GAU Research Journal **46** (3) : 202–209 (July 2021)

Phytochemical Screening and GC-MS Analysis of *Clausena dentata* (Willd.) Roem

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Received: 09 December, 2020 Accepted: 26 July, 2021

ABSTRACT

The present study is aimed to screen phytochemicals present in*Clausena dentata*. Methanol is used as a solvent system and leaves are considered for this study. Secondary metabolites are chemical compounds responsible for all functions in plants. Leaves of *Clausena dentata* studied for the presence of steroid, terpenoid, flavonoid, coumarins, carbohydrates, saponins, tannins, cardiac glycosides, phenols and alkaloids. The present study showed that steroid, flavonoid, carbohydrates, tannins, cardiac glycosides, phenolsand alkaloids were found to be present whereas coumarins and saponins was absent. The present study also deals with GC-MS analysis which results in observation of 36 peaks with different retention time, peak area and peak height. Each peak represents particular chemical compounds present in this studied plant.

Key words: Clausena dentata, Methanol, Phytochemicals, Secondary metabolites, GC-MS analysis,

INTRODUCTION

Clausena dentata (Willd.) Roem is a small tree, which belongs to the family Rutaceae. The essential oil and its components of leaf act as mosquito repellent and displayed larvicidal activity against Aedes aegypti and Culex quinquefasciatuslarvae (Rajkumar et al., 2010). These plants are aromatic trees, shrubs, and a few herbs and are distributed throughout the humid and temperate regions of the world. The leaf juice is applied to the injured eye in Tamil Nadu (Apparananthamet al.,1986). The stem bark of Clausena dentata is used in veterinary medicine for the treatment of wounds and sprains (Chopra et al.,1956). Even though C. dentata has a lot of potential medical uses, the study of pharmacological properties is very scarce (Subba Rao et al., 1976). The enzymatic antioxidants are produced itself in our body, whereas most of non-enzymatic antioxidants are obtained from either natural plants or synthetics which are used for the treatment for various diseases (Lee et al.,2004). A number of plants belong to the family Rutaceae are of medicinally valuable and furnish several drugs and pharmaceutical products.

Phytochemicals are chemical compounds which are used traditionally in various medicinal preparations to cure various ailments of human beings.They are of plants origin and may be primary metabolites or secondary metabolites. Naturally, many plants are found to possess various secondary metabolites, like alkaloids, tannins, glycosides, volatiles oils and contain

minerals and vitamins, which possess different therapeutic uses (Wang *et al.*,2005). Fiorentino*et al.*, (2007) the phytocompounds studies have revealed that the leaf extract contains phenolic antioxidant compounds, such as flavonoids, phenolic glycosides, tannins, gallic acid derivatives, anthocyanins.Gas chromatography-mass spectroscopy (GC-MS) is a simultaneously analytical techniqueused for the identify compounds in traditional medicines and medicinal plants.GC-MS studies in recent years have been increasingly useful for the analysis of medicinal plants as this technique has proved to bea important method for the analysis of non-polar components and volatile essential oil, lipids, fatty acids (Jie*et al.*, 1988) and alkaloids (Betz *et al.*, 1997).Considering the importance of the plant, the present study was undertaken tostudy its secondary metabolite constitution and GC-MS analysis in leaves of *Clausena dentata*.

MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACTION

The fresh leaves of *Clausena dentata* were collected from Pachaimalai hill, Salem District, Tamil Nadu, India. The healthy leaves were washed thoroughly in tap water and also rinsed using distilled water and then air dried under shade in room temperature for two weeks. The dried leaves were grinded and fine powdered by using electric mixer for extraction. Two fifty grams (250g) of powdered plant materials were soaked in 750ml of methanol and incubated for 48hrs. The soaked material were filtered by whatman filter paper no.1 for the extraction. The filtrate was concentrated using a vaccum rotary evaporator (Superfit-ROTAVAP, India). The percentage yield of extracts was calculated (Rashmi Tambe*et al.*, 2014).

