IJPSR (2017), Vol. 8, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 October, 2016; received in revised form, 13 December, 2016; accepted, 16 December, 2016; published 01 May, 2017

PHYTOCHEMICAL SCREENING AND GC-MS STUDIES OF *SYZYGIUM DHANESHIANA* LEAF AND BARK EXTRACTS

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Keywords:

S.dhaneshiana, GC-MS analysis, Phytochemical screening, Cetene

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ABSTRACT: *Syzygium dhaneshiana* is a new plant species of Syzygium is described from the Southern Western Ghats, Kerala, India. The medicinal values of the plant is so far unexplored, hence the study forms a basis for the bioactive components present in the plant. The aim of this study is to screen the phytochemicals present in the leaf and bark of the plant and the analysis of bioactive components present in it. The leaves and bark were successively extracted using petroleum ether, chloroform, ethanol and water based on the increasing order of polarity. The result reveals the presence of Carbohydrates, protein, amino acid, alkaloids and phenols in all the extracts. Saponin, fixed oils and fats were present in the leaf extracts. The GCMS analysis reports shows the presence of 17 Compounds in the leaf ethanolic and 19 in leaf chloroform extract of *Syzygium dhaneshiana* by comparing their retention time and their mass spectra. The bark ethanolic and chloroform comprises of 14 &16 Compounds respectively. Among the identified compounds major bioactive compounds are 2-Fluoroformyl-3,3,4,4-tetrafluoro-1,2-oxazetidine, 2,4, di tert-butylphenol, Cetene, E- 15. Heptadecenal, Neophytadiene, phytol and Nonacos-1-ene. The presence of bioactive compounds confirms that *Syzygium dhaneshiana* is a medicinal source and it may proceed to discover a novel drug.

INTRODUCTION: India is known for its wealth of medicinal plants which are found in its diverse climatic and physiographic conditions. Medicinal plants are widely used for the management of different disease conditions and to play a beneficial role in human health [1]. More over traditional medicine is still predominant means of healthcare in developing countries where 80% of the total population depends on the medicinal plants for their well-being [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(5).2277-81

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (5).2277-81

Medicinal plants are valuable natural resources and regarded as potentially safe drugs to play an important role in the modern medicine [3]. Therefore, the increased use of herbal remedies which contains complex mixtures of natural products need intensified scientific studies.

Syzygium dhaneshiana is a new plant species is described from the Southern Western Ghats of Kerala, India. [4] the plant is similar to *Syzygium gardneri Thw*. but it differs by several charactors as that of *Syzygium gardneri.* The plant is distributed in the Nedumpoyil Ghat area in Kannur district and Chanthanathodu area of forest range in Western Ghats. The ethno botanical reports reveals that the bark of the plant have antimicrobial activity and the plant has antipyretic property also. The fruit of the

plant is edible. Hence the plant is obligatory to screen the secondary metabolites and to screen the bioactive components by GC-MS method.

MATERIALS AND METHODS:

Collection and identification of plant material: Different parts of *Syzygium dhaneshiana* were collected during the month of June 2014 from Nedumpoyil Ghats in Kannur district of Kerala, India. The plant was identified and confirmed from Dr. Ratheesh Narayanan, Assistant professor, Payyannur College, Kannur, Kerala. The fresh materials were washed under running tap water to remove the surface pollutants and were shade dried at room temperature. The leaf and bark were separately homogenized in to fine powder using mixer (MG 172; Preethi Kitchen Appliances Pvt. Ltd., Chennai, India) and used for further analysis.

Extraction of plant material: The powdered plant materials such as leaf and bark were packed in small thimbles separately and extracted using soxhlet apparatus (3840; Borosil Glass works Ltd., Mumbai, India) with organic solvents such as petroleum ether, chloroform and ethanol in the increasing order of polarity. The thimble was air dried before extracting with the next solvent. Finally macerated using hot water with constant stirring for 24 hours using the orbital shaker (Rivotek; Riviera Glass Pvt. Ltd., Mumbai, India) and the extract was filtered. The extracts were concentrated, air dried and stored at -20 ºC in the deep freezer (RQV- 300; plus, REMI electrotechnik Ltd., Thane, Maharashtra, India) for further analysis.

