevenger trap apparatus. Separated berry essential oil was used for evaluating the physical and chemical properties including therapeutic activity (antioxidant activity) using DPPH scavenging assays. The berry oil exhibited 76.45% of antioxidant activity. The oil was subjected to GC-MS analysis. The main constituents present in the berry oil were γ -elemene, 1,3-benzodioxol, eugenol and caryophyllene. As it is a red listed plant it become very important to preserve the plant for further studies and for the future generations to make use of its valuable medicinal properties for the humanity. The present study gave a high degree of evidence and encouragements to demonstrate the plant's promising health benefits.

INTRODUCTION: *Cinnamomum* is a large genus consisting of evergreen aromatic trees and shrubs belonging to the Laurel family, Lauraceous. The genus contains over 300 species, distributed in tropical and subtropical regions of America and Asia. *Cinnamomum riparium* Gamble growing along the streams in the Travancore hills, Coorg, Malappuram, Kannur and in Western Ghats has been traditionally used as a medicine.

Phytochemicals are naturally produced chemical compounds and reported to have various biological effects, such as ant mutagenic, ant carcinogenic, antioxidant, antimicrobial, and anti-inflammatory. Antioxidants are the chemical substances that reduce or prevent oxidation and have the ability to counteract the damaging effects of free radicals in tissues, and thus are believed to protect against cancer, heart disease and several other diseases. They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen.¹ The present study was done to explore the antioxidant activity from essential oils from the berries of *Cinnamomum riparium*


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FIG. 1: CINNAMOMUM RIPARIUM LEAVES



FIG. 2: CINNAMOMUM RIPARIUM FRUITS

Plant Description and pharmacognosy:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Habit: Trees up to 7 m tall.

Regional name: Malayalam: Aattuvayana

Leaves: Leaves simple, opposite to subopposite; Petiole to 1 cm long, planoconvex in cross section, glabrous; lamina 18 x 1.5 cm, linear-lanceolate, apex tapered and acute or slightly acuminate, base acute to rounded, glabrous, chartaceous; trinerved basal or suprabasal, laterals not reaching leaf apex; tertiary nerves horizontally percurrent; higher order nerves minutely reticulate.

Inflorescence / Flower: Inflorescence auxiliary or subterminal paniced cymes, few to more flowered; peduncle slender.

Fruit and Seed: Berries are green in color and turns to deep purple on ripening.²

MATERIALS AND METHODS:

Plant Material: Approximately 1kg of fresh berries of *Cinnamomum riparium* Gamble (Lauraceae) collected during March-June 2015 was used for the study. The berries Fig. 3 were collected from the riverbanks of Periyar, Paniyeli poru, Ernakulam District, Kerala and authenticated by Dr. Mathew Dan - senior scientist E1, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Trivandrum, Kerala, India and herbarium voucher specimens (Collection No.83480) was deposited.



FIG. 2: CINNAMOMUM RIPARIUM SEEDS

Volatile oil Extraction: 100grams of fresh berries were crushed and taken in 1L distillation flask, and soaked over night with 500 ml of distilled water and Clevenger trap was connected and distilled by steam distillation. The essential oil and hydrosols were carefully separated till the Plant material was completely exhausted out with traces of essential oils. Calculated the percentage of yield of oil and was filled in amber colored glass container and kept for further studies.³

Determination of Physical and Chemical Properties:

Physical Properties: Determination of color, odor, taste and appearance of berry essential oil was conducted. Microscopic studies of berries including seed were done.

Chemical Properties:

GC-MS Analysis: GC-MS analysis of the essential oil was performed using Acq Method coupled with a HP 5975 B mass selective detector. HP 530m x

0.32mm x 0.25 μ capillary column was used with helium as a carrier gas and an injection volume of 1 μ l was employed (split ratio of 10:1). During the temperature programming was as follows 40 °C for 3 min and rise at 5C / min to 280 °C, isotherm for 10 min; Post run 10 min at 300 °C. GC-MS operation condition: injector temperature- 220 °C; transfer line - 240 °C; oven temperature programmed: 40 °C- 300 °C (3° C min⁻¹); carrier gas -He at 1.4mL min⁻¹ Mass spectra: Electron impact (EI +) mode 70ev, ion source temperature 230°C. Retention time and mass spectra were compared with the libraries (MP and NIST) and co injecting with standards.⁴

Therapeutic Properties:

Antioxidant Studies:

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH): The free radical scavenging activity of berry oil from *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 colored 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 the decrease in absorbance.

