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Research article

Comparative study of qualitative phytochemical and quantitative GC-MS analysis of *Olea dioica* Roxb. from different forest types of Western Ghats, Karnataka, India

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Abstract: *Olea dioica* is a medicinal angiospemic tree of Western Ghats India. Roots of the plant used for cancer and snake bite treatment in siddha and bark, fruit paste is used in the treatment of rheumatism; decoction of the bark is used to wash old wounds and given in fever. Plant material was collected from different forest types of Western Ghats, Karnataka were air dried subjected for soxhlet extraction and these extracts were subjected for preliminary phytochemical and GC-MS analysis using standard procedures. The results obtained was compared with the different forest types of Western Ghats revealed that the Sagara forest area showed higher yield of crude extract, with higher secondary metabolites compared to the Chakra and Kigga forest area. which may be due to the influence of climatic factors like stress, temperature, rainfall, humidity, wind speed, light intensity, the supply of water, minerals, and CO_2 etc.

Keywords: *Olea dioica* - Western Ghats - Phytochemical analysis - GC-MS - Benzeneethanol - 4-hydroxy.

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INTRODUCTION

Secondary metabolites play a major role in the survival of the plant in its environment. Secondary metabolites synthesis triggered in response to predators and diseases, stress and also in attraction of pollinators (Harborne 1978, Wink 1988).

A wide array of external stimuli are capable of triggering changes in the plant cell which leads to a cascade of reactions resulting in the formation and accumulation of secondary metabolites which helps the plant to overcome the stress. The biotic and abiotic elicitors trigger enhancement of the secondary metabolite production. The stimuli are perceived by receptors, which result in the activation of the secondary messengers. These then transmit the signals into the cell through the signal transduction pathways leading to gene expression and biochemical changes and the synthesis of secondary metabolites (Sudha & Ravishankar 2002).

Many secondary metabolites like glycosides (Wang *et al.* 2010), alkaloids (Christiansen *et al.* 1997, Szabo *et al.* 2003), Flavonoids (Larson 1988, Nogues *et al.* 1998), Phenols (Hernández *et al.* 2006), Terpenoids (Nacif & Mazzafera 2005), Chlorogenic acid is an important intermediate in lignin biosynthesis (Del-Moral 1972) etc., were observed whenever stress was induced in the plant.

Botanical classification of *Olea dioica* Roxb.

Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Oleaceae
Genus:	*Olea*
Species:	*dioica* Roxb.

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Olea dioica Roxb. Is an important ethno-medicinal tree belonging to the family Oleaceae. The tree grows up to 15 m tall. Bark of the tree is brownish, rough; blaze pale brown. Young branchlets are subquadrangular, lenticellate, glabrous. Leaves are simple, opposite, decussate; petiole 0.6–1.3 cm long, canaliculate; lamina 7.5–17.5 cm long & 2.3–7.5 cm wide, elliptic to elliptic-oblong, apex gradually acuminate to subacute, base acute or attenuate, margin distantly serrate (with strong teeth) or entire, coriaceous to subcoriaceous, glabrous; midrib flat above, usually reddish when dry; secondary nerves 8–12 pairs; tertiary and higher order nerves obscure or faintly impressed. Inflorescences are axillary & divaricate panicles; flowers polygamodioecious, cream-white; pedicel 0.4 cm long. Fruit is drupe, ellipsoid, blue when ripe; one-seeded. Roots of the plant have medicinal properties and are used for treatment of cancer and snake bite in siddha medicine. In Maharashtra, the tribes use *Olea dioica* fruits for treatment of skin disease. Bark and fruit paste are used in rheumatism; decoction of the bark is used to wash old wounds and given to counter fever (Pullaiah 2006). Ripe fruits are traditionally used by the tribes in Kerala forest (Yesodharan & Sujana 2007). *Olea dioica* leaf ethanolic extract showed appreciable antibacterial and antifungal activity (Ashwathanarayana & Naika 2015).

Despite of many work on this genus *Olea dioica* Roxb. a very important medicinal plants were unexplored for many pharmacological activities which is traditionally used by the folklore and tribes. Therefore, the aim of the study was to provide data on the expression of secondary metabolites in *Olea dioica* Roxb. collected from different type of forest of Western Ghats, Karnataka with slightly different climatic conditions.