Extract Recovery Percent:

The amount of extract recovered after successive extraction was weighed and the percentage yield was calculated by the following formula,

$$\text{Extract Recovery Percent} = \frac{\text{Amount of extract (g)}}{\text{Amount of plant sample (g)}} \times 100$$

Qualitative Phytochemical Screening: The different extracts of the plant were subjected to preliminary phytochemical screening by using standard methods [5]. Major metabolites such as Carbohydrates, Proteins, Amino acids, Alkaloids, Saponins, Phenolic compounds, Tannins, Flavonoids, Glycosides, Flavanol glycosides, Cardiac glycosides, Phytosterols, Fixed oils & fats, and Gums & mucilages were analyzed.

GC-MS analysis: GC-MS analysis of *S.dhaneshiana* leaf and bark extracts were performed using Themo Scientific Trace 1300 Gas chromatograph equipped with ISQ- QD Mass spectrometer with TG-5MS column (30mm × 0.25mm ID ×0.25µm). Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/minute and an injection volume of 1µl was employed. An injection port temperature of 280 ºC and an ion-source temperature of 200 ºC was hired for this study. The oven temperature was programmed from 60 ºC for 3 minutes with an increase of 5 ºC /minute to 240ºC with a hold time of 3 minutes. Then temperature was increased at a rate of 35 ºC/min till 280 ºC with a hold time of 5 minutes. Scan interval was programmed for 0.2 seconds with a mass range of 40-450 amu. Total GC running time was 45 minutes. The components in the extract were identified based on the mass spectra of NIST library data in reference with RSI and SI value.

RESULTS AND DISCUSSION: The percentage yield of leaf and bark extracts of *Syzygium dhaneshiana* in different solvents are presented in **Table 1.** The maximum yield was obtained in ethanol extract of bark and was 4.9%. In the case of leaf, the maximum yield was obtained in chloroform (1.32%). Preliminary phytochemical screening of bark and leaves of *S. dhaneshiana* are presented in **Table 2**. GC-MS chromatogram of ethanolic extract of bark of the plant revealed 14 peaks indicating fifteen phytochemical constituents. The major compounds identified with their retention time, molecular formula, molecular weight and peak area are presented in **Table 3**.

Among them Quinic acid, Hexadecanoic acid and Eicosane are the major compounds. Fourteen compounds were identified in the chloroform extract of *S. dhaneshiana* bark extract. Among them 2, 4, di-tert- butyl phenol was the major compound. The phytochemical constituents present in the chloroform extract of bark are presented in **Table 4**. The phytochemicals identified in the leaf ethanol extract of *S. dhaneshiana* revealed 15 major compounds are presented in **Table 5**.

Among the identified compounds Cetene, Neophytedine and Phytol are the significant compounds. The leaf chloroform extract of the plant comprises of 17 important phytoconstituents are tabulated in **Table 6**. 1-Hexadecanol, 2,4-Di-tert-butylphenol, Tridecanoic acid, n-Hexadecanoic acid and Octadecanoic acid are the major compounds identified in the leaf chloroform extract.

Alkaloids	+++	+++
Saponins	+++	-
Phenolic compounds	+++	+++
Tannins	-	-
Flavonoids	-	-
Glycosides	-	-
Flavonol Glycosides	-	-
Cardiac glycosides	-	-

TABLE 1: EXTRACT YIELD PERCENTAGE OF *SYZYGIUM DHANESHIANA* (g/100g DRIED POWDER)

Solvents	Leaf	Bark
Petroleum Ether	0.661	0.1
Chloroform	1.326	0.52
Ethanol	1.16	4.9
Hot water	1.24	1.6