PHYTOCHEMICAL EVALUATION

It is important to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms (Harborne 1994). Qualitative phytochemical screening of leaf extract with methanol was performed as per the protocols stated by Kokate (1996),Trease*et al.*,(1989), Harborne (1998),Sofowora, (1993), Mace *et al.*,(1963). The extract profile established was performing for different phytochemical screening tests for composition of chemical. The extract using standard methods for identification of steroid, terpenoid, flavonoid, coumarins, carbohydrates, saponins, tannins, cardiac glycosides, phenols and alkaloids.

1. Test for sterols

Libermann-burchard Test: To 2ml of plant extract add 1ml of acetic acid. This mixture is cooled and few drops of concentrated sulphuric acid were added. Colour change from violet to blue or bluish green indicates the presence of steroids.

2. Test for carbohydrates

Fehling's Test: Take 1ml of plant extract and add few drops of diluted Hydrochloric acid. Heat the content in the water bath. After few minutes, add 6-7 drops of Fehlings A and B solution. Red colour precipitate obtained which shows the presence of carbohydrates.

3. Test for Cardiac Glycosides

Take 1ml of plant extract add 2ml of glacial acetic acid and 2 drops of 5% ferric chloride solution. Then it was treated with 1ml of Concentrated Sulphuric acid. Formation of Brown ring at the interface indicates the presence of Cardiac Glycosides.

4. Test for Flavonoids

Take 2ml of plant extract add few drops of 10% sodium hydroxide solution. Intense yellow colour formed and it turned to colourless on addition of few drops of concentration sulphuric acid which indicates the presence of flavonoids.

5. Test for Terpenoids

Take 2ml of plant extract add 2ml of chloroform and 3ml of concentrated sulphuric acid. This form a layer and formation of reddish brown colour indicates the presence of

terpenoids.

6. Test for Tannins

Take 1.0ml of extract 1.0ml of 5% ferric chloride was added. A blue colour indicates condensed tannins green colour indicates the presence of tannins.

7. Test for Alkaloids

To 2ml of leaf extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

8. Test for Coumarins

To 1mL of Leaf extract, 1mL of 10% NaOH was added. Formation of yellow colour indicates presence of coumarins.

9. Test for Phenols

To 1ml of the leaf extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

10. Test for Saponins

Foam test: To the 2.0ml of extract 5ml of distilled water is added and shake vigorously for a stable persistent froth.

PREPARATION OF SAMPLE FOR GC-MS ANALYSIS

About 30gm of Clausena dentata shade dried plant leaf was soaked in 95% methanol for 12hrs. The extract was then filtered through whattman filter paper No. 1 along with 2 gm sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95% methanol along with sodium sulphate. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phyto-components in the plant material. 2µl of this solution was employed for GC-MS analysis (Merlin et al., 2009).

GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS

GC-MS analysis was carried out by using Agilent 7890B, Gas chromatography associated to 5977A Mass Spectrometer. Column HP-5MS fused capillary column (30 x 250µm x 0.25µm) composed of 5% Phenyl Methyl siloxane. Helium (99.999%) was used as carrier gas at a constant flow of 1ml per min and injection volume of 2µl was adopted (split less mode). The injected sample was detected by Turbo mass gold detector with the aid of Turbo mass 5.2 software. During 36 minute GC extraction process, the oven temperature was programmed from of 110°C with an increase of 10°C/min up to 200°C, then 5°C/min up to 280°C (9 minutes hold). The injector temperature was set at 250°C (mass analyzer). Mass spectra were taken at 70eV and fragments from 45-450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST (National Institute of Standards and Technology). Compounds identification: Identification was based on the molecular structure, molecular moss and calculated fragment.