MATERIALS AND METHODS

Study site

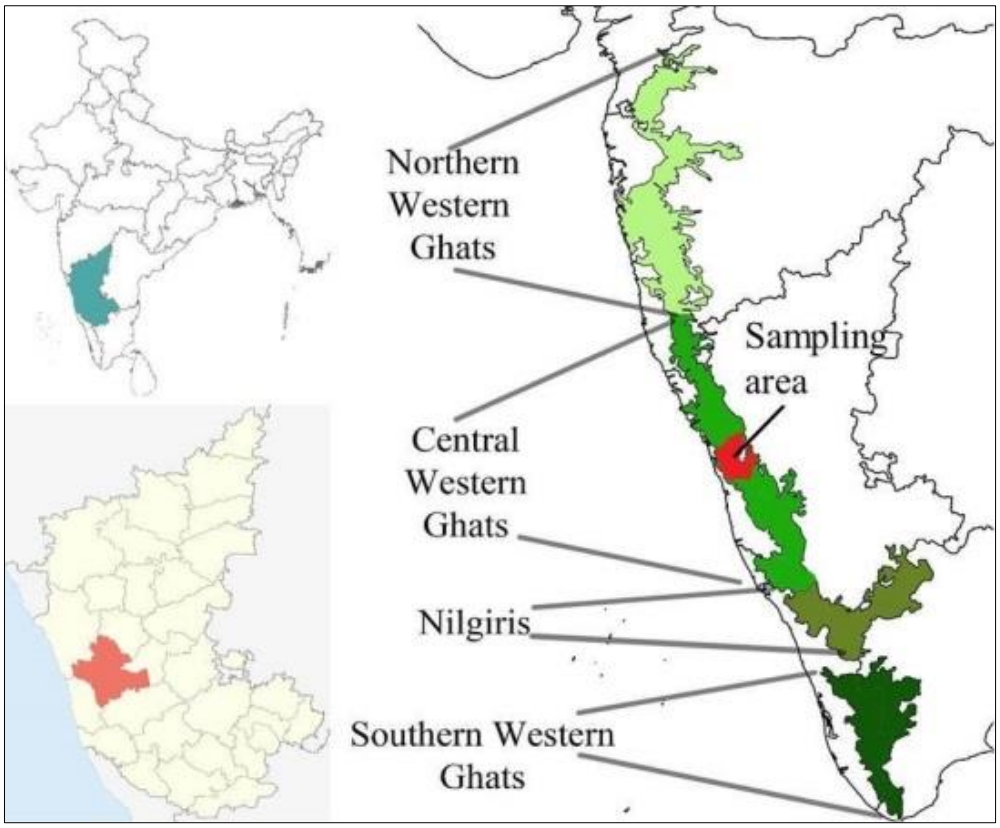

Figure 1. Location of sampling site.

The study site was three different areas with different forest types (Kigga- everngreen, Chakra- semi evergreen, Sagara- moist deciduous) situated in Shimoga and Chikmagalur district within the Western Ghats, Karnataka, India with altitude range 630–840 m (Kigga- N 14° 8'9.4992" E 74° 57'33.0480", Chakra- N 13° 25'5.1636" E 75° 10' 26.1804", Sagara- N 13° 48'28.3212" E 74° 58' 1.3980") (Fig. 1).

Sample collection

The plant samples were collected from Kigga forest, Chakra forest, Sagara forest, Karnataka. The botanical identification of the plant was done by Prof. K G Bhat, Udupi and the voucher specimen was conserved under the reference number KU/AB/RN/AS/001.

Purification and extraction

The plant samples were shade dried for about 30 to 45 days and mechanically powdered. Powdered material was subjected to soxhlet extraction with ethanol, air dried and kept in an air tight bottles.

Qualitative tests on plant material for secondary metabolites

Extracted plant samples were screened (Gartlan *et al.* 1980) for the presence of tannins, alkaloids, saponin, glycosides, flavonoids, steroids/sterols and phenols using the methods described by Harborne (1998).

Alkaloids

Hager's test- test solution was treated with few drops of Hager's reagent (saturated picric acid solution). A positive result for the alkaloids presence was shown by the formation of yellow precipitate.
Mayer's test- test solution was treated with Mayer's reagent cream colour precipitate is formed.

Saponins

Foam test- test solution was mixed with water and shaken well and observed for the formation of foam froth which is stable for 15 minutes for a positive result.

Tannins

Ferric chloride test- Plant material was boiled with water for a few minutes, this was filtered and diluted with more water. Bluish-black colour formation after addition of few drops of ferric chloride, is indicative of the presence of tannins.
Gelatin test- test solution treated with gelatin solution gives white precipitate indicating the presence of tannins.

Flavonoids

Ferric chloride test- test solution when treated with few drops of ferric chloride solution would results in the formation of blackish red colour indicating the presence of flavonoids.
Alkaline reagent test- test solution treated with sodium hydroxide solution shows increase in the intensity of yellow colour which would become colourless by the addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.
Lead acetate test- test solution treated with few drops of lead acetate (10%) results in the formation of yellow precipitate.
Shinda test- test solution and add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid magenta red colour formed.

Steroids / Sterols

Liebermann burchard test- crude extract mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube and then observed for the formation of a brown ring at the junction of two layers. Green colouration of the upper layer and the formation of deep red colour in the lower layer would indicates a positive test for steroids and sterols respectively.