TABLE 2: PHYTOCHEMICAL SCREENING OF *SYZYGIUM DHANESHIANA* LEAF AND BARK EXTRACTS

Phytochemicals	Leaf	Bark
Carbohydrates	++	++
Proteins	++	+
Amino acids	+	+

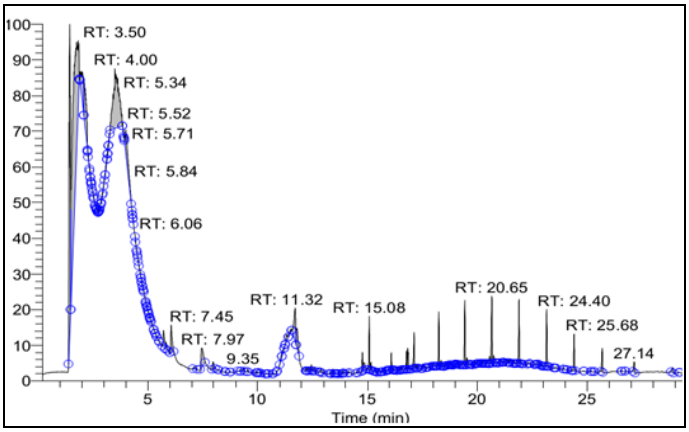

FIG. 1: GC/MS ANALYSIS OF ETHANOL EXTRACTS OF *S. DHANESHIANA* BARK

TABLE 3: GC/MS ANALYSIS OF ETHANOL EXTRACTS OF *S.DHANESHIANA* BARK

S.no	R.T	Name of the compound	Peak area (%)	Molecular Formula	Molecular Weight
1	1.62	2-Fluoroformyl-3,3,4,4-tetrafluoro-1,2-oxazetidine	30.53	$C_3F_5NO_2$	177
2	6.06	2,6-Octadienal, 3,7-dimethyl-, (E)	1.46	$C_{10}H_{16}O$	152
3	11.71	Quinic acid	2.08	$C_7H_{12}O_6$	192
4	15.08	Hexadecanoic acid	1.10	$C_{16}H_{32}O_2$	256
5	17.12	Eicosane	1.01	$C_{20}H_{42}$	282
6	24.42	Eicosane 10 methyl	1.04	$C_{21}H_{44}$	296
7	24.4	Tritetracontane	1.04	$C_{43}H_{88}$	604
8	21.08	Nonadecane, 9-methyl-	0.18	$C_{20}H_{42}$	282
9	7.45	1,2,3- benzenetriol	1.72	$C_6H_6O_3$	126
11	12.56	Tetradecanoic acid	0.12	$C_{14}H_{28}O_2$	228
12	15.08	Hexadecanoic acid, ethyl ester	1.10	$C_{18}H_{36}O_2$	284
13	15.16	Cyclopropane octanoic acid	0.16	$C_{22}H_{38}O^2$	334
14	20.65	Eicosane	1.3	$C_{20}H_{42}$	282