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS

The phytochemical screening of leaf extract ofClausena dentata was carried out to study the presence or absence of various secondary metabolites as shown in Table 1. Methanol was used as a solvent system. The present study showed that steroid, flavonoid, carbohydrates, tannins, cardiac glycosides and phenols were found to be present whereas Coumarins and saponinswere absent. According to Pandith et al.,(2014), phytochemicals are chemical compounds which have complimentary and overlapping mechanism of action in the body including antioxidant effects modulation of detoxification enzymes, stimulation of the immune system and modulation of hormone metabolism. They are responsible for many activities such as antioxidant, antifungal,

antibacterial, antitumour, antidiabetic etc., studied that phytochemicals are used as a reagents for medicinal uses (Krishnarajuet al.,1995).

Table 1. PHYTOCHEMICAL ANALYSIS OF METHANOLIC LEAF EXTRACT OF *CLAUSENA DENTATA*

S.No	Phytochemical Constituents	*Clausena dentata* Leaf
1.	Steroid	+
2.	Terpenoid	+
3.	Flavonoid	+
4.	Coumarins	-
5.	Carbohydrates	+
6.	Saponins	-
7.	Tannin	+
8.	Cardiac Glycosides	+
9.	Phenol	+
10.	Alkaloid	+

GC-MS ANALYSIS

GC-MS analysis of methanolic leaf extract of *Clausena dentata* shows 36 peaks which indicate the presence of 36 major secondary metabolites. The result revealed the presence of 36 different chemical constituents with peak area percentage were 1H-Pyrrole, 1-methyl – (2.31%), Levoglucosenone – (0.09%), 2-t-Butyl-5-propyl-[1,3]dioxolan-4-one – (0.85%), L-Proline, 1-methyl-, methyl ester – (0.21%), cis-Linaloloxide – (0.14%), 2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis – (0.07%), 2,5-Dimethyl-4-hydroxy-3(2H)-furanone – (0.15%), l-4-Hydroxylysine lactone – (1.72%), Dodecane – (0.56%), Benzaldehyde, 4-(1-methylethyl) – (0.70%), 4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid – (0.77%), 2-Methoxy-4-vinylphenol – (0.57%), Pyrrolidine, 1,1'-methylenebis – (11.60%), Caryophyllene – (0.38%), Piperidine, 1-nitroso – (2.38%), 3',5'-Dimethoxyacetophenone – (0.54%), Caryophyllene oxide – (0.14%), N-(Cyclohexyl)succinimide – (0.30%), 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol – (0.22%), Tetradecanoic acid – (0.26%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol – (0.28%), Hexadecanoic acid, methyl ester – (0.28%), Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester – (0.27%), n-Hexadecanoic acid – (6.81%), 2-Allyl-4-methylphenol – (16.69%), 10,13-Octadecadiynoic acid, methyl ester – (16.69%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) – (0.45%), Phytol – (3.15%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) – (16.69%), 3,7,7-Trimethyl-1-penta-1,3-dienyl-2-oxabicyclo[3.2.0]hept-3-ene – (0.33%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester – (0.10%), Diisooctyl phthalate – (1.65%), Squalene – (2.15%), 1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E) – (0.18%), Neodiosgenin(3β,25S) acetate – (0.24%), Vitamin E – (9.92%)(Table 2). The GC-MS spectrum confirm the presence of 36 components with the retention time 2.69, 4.52, 6.03, 6.93, 7.46, 7.72, 7.89, 8.21, 9.27, 9.49, 10.30, 11.44, 12.13, 12.64, 13.29, 14.70, 14.83, 15.05, 15.82, 16.98, 17.45, 18.36, 18.67, 19.21, 19.60, 20.08, 20.16, 20.32, 20.98, 22.21, 24.00, 24.17, 27.64, 29.76, 33.28 and 36.08, respectively **(Fig. 1)**.Hymavathi*et al.*, (2009) carried out a bioassay

guided fractionation and chemical investigation of the stem bark of *Premna tomentosa* and reported the presence of fouricetexane diterpenes (icetexatriene-10,11,12,16-tetrol, 8,11,13 icetexatriene-10,11,16-triol, 8,11,13 icetexatriene7,10,11,16-tetrol and 7,10-epoxy-8,11,13 icetexatriene11,12,16-triol) and other known compounds such as coniferaldehyde, syringaldehyde, lupeol, betulin, and 2-(4-methoxyphenyl)- 2-butanone.