Glycosides

Keller killiani test- Test solution was treated with some drops of glacial acetic acid and ferric chloride solution then mixed. Concentrated sulphuric acid was added and observe for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicates positive test for glycosides.
Bromine water test- test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Phenols

Ferric chloride test- test solution and add 0.5 ml of ferric chloride results in the formation of intensive colour.
Glacial acetic acid test- test solution and add few drops of 5% glacial acetic acid and 5% of sodium nitrate result in the formation of muddy yellow or Niger brown or deep chocolate colour precipitate.

RESULTS

Comparison of physical parameters of Olea dioica collected from different places of Western Ghats, Karnataka

Physical parameters are compared within the forest types, in that, Kigga forest situated in higher altitude 841m above the sea level compared to other to (Chakra- 640 m and Sagara- 613 m) and Kigga is highest rain fall area (1905 mm) compared to other two (Chakra- 1544 mm and Sagara- 843 m), forest type of three

sampling site varies from evergreen to semi evergreen to moist deciduous or mixed of two or more forest type observed. Kigga forest has least average annual temperature (20–30 °C) compared to other two (Chakra- 23–34 °C and Sagara- 26–36 °C). Wind speed of Sagara and Chakra forest (5 km.h^{-1}) area is more compared to Kigga (4 km.h^{-1}). Wind direction of Kigga forest is east and north east side while wind direction of chakra forst is east and south east and Sagara forest wind blow towards east direction. Average humidity of Kigga forest (95%) is more compared to the rest of two forest types (Chakra- 80% and Sagara- 79%). Average precipitation also high in case of Kigga forest (1200 mm) compared to the rest of two forest types (Chakra- 1180 mm and Sagara- 911 mm). All the data collected form metrological stations of Shimoga and Chikamagaluru (Table 1).

Table 1. Comparison of physical parameters of *Olea dioica* collected from different places of Western Ghats, Karnataka (represented from IIRs 2013).

S.N.	Parameters	Kigga forest	Chakra forest	Sagara
1	Altitude (m)	841m	640m	613 m
2	Rainfall (mm)	1905	1544	843
3	Forest type	Evergreen Semi evergreen	Evergreen Semi evergreen	Semi evergreen, Moist deciduous
4	Average temperature (ºC)	20–30 °C	23–34 °C	26–36 °C
5	Average humidity	95%	80%	79%
6	Average precipitation (mm)	1200	1180	911
7	Wind speed	4 km.h^{-1}	5 km.h^{-1}	5 km.h^{-1}
8	Wind direction	E NE	E SE	E
9	Latitude longitude	N 14° 8' 9.4992" E 74° 57' 33.0480"	N 13° 25' 5.1636" E 75° 10' 26.1804"	N 13° 48' 28.3212" E 74° 58' 1.3980"

Compound yield of Olea dioica plant with respect to different climatic zones of Western Ghats, Karnataka.

For soxhlet extraction 750 grams of different plant parts like Matured leaves, Immature leaves, Flower, immature fruit, mature fruit, Immature seed, Matured seed, Inner bark, Outer bark & Roots were used and run using ethanol. The compound yield was compared between three forest types (Kigga, Cakra and Sagara) reveals that the Sagara forest yields highest extract compared to chakra and Kigga. The moderate *Olea dioica* extract yield is observed in chakra and least extract of *Olea dioica* was observed in Kigga forest (Table 2; Fig. 2).

Table 2. Extract yield of ethanolic extracts of *Olea dioica* collected from different places of Western Ghats, Karnataka.

S. N.	Solvent used	Quantity of sample used	Plant parts	Quantity of yield of extract (gram)		
				Kigga forest	Chakra forest	Sagara forest
1	Ethanol	750 grams	Matured leaves	14.32	15.33	17.31
2			Immature leaves	10.56	9.32	14.35
3			Flower	9.54	10.43	15.67
4			immature fruit	12.34	14.54	17.22
5			mature fruit	15.34	13.43	16.98
6			Immature seed	11.22	12.65	15.35
7			Matured seed	14.32	15.97	19.65
8			Inner bark	19.67	20.87	20.45
9			Outer bark	20.32	23.44	29.67
10			Root	19.67	21.83	30.54

Distribution of secondary compounds in plant parts with respect to different climatic zones of Western Ghats, Karnataka.

1. Kigga forest: *Olea dioica* plant parts collected form Kigga forest subjected for qualitative phytochemical analysis which revealed the presence of many secondary metabolites. Alkaloids were present only in matured leaves, inner bark, outer bark and root. Saponins were present in all the parts except immature leaves and immature seed. Tannins were distributed in matured leaves, immature leaves, inner bark and roots. Flavonoids

were present in all the parts examined except immature leaves and immature seed. Steroids were present in matured leaves, matured fruit and matured seed. Glycosides were present in matured leaves, matured seeds, inner bark, outer bark and roots. Phenols were absent in immature leaves and immature seed (Table 3).