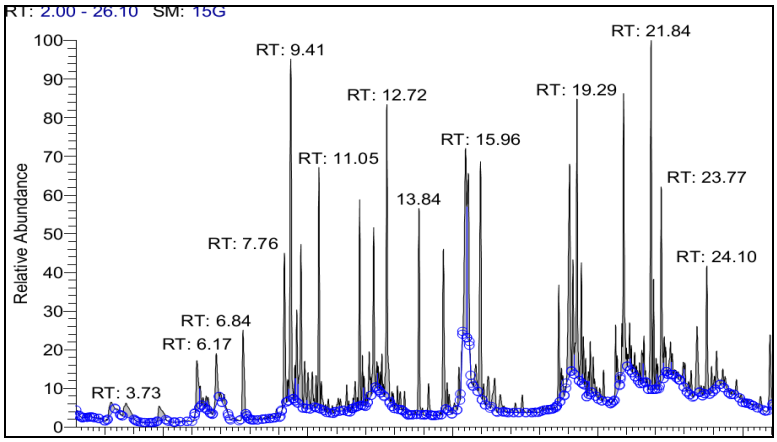

FIG. 2: GC/MS ANALYSIS OF CHLOROFORM EXTRACTS OF *S.DHANESHIANA* BARK

TABLE 4: GC/MS ANALYSIS OF ETHANOL EXTRACTS OF *S.DHANESHIANA* BARK

S.no	R.T	Name of the compound	Peak area (%)	Molecular Formula	Molecular Weight
1	9.19	Dodecane 2,6,11- trimethyl	2.32	$C_{15}H_{32}$	212
2	9.41	2,4, di – tert-butylphenol	5.19	$C_{14}H_{22}O$	206
3	9.62	Benzoic acid,4- ethoxy- ethylester	1.17	$C_{11}H_{14}O_3$	194
4	10.38	Cetene	2.67	$C_{16}H_{32}$	224
5	12.72	10- Heneicosene	3.88	$C_{21}H_{42}$	294
6	13.84	Pthalic acid, hept – 4yl- isobutyl ester	2.59	$C_19H_{28}O_4$	320
7	15.68	n- hexadecanoic acid	2.89	$C_{16}H_{32}O_2$	256
8	15.96	Nonacos -1-ene	3.70	$C_{29}H_{58}$	406
9	19.03	Octadecanoic acid	3.98	$C_{18}H_{36}O_2$	284
10	19.15	Heneicosane	1.18	$C_{21}H_{44}$	296
11	21.84	Dicyclohexyl phthalate	4.19	$C_{20}H_{26}O_4$	330
12	21.93	Pthalicacid di(2-propylpentyl)ester	0.98	$C_{24}H_{38}O_4$	390
13	23.77	1- Heptacosanol	1.67	$C_{27}H_{56}O$	396
14	25.95	Octatriacontyl trifluoroacetate	1.18	$C_{40}H_{77}F_3O_2$	646

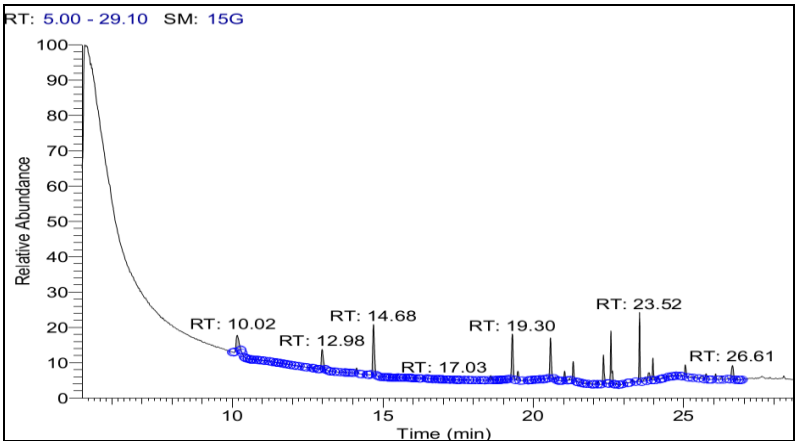

FIG. 3: GC/MS ANALYSIS OF ETHANOL EXTRACTS OF *S.DHANESHIANA* LEAF

TABLE 5: GC/MS ANALYSIS OF ETHANOL EXTRACTS OF *S.DHANESHIANA* LEAF

S.no	R.T	Name of the compound	Peak area (%)	Molecular Formula	Molecular Weight
1	10.16	Cetene	5.65	$C_{16}H_{32}$	224
2	12.98	2,4, Di – tert-butylphenol	5.21	$C_{14}H_{22}O$	206
3	14.12	Dodecanoic acid	1.26	$C_{12}H_{24}O_2$	200
4	18.58	Tetradecanoic acid (Myristic acid)	0.99	$C_{14}H_{28}O_2$	228
5	19.30	E- 15. Heptadecenal	9.25	$C_{17}H_{32}O$	252
6	19.49	Dodecane 2,6,11- trimethyl	1.88	$C_{15}H_{32}$	212
7	20.57	Neophytadiene	7.81	C_20H_{38}	278
8	21.32	3,7,11,15 – Tetramethyl – 2-hexadecen-1-ol	3.57	$C_{20}H_{40}O$	296
9	22.33	n- hexadecanoic acid	4.90	$C_{16}H_{32}O_2$	256
10	23.52	Phytol	7.88	$C_{22}H_{42}O_2$	338
11	23.71	Ethyl iso- allocholate	1.06	$C_{26}H_{44}O_5$	436
12	23.82	12- Methyl-E,E- 2,13- Octadecadiene-1-ols	2.10	$C_{19}H_{36}O$	280
13	23.97	Pentacos – 1- ene	2.76	$C_{25}H_50$	350
14	25.04	10 – Heneicosene	2.58	$C_{21}H_{42}$	294
15	25.63	Octadecane,3 – ethyl- 5 –(2-ethylbutyl)	1.07	$C_{26}H_{54}$	366