3ml of sample kept in dark for 30 min and absorbance measured at 517nm by UV visible spectrophotometer. The blank used was a mixture of methanol and sample. The control was a mixture of DPPH radical solution in methanol. 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^{5, 6, 7}

DPPH radical scavenging capacity (%)

$$= [(A_0 - A_1) / A_1] \times 100$$

A₀ = Absorbance of the sample

A₁ = Absorbance of the blank

RESULTS AND DISCUSSION:

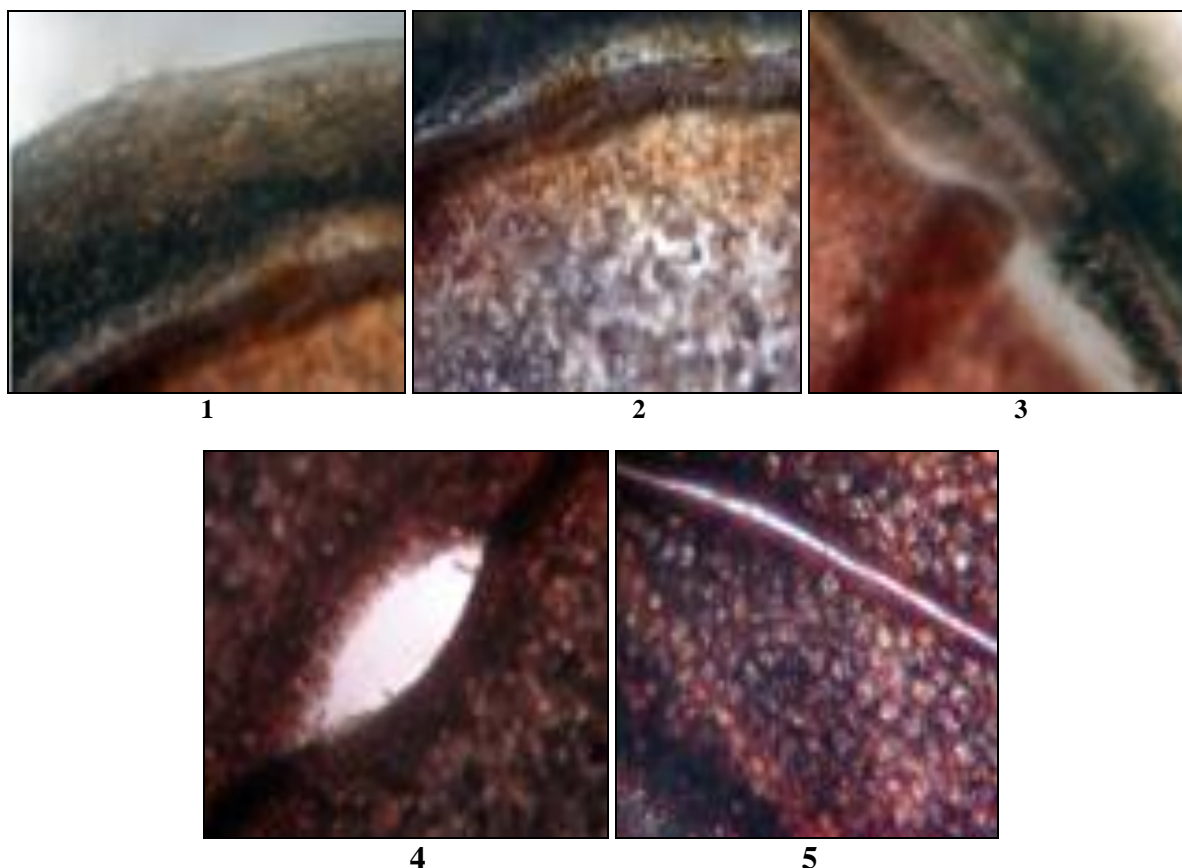


FIG. 4: T.S OF FRESH BERRY OF *CINNAMOMUM RIPARIUM*

Yield of the Oil: The percentage yield of berry oil obtained is 1.2% v/w.

Physical Properties:

Colour: Almost clear

Odour: Spicy, sweet and betel leaf like

Taste: Spicy, pungent and betel leaf like

Appearance: Free flowing, water like

GC-MS Analysis: GC-MS analysis of berry oil of *C. riparium* was carried out. Showed twenty eight major compounds. The relative percentage of individual components was obtained from the % peak area from GC-MS data (Table 1). Graphical representation of major 7 compounds are given in Fig. 5.