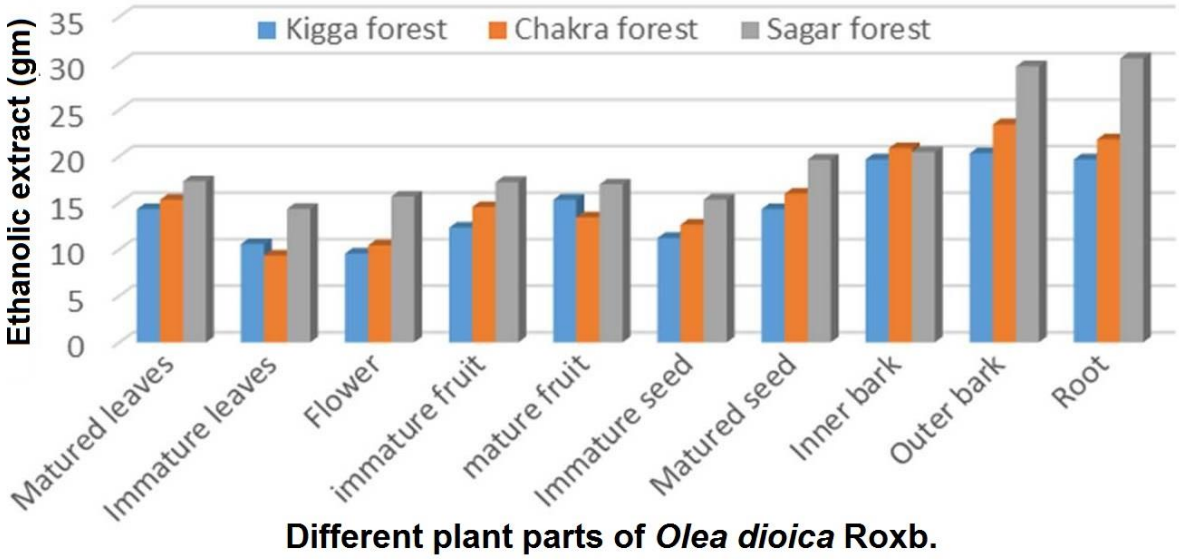

Figure 2. Extract yield of ethanolic extracts of *Olea dioca* collected from different places of Western Ghats, Karnataka.

Table 3. Presence of secondary metabolites in *Olea dioica* collected near Kigga, Chakra and Sagara forests (Qualitative assay).

S. N.	Plant parts used	Alkaloids			Saponins			Tannins			Flavonoids			Steroids/sterols			Glycosides			Phenols		
		Kigga	Chakra	Sagara	Kigga	Chakra	Sagara	Kigga	Chakra	Sagara	Kigga	Chakra	Sagara	Kigga	Chakra	Sagara	Kigga	Chakra	Sagara	Kigga	Chakra	Sagara
1	Matured leaves	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Immature leaves	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	+
3	Flower	-	-	-	+	+	+	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+
4	Immature fruit	-	-	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	+	-	+
5	mature fruit	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+
6	Immature seed	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+
7	Matured seed	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
8	Inner bark	+	+	+	+	-	+	+	+	+	+	-	+	-	-	-	+	-	-	+	-	-
9	Outer bark	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+
10	Root	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	-	+	+	+

Note: +, presence; -, absence.

2. Chakra forest: *Olea dioica* plant parts collected form Chakra forest has different metabolite confirmation, alkaloids present in immature leaves, inner bark, outer bark and root parts. Saponins were present in all the parts except immature leaves, inner bark and immature seeds. Tannins were distributed in matured leaves, matured fruit, and inner bark. Flavonoids were present in parts like matured leaves, flower, matured fruit, matured seed, outer bark and root except immature leaves, immature seeds and inner bark. Steroids were present in matured leaves, flower, matured fruit, matured seed and outer bark. Glycosides were present in matured leaves, flower,

immature fruit, immature seed, mature seed, outer bark and roots. Phenols were absent in immature leaves, immature fruit, immature seed and inner bark, but in all the plant parts phenols were present (Table 3).

3. Sagara forest: *Olea dioica* plant parts collected form Sagara forest subjected for qualitative phytochemical analysis revealed the confirmation of different secondary metabolites compared to other study sites. alkaloids present in all the plant parts except flower, immature seed and outer bark. Saponins were present in all the parts except immature leaves, immature fruit, immature seeds and outer bark. Tannins were distributed in matured leaves, immature leaves, mature fruit, inner bark and outer bark. Flavonoids were present in all parts. Steroids were present in matured leaves, flower, matured fruit, matured seed, and outer bark. Glycosides were present in matured leaves, flower, mature fruit, immature seed, mature seed and outer bark. Phenols were absent in inner bark, but in all the plant parts phenols were present (Table 3).