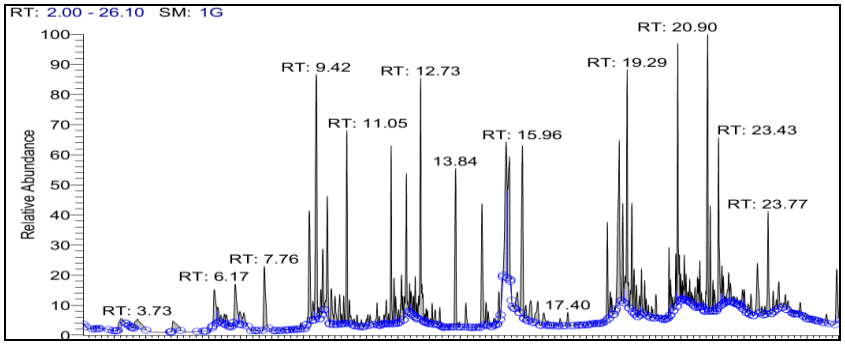

FIG. 4: GC/MS ANALYSIS OF CHLOROFORM EXTRACTS OF *S. DHANESHIANA* LEAF

TABLE 6: GC/MS ANALYSIS OF CHLOROFORM EXTRACTS OF *S. DHANESHIANA* LEAF

Sl. no:	R.T	Name of the compound	Peak area (%)	Molecular Formula	Molecular Weight
1	6.17	Dodecane, 2,6,11-trimethyl-	1.05	$C_{15}H_{32}$	212
2	7.76	1-Hexadecanol	1.58	$C_{16}H_{34}O$	242
3	9.09	Guaia-3,9-diene	0.01	$C_{15}H_{24}$	204
4	9.42	2,4-Di-tert-butylphenol	4.98	$C_{1}4H_{22}O$	206
5	9.62	Benzoic acid, 4-ethoxy-, ethyl ester	0.88	$C_{11}H_{14}O_3$	194
6	10.38	Hexadecen-1-ol, trans-9-	2.52	$C_{16}H_{32}O$	240
7	13.84	Phthalic acid, hept-4-yl isobutyl ester	2.42	$C_{19}H_{28}O_4$	320
8	13.96	Neophytadiene	0.08	$C_{20}H_{38}$	278
9	15.44	Tridecanoic acid	3.67	$C_{13}H_{26}O_2$	214
10	15.55	n-Hexadecanoic acid	2.99	$C_{16}H_{32}O_2$	256
11	15.96	Nonacos-1-ene	3.52	$C_{29}H_{58}$	406
12	18.66	Hexadecane, 2,6,11,15-tetramethyl-	1.20	$C_{20}H_{42}$	282
13	19.04	Octadecanoic acid	3.92	$C_{18}H_{36}O_2$	284
14	19.15	Heneicosane	1.16	$C_{21}H_{44}$	296s
15	19.44	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	1.40	$C_{20}H_{30}O$	286
16	20.90	Tricosyl heptafluorobutyrate	3.15	$C_{27}H_{47}F_7O_2$	536
17	22.19	1-Heptacosanol	2.39	$C_{27}H_{56}O$	396

CONCLUSION: The chemical analysis of this plant has been evaluated first time. The results of GC-MS analysis and preliminary phytochemical screening designated that the bark and leaves contained numerous bioactive phytoconstituents belonging to various classes. The presence of various chemical compounds confirms that the plant hold medicinal value and further plan of study includes isolation and purification of chemical compounds.

AKNOWLEDGEMENT: The authors are thankful to Department of Science And Technology, New Delhi, India for providing financial assistance.

CONFLICT OF INTEREST: The authors declare no known conflict of interest.

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How to cite this article:
Krishna CS, Chandra R and Khaleel KM: Phytochemical screening and GC-MS studies of *Syzygium dhaneshiana* leaf and bark extracts. Int J Pharm Sci Res 2017; 8(5): 2277-81.doi: 10.13040/IJPSR.0975-8232.8(5).2277-81.