TABLE 1: THE RELATIVE PERCENTAGE OF INDIVIDUAL COMPONENTS WAS OBTAINED FROM THE % PEAK AREA FROM GC-MS DATA

S. No	Compounds	Retention time	% of Total
1	Copaene	7.878	0.242
2	Cis- β terpineol	8.137	0.149
3	Caryophyllene	10.576	5.627
4	3-cyclohexen-1-ol	11.423	0.578
5	α -caryophyllene	12.450	0.359
6	1,4,7-cycloundecatriene	12.800	0.041
7	aristolene	12.911	0.026
8	7-methanoazulene1-H- cyclopentane	13.517	0.247
9	Naphthalene	13.622	0.331
10	3-cyclohexane-1- γ -elemene	13.738	0.624
11	γ -elemene	14.002	1.191
12	1,5-cyclodecadiene	15.878	0.044
13	Cyclooctasiloxane	16.122	0.089
14	1,3benzodioxol	17.578	73.107
15	1,14-tetradecanediol	18.342	0.028
16	Caryophyllene oxide	19.468	0.039
17	Veridiflorol	21.482	0.045
18	Benzene	22.101	0.016
19	2-ethyl-5-n-propylphenol	22.315	0.044
20	Spathulenol	22.633	0.034
21	Eugenol	23.322	1.805
22	Hexadecanoic acid	23.860	0.069
23	1,3-benzodioxole	24.315	0.044
24	Silane	24.720	0.133
25	Octadecanoic acid	26.543	0.060
26	Methyl ester	27.234	0.086
27	Phenol	27.393	0.084
28	n-hexadecanoic acid	28.369	0.062

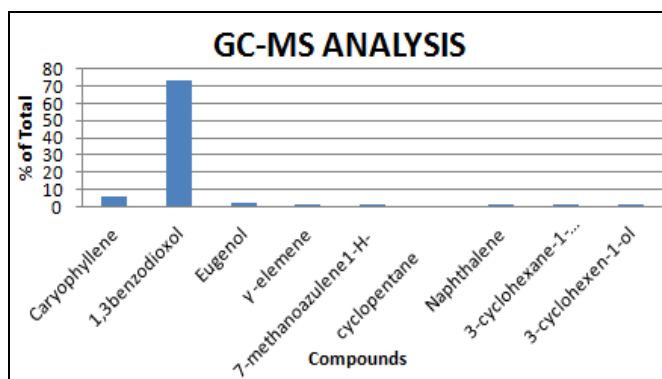


FIG. 5: BAR DIAGRAM OF MAJOR COMPOUNDS OF GC-MS

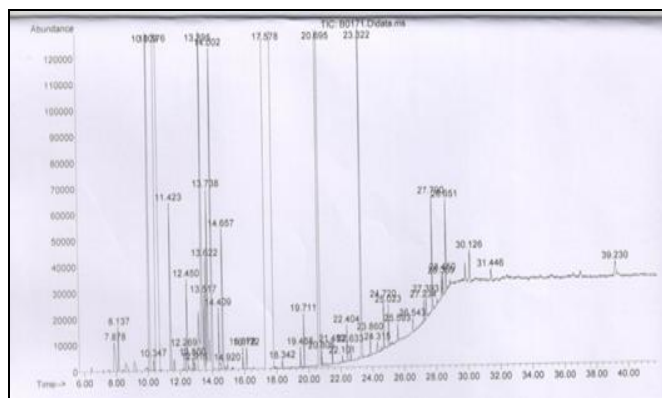


FIG. 6: GC-MS SPECTRA OF ESSENTIAL OIL SEPARATED FROM BERRY OF CINNAMOMUM RIPARIUM

Antioxidant Study: DPPH radical Scavenging Assay: The scavenging activity of berry oil was performed as per the accepted method. The berry oil exhibited 76.45% of antioxidant activity.

CONCLUSION: As the plant is in the red list of IUCN it is our responsibility to protect the plant for the future. The present study revealed its chemical and therapeutic properties. The research should be entertained on this plant in various dimensions. The continuation research work on the same plant is on pipeline with me.

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CONFLICTS OF INTEREST: Nil

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