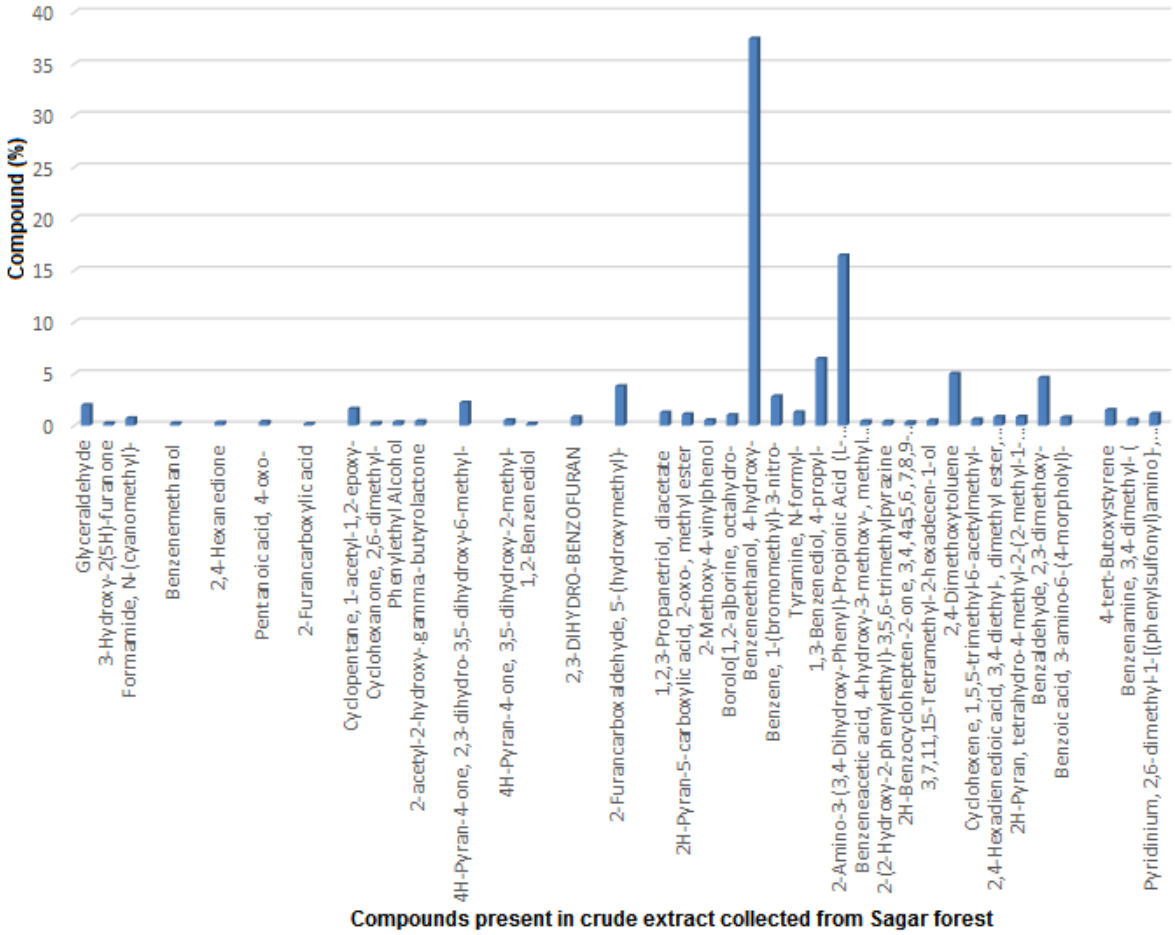

Figure 3. GC-MS of crude ethanolic extract collected from Sagara forest showing percentage of different compounds.

Table 4. Presence of metabolites in GC-MS analysis of crude ethanolic extract collected from different sampling sites with their properties.

S. N.	Chemical compound present	Average Percentage	Properties of the compound	Kigga forest (18 compounds present)	Chakra forest (33 compounds present)	Sagara forest (38 compounds present)
1	Glyceraldehyde	1.97	Parental compound of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme induced under environmental stress	+	+	+
2	3-Hydroxy-2(5H)-furanone	0.23	anti-oxidants and anti-inflammatory	-	+	+
3	Formamide, N-(cyanomethyl)-	0.71	Hematoxic to animals	-	+	+
4	Benzenemethanol	0.23	Bacteriostatic agent	-	-	+