Similar types of compounds were identified among the thirty six compounds of this present studySermakkani*et al.*, (2012) identified 17 compounds with9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- as the major compounds in the methanol extract of leaves of *Cassia italic.* The compounds were found to have anti-inflammatory, cancer preventive, insectifuge, hypocholesterolemic, antihistaminic, nematicide, hepatoprotective, anticoronary properties anteczemic, antiacne.GC-MS analysis of ethanol extract of *Caesalpinia sappan*revealved the presence of 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and di-isooctyl ester (Sarumathy*et al.*, 2011).

Rajkumar and Jabanesen, (2010) The leaf essential oil of the *Clausena dentata* revealed presence of 14 major compounds was biofloratriene, sabinene, β-bisabolol and borneol. Parasuraman *et al.*, (2009) Octadecanoic acid and n-Hexadecanoic acid were identified in theleaves of *Cleistanthuscollinus.*Praveen kumar*et al.*, (2010) Hexadecanoic acid, Phyto, 9, 12 – Octadecadienoic acid, n-hexadecanoic acid, Squalene and 9, 12, 15-Octadecatrienoic acid was identified in the ethanol leaf extract of *Vitex negundo.*

Table 2. IDENTIFICATION OF COMPOUNDS FROM METHANOLIC LEAF EXTRACT OF *CLAUSENA DENTATA* BYGC-MS ANALYSIS

S.No	RT (min)	Name of Compounds	Molecular Formula	Molecular Weight	Peak Height	Peak Area %
1.	2.69	1H-Pyrrole, 1-methyl	C_5H_7N	81	7757003	2.31
2.	4.52	Levoglucosenone	$C_6H_6O_3$	126	912877	0.09
3.	6.03	2-t-Butyl-5-propyl-[1,3]dioxolan-4-one	$C_{10}H_{18}O_3$	186	3607323	0.85
4.	6.93	L-Proline, 1-methyl-, methyl ester	$C_7H_{13}NO_2$	143	3823232	0.21
5.	7.46	cis-Linaloloxide	$C_{10}H_{18}O_2$	170	2779879	0.14
6.	7.72	2-Furanmethanol, 5- ethenyltetrahydro α,α,5-trimethyl-, cis	$C_{10}H_{18}O_2$	170	1698163	0.07
7.	7.89	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128	1630109	0.15
8.	8.21	l-4-Hydroxylysine lactone	$C_6H_{12}N_2O_2$	144	31338046	1.72
9.	9.27	Dodecane	$C_{12}H_{26}$	170	13485501	0.56
10.	9.49	Benzaldehyde, 4-(1-methylethyl)	$C_{10}H_{12}O$	148	25773320	0.70
11.	10.30	4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid	$C_6H_{11}NO_3$	145	12762893	0.77
12.	11.44	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	11964512	0.57
13.	12.13	Pyrrolidine, 1,1'-methylenebis	$C_9H_{18}N_2$	154	57403450	11.60
14.	12.64	Caryophyllene	$C_{15}H_{24}$	204	15464808	0.38