5	2,4-Hexanedione	0.29	Antimicrobial activity	-	+	+
6	Pentanoic acid, 4-oxo-	0.37	Anti-inflammatory and medication for cancer; anti-bacterial	-	+	+
7	2-Furancarboxylic acid	0.16	Bactericide and fungicide	-	+	+
8	Cyclopentane, 1-acetyl-1,2-epoxy-	1.62	Unknown	+	+	+
9	Cyclohexanone, 2,6-dimethyl-	0.26	Cytotoxic, Antimicrobial, Anticancer, Anti-malarial activity	-	-	+
10	Phenylethyl Alcohol	0.33	Antimicrobial	-	+	+
11	2-acetyl-2-hydroxy-.gamma.-butyrolactone	0.41	Aecreational intoxicant,	-	+	+
12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2.22	Sedative, Cytotoxicity, Anticancer, Anti-inflammatory	+	+	+
13	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	0.52	Antioxidant	-	+	+
14	1,2-Benzenediol	0.17	Pesticides, Carcinogen, Cytotoxic	-	-	+
15	2,3-DIHYDRO-BENZOFURAN	0.82	Entactogen agent	-	+	+
16	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	3.81	Mutagenic	+	+	+
17	1,2,3-Propanetriol, diacetate	1.25	Antifungal agent	+	+	+
18	2H-Pyran-5-carboxylic acid, 2-oxo-, methyl ester	1.08	Unknown	+	+	+
19	2-Methoxy-4-vinylphenol	0.52	Flavoring agent	-	-	+
20	Borolo[1,2-a]borine, octahydro-	1.01	Unknown	+	+	+
21	Benzeneethanol, 4-hydroxy-	37.44	Antioxidant	+	+	+
22	Benzene, 1-(bromomethyl)-3-nitro-	2.82	Unknown	+	+	+
23	Tyramine, N-formyl-	1.28	Unknown	-	+	+
24	1,3-Benzenediol, 4-propyl-	6.46	Antioxidant	+	+	+
25	2-Amino-3-(3,4-Dihydroxy-Phenyl)-Propionic Acid (L-Dopa)	16.47	psychoactive drug, cytotoxic to rats	+	+	+
26	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	0.41	Cytotoxic	-	+	+
27	2-(2-Hydroxy-2-phenylethyl)-3,5,6-trimethylpyrazine	0.38	unknown	-	+	+
28	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro-	0.32	unknown	-	+	+
29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.49	chemical deterrents against predation	+	+	+
30	2,4-Dimethoxytoluene	5.01	insect repellant activity	+	+	+
31	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	0.6	unknown	-	-	+

32	2,4-Hexadienedioic acid, 3,4-diethyl-, dimethyl ester, (E,Z)-	0.84	unknown	+	+	+
33	2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	0.85	Flavor Ingredients, fragrance chemical	-	+	+
34	Benzaldehyde, 2,3-dimethoxy-	4.63	Antioxidants, Flavor Ingredients	+	+	+
35	Benzoic acid, 3-amino-6-(4-morpholyl)-	0.79	unknown	+	+	+
36	4-tert-Butoxystyrene	1.51	unknown	+	+	+
37	Benzenamine, 3,4-dimethyl- (	0.59	precursor for vitamin B2, with modest toxicity	-	+	+
38	Pyridinium, 2,6-dimethyl-1-[(phenylsulfonyl)amino]-, hydroxide	1.14	unknown	+	+	+

Note: +, presence; -, absence.

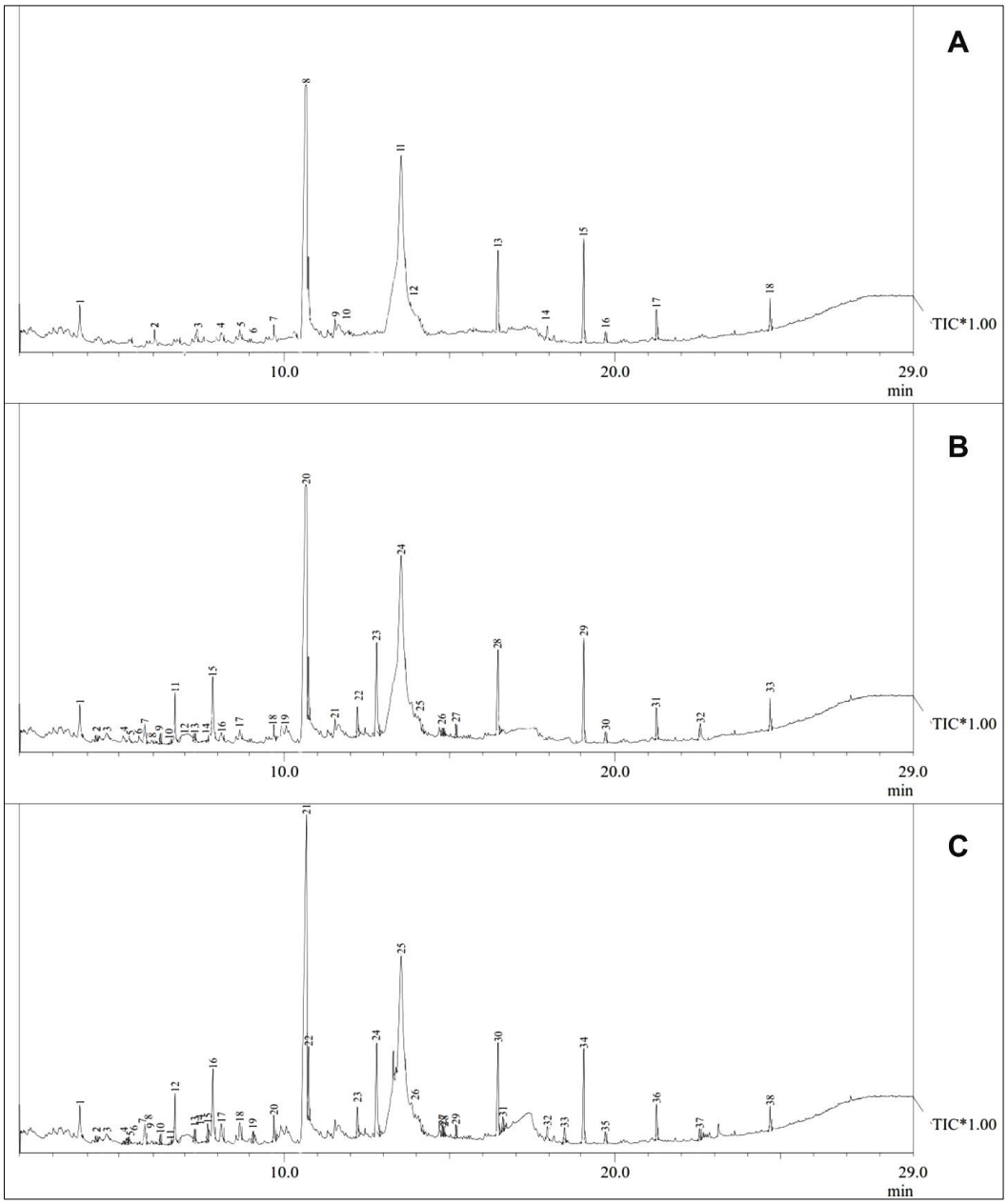

Figure 4. GC-MS of the *Olea dioica* crude extract: **A,** Kigga forest; **B,** Chakra forest; **C,** Sagara forest.

GC-MS analysis of ethanolic crude extract collected from different sampling sites

In GC-MS analysis of medicinal *Olea dioica* ethanolic extract revealed the presence 18 compounds form Kigga forest, 33 compounds from chakra forest, 38 compounds from Sagara forest in that major compounds present in all the sampling site was Benzeneethanol, 4-hydroxy - (37.44), 1,3-Benzenediol, 4-propyl - (6.46), 2-Amino-3-(3,4-Dihydroxy-Phenyl)-Propionic Acid (L-Dopa) - (16.47), 2,4-Dimethoxytoluene - (5.01), Benzaldehyde, 2,3-dimethoxy - (4.63) in average percentages. GC-MS analysis of ethanolic crude extract revealed that it has Benzeneethanol, 4-hydroxy - (37.44%) has anti-oxidant and cytotoxic properties and 2-Amino-3-(3,4-Dihydroxy-Phenyl)-Propionic Acid (16.47%) commonly called as L-Dopa which is used as psychoactive drug and also cytotoxic properties (Table 4; Figs. 3–4).

DISCUSSION

Presence of secondary metabolites in Olea dioica collected near Kigga, Chakra and Sagara forest (Qualitative assay)

Alkaloids were absent from semi ripened and ripe fruit, which may declines the seed dispersal chances through animals (McKey 1974) in all the other parts alkaloids were present main defense compound and main compound released during stress conditions.

Saponins were found in all plant parts examined. The lowest occurrence of saponins was found in tree twigs. Most of the saponins observed were poisonous to fishes, pests etc. but its expression to the physical test is unknown. Saponins are widespread in plant, studies also revealed that saponins and tannins should not co-occur in plant parts but in our experiments plant parts like leaf, fruit, bark and root parts both tannin and saponins were present which has to be investigated.

It is belief that condensed tannins have evolved mainly in defense against microbes and fungi (Azaizeh *et al.* 1990). In our study area there is varied forest type with or without human interference and for about 4 months of rainy season also triggers favorable conditions for common pathogens as well as opportunistic to infect plants so, the plants must produce metabolites like condensed tannins against these pathogens to survive and propagate. Having powerful antioxidant activity and anticancer properties of hydrolysable tannins its plant defense activity was not clearly known.