15.	13.29	Piperidine, 1-nitroso	$C_5H_{10}N_2O$	114	20357370	2.38
16.	14.70	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180	14121173	0.54
17.	14.83	Caryophyllene oxide	$C_{15}H_{24}O$	220	6976303	0.14
18.	15.05	N-(Cyclohexyl)succinimide	$C_{10}H_{15}NO_2$	181	10490545	0.30
19.	15.82	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	$C_{14}H_{22}O$	206	7974338	0.22
20.	16.98	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	8415588	0.26
21.	17.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	15443732	0.28
22.	18.36	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	15953448	0.28
23.	18.67	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292	7516696	0.27
24.	19.21	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	124675075	6.81
25.	19.60	2-Allyl-4-methylphenol	$C_{10}H_{12}O$	148	380069483	16.69
26.	20.08	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	376215835	16.69
27.	20.16	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292	31001337	0.45
28.	20.32	Phytol	$C_{20}H_{40}O$	296	184997280	3.15
29.	20.98	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	278	274028700	16.69
30.	22.21	3,7,7-Trimethyl-1-penta-1,3-dienyl-2-oxabicyclo[3.2.0]hept-3-ene	$C_{14}H_{20}O$	204	16350664	0.33
31.	24.00	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	3204032	0.10
32.	24.17	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	75581899	1.65
33.	27.64	Squalene	$C_{30}H_50$	410	62444741	2.15
34.	29.76	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-	$C_{20}H_{34}O$	290	4199517	0.18
35.	33.28	Neodiosgenin(3β,25S) acetate	$C_{29}H_{44}O_4$	456	4130301	0.24
36.	36.08	Vitamin E	$C_{29}H_{50}O_2$	430	88934792	9.92

CONCLUSION

In the present study, methanolic leaves extract of *C. dentata*showed more number of secondary metabolites such assteroid, flavonoid, carbohydrates, tannins, cardiac glycosides and phenols were present. Coumarinsand saponins were found to be absent. Gas chromatography and mass spectroscopy analysis showed the existence of 36 phytochemical constituents which contribute the activities like anti-inflammatory, anticancer, hypercholesterolemia and other activities. GC-MS report can be used as drug detection and biochemical markers in the pharmaceutical industries to recognize the authentic plants and separate fromits adulterants.Further study is required for possible development of novel drugs using some of the bioactive compounds found in *C. dentata*.