We observed tannins mainly in matured plant parts like inner bark, entire bark and root and matured leaf and fruit parts because plant should protect these parts otherwise it will be dead in the growth stage and will in turn affects the plant growth. In immature leaf, flower, immature fruit, matured fruit, immature seed and matured seeds we observed nil tannins because the tannins will resist water, seeds must be germinate as soon as possible so it must be less defense compounds and rich in other nutritive compounds which attracts the animals as well as microbes, in other parts the absence of tannin is unknown. In some matured and immature leaf parts tannins were observed which may hydrolysable tannins and in matured fruit we observed tannins which may be condensed tannins not hydrolysable tannins (Mali & Borges 2003).

In entire bark, root parts we observe nil tannins, because they protected by high levels of fiber and lignin therefore do not require protection from other compounds. But Inner bark shows positive results for tannin reason is unknown. We observed tannins mainly in matured plant parts like inner bark, entire bark, matured fruit, matured leaf and root parts. In immature leaf, flower, immature fruit, immature seed and matured seeds we observed nil tannins. Inner bark and entire bark tannins were observed may help in regulating the growth of phloem, xylem, cortex and epidermis. Tannins has main role in controlling the pest invading stem root etc. Different climatic zones of Western Ghats will not affect the presence of tannins in different plant parts of *Olea dioica* but in Kigga forest we observe less tannins form all the parts.

In our study flavonoids mainly present in all the parts except immature leaves and immature seeds. Flavonoids mainly present in flowers because they are the important plant pigments for flower colouration and pigmentation in petals intended to attract pollinator insects and animals. Flavonoids also present in matured leaves, roots, matured seed, inner and outer bark because flavonoids has many tasks like UV filtration, symbiotic nitrogen fixation, chemical messengers, physiological regulators, and cell cycle inhibitors. Some flavonoids have inhibitory activity against pathogens that cause plant diseases, e.g. *Fusarium oxysporum.* Flavonoids also have protective functions in drought stress.

Sterols and Steroids were helps plant to tolerance/resistance a wide range of biotic and abiotic stresses, like drought, salinity, heat, cold, virus infection, and pathogen attack (Divi & Krishna 2009). In our study the

research plant steroids were mainly found in matured leaves, flower, matured seeds, matured fruit and inner bark which may be localized in the this part against the physical stress and also pathogenic defense.

Glycosides mainly found in matured leaves, matured seed, inner bark, outer bark, roots. Glycosides appear to be very much less as defense chemicals than alkaloids, saponins and phenolics.

Phenols were found in in all the parts except immature leaves and immature seeds. Phenols were main defense secondary metabolites against almost all pathogens (phytoalexins) and major compound active during the physical stress.

Climatic factors affecting on the bio synthesis of secondary metabolites

Altitude matters in case of plant to encounter stress. Sagara forest is nearer to coastal area compared to Kigga and chakra has less altitude with high stress compared with higher altitude plants. Rainfall data was evaluated showed that the Kigga forest was well rain fed area with evergreen type of forest compared to Chakra and Sagara which has semi- evergreen and moist deciduous forest types. Sagara forest has least rain fall which in turn triggers the stress tolerating machineries with the other stress factors like high temperature, less humidity, less precipitation and high wind speed compared to Kigga and chakra forest. Due to stress plants trigger the production of stress tolerating metabolites for its existance. GC-MS analysis was compared with the three forest types showed that many secondary metabolite present in the extract has some role in tolerating physical and biological stress.

GC-MS analysis

In GC-MS analysis of *Olea dioica* ethanolic extracts revealed the presence of 18 compounds collected form Kigga forest, 33 compounds collected from chakra forest, 38 compounds collected from Sagara forest (Table 4; Figs. 3–4). The major and the common compounds present in all the sampling site was Benzeneethanol, 4-hydroxy - (37.44), 1,3-Benzenediol, 4-propyl - (6.46), 2-Amino-3-(3,4-Dihydroxy-Phenyl)-Propionic Acid (L-Dopa) - (16.47), 2,4-Dimethoxytoluene - (5.01), Benzaldehyde, 2,3-dimethoxy - (4.63) in average percentages. These compounds has many medicinal properties as showed in table 4 along with other unexplored metabolites has some medicinal value yet to be discovered.

CONCLUSION

The results obtained was compared with the different zones of Western Ghats revealed that, the Sagara forest area shows higher yield of crude extract, compared to the Chakra and Kigga forest area and preliminary phytochemical analysis also revealed that the presence of secondary metabolites was higher in case of Sagara forest area compared to the Chakra and Kigga forest area. Climatic factors like stress, temperature, rainfall, humidity, wind speed, light intensity, the supply of water, etc. were the main reason which triggers the stress in the Sagara forest to synthesize more secondary metabolites to tolerate it and to survive in that condition.

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