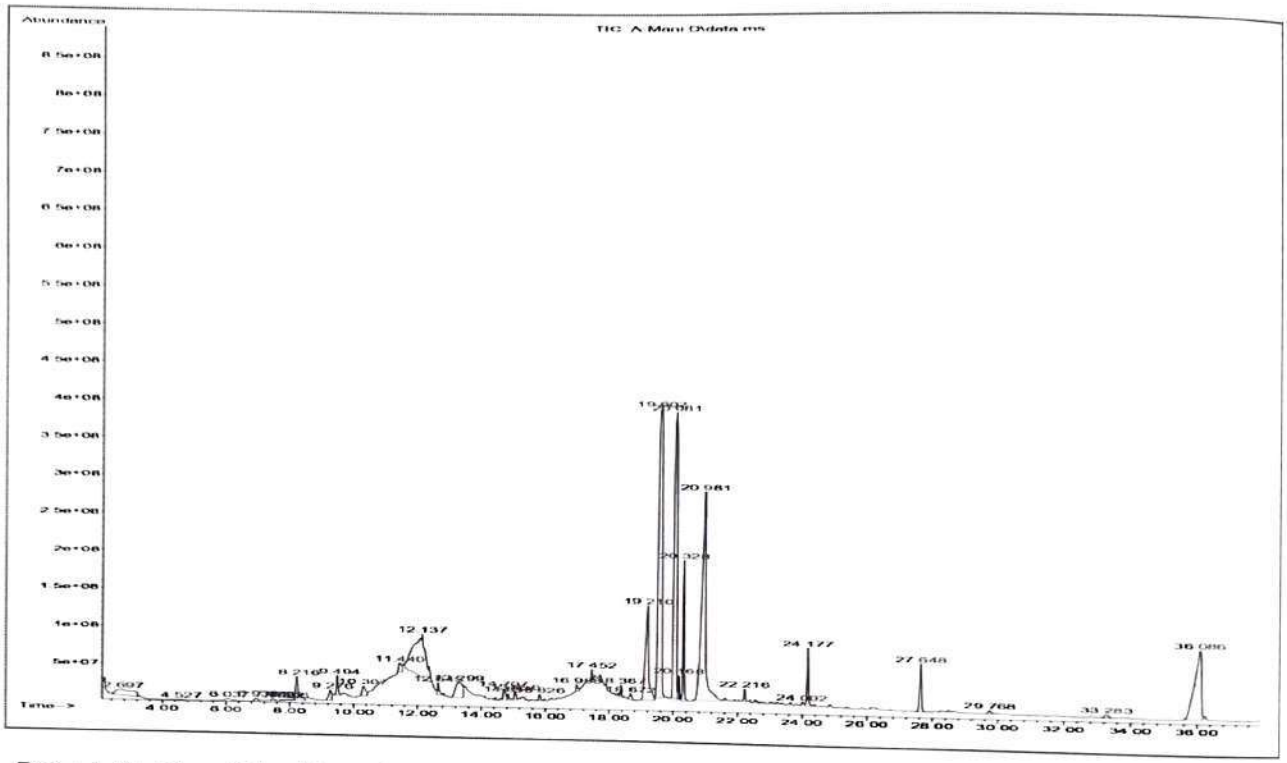
ACKNOWLEDGEMENT

The first author greatly acknowledges the Ministry of Earth Science (MoES), Government of India, New Delhi for providing financial support through a R&D project (Ref. No. MoEs/16/25/2013/RDEAS dated 03.07.2014). Authors are also extended the sincere gratitude to Department of Biotechnology, School of Bioengineering, SRM-IST (SRM University), Kattankulathur, for providing lab facilities to analysis the sample.

REFERENCES

Apparanantham, T. and Chelladurai. V. 1986. Glimpses of folk medicines of Dharmapuri forest division, Tamil Nadu. *Ancient Science of Life*,**5**: 412-413.

Fig 1. CHROMATOGRAM SHOWING RETENSION TIME OF COMPOUNDS FOUND IN LEAVES OF *CLAUSENA DENTATA*



Betz, J.M., Gay, M.L., Mossoba, M.M., Adams, S. and Portz, B.S. 1997. *Journal of AOAC International*, 80: 303.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. Glossary of Indian Medicinal Plants. New Delhi, India: C. S. I. R; p. 246.

Fiorentino, A., Castaldi, S., D'Abrosca, B., Natale, A., Carfora, A., Messere, A., Monaco, P., 2007. Polyphenol form the hydroalcoholic extract of *Arbutus unedo* living in a monospecific Mediterra-nean woodland. *Biochemical Systematics and Ecology*, 35: 809-811.

Harborne, J.B. 1994. Phytochemical methods: a guide to modern techniques of plant analysis. 2nd edn. 1994. Chapman and Hall, London 1-35.

Harborne, J.B. 1998. Phytochemical method: A Guide to Modern Techniques of plant Analysis. 2nd edn. *Chapman and Hall Publishers*, London, U.K.

Hymavathi, A., Suresh Babu, K., Naidu, G.M., Ramakrishna. S., Prakash, V.D. and MadhusudanaRao, J. 2009. Bioactivity-guided isolation of cytotoxic constituents from stem-bark of *Premna tomentosa*. *Bioorganic and Medicinal Chemistry Letters*, 19, 5727-5731.

Jie, M.S.F. and Choi, C.Y.C. 1991. *The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine*, 3: 122.

Kokate, C.K. 1996. Practical Pharmacognosy. 4th edn. VallabPrakashan, New Delhi. Pp10-107.

Krishnaraju, A.V., Rad, T.V.N. and Sundaraju, D. 1995. Assessment of bioactivity of India medicinal plants using brine Shrimp (Artemisalina) lethality assay. *International Journal of Applied Science and Engineering*, 2pp. 125-134.

Lee, J., Koo, N. and Min, D.B. 2004. Reactive oxygen species, aging and antioxidative

nutraceuticals. *Comprehensive reviews in food science and food safety,* 3(1):21-33.

Mace, M.E. 1963. Histochemical localization of phenol in healthy and diseases tomato roots. *Phytopatholoy,*16: 915-925.

Maria Jancy Rani, P., Kannan, P.S.M. and Kumaravel, S. 2011. GC-MS Analysis of *Lantana camara* L. leaves. *Journal of Pharmaceutical Research and Development,* 2(11): 63-66.

Merlin, N.J., Parthasarathy, V., Manavala, R. and Kumravel., S.S. 2009. Chemical investigation of aerial parts of Gmelina asiatica Linn by GC-MS.*Pharmacognosy Research*,1(3), 152-156.

Pandith, S.A., Hussain, A., Bhat, W.W., Dhar, N., Qazi, A.K., Rana, S., Razda, S., Wani, T.A., Shah, M.A. and Bedi, Y.S., Hamid, A. and Lattoo, S.K. 2014. Evaluation of anthraquinones from Himalayan rhubarb (Rheum emodi Wall. ex Meissn) as antiproliferative agents. *South African Journal of Botany,*95: 1-8

Parasuraman, S., Raveendran, R. and Madhavrao, C. 2009. GC-MS analysis of leaf extracts of *Cleistanthuscollinus*Roxb. (Euphorbiaceae). *International Journal of Pharmacological Science,* 1(2): 284-286.

Praveen kumar, P., Kumaravel, S. and Lalitha, C. 2010. Screening ot antioxidant activity, total phenolics and GC-MS study of *Vitex negundo. African Journal of Biochemistry Research,* 4(7): 191-195.

Rajkumar, S. and Jabanesan, A. 2010. Chemical composition and larvicidal activity of leaf essential oil from Clausena dentata (Willd.) M. Roem (Rutaceae) against the chikungunya vector, Aedes aegypti L. (Diptera: Culicidae). *Journalof Asia-Pacific Entomology.* b; 13: 107-109.

Rajkumar, S. and Jebanesan, A. 2010. Prevention of Dengue fever through plant based mosquito repellent *Clausena dentata* (Willd.) M. Roem (Family: Rutaceaece) essential oil against *Aedes aegypti* L. (Diptera: Culicidae) mosquito. *European Review for Medical and Pharmacological Sciences,* 14(3): 231-234.

Rashmi Tambe, R.G., Singhal, Kiran Bhise. and Maushmi Kulkarni, 2014. Phytochemical screening and HPTLC fingerprinting of leaf extracts of *Psidium guajava* Linn. *Journal of Pharmacognosy and Phytochemistry,*3(1); 52-56.

Sarumathy, K., Vijayayakanthia, T. and Dhana Rajan, M.S. 2011. A Protective effect of *Caesalpinia sappan(CS)* on acetaminophen induced Nephrotoxicity and oxidative stress in male *albino rats. Journal of Pharmacology and Toxicology,* 1(2): 11-21.

Sermakkani, M. and Thangapandian, V. 2012. GC-MS Analysis of *Cassia italica*leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research,* 5(2): 90-94.

Sofowora, A. 1993. Medicinal plants and traditional medicinal in Africa, spectrum books, *Ibadam.* Pp.150.

Subba Rao, G.S., Srinivasa Rao, K. and Ravindranath, B. 1976. Structure of diclausenan A and B. *Tetrahedron Letters,* 197613:1019-20.

Trease, G.E. and Evans, M.D. 1989. A text book of Pharmacognosy 13thedn. Baillier, Tindal and Caussel, Landon. Pp.144. 144-148.

Wang, Y.S., He, H.P., Yang, J.H., Shen, Y.M., Zhou, J. and Hao, X.J. 2005. A New Cyclopeptide from *Clausenaanisum-olens. Helvetica ChimicaActa*, 88, 2345-2348.