ORIGINAL ARTICLE Open Access

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences

GC-MS analysis of methanolic extract of stem and root bark of *Kirganelia reticulata* for bioactive components

R. A. Reddy[1], V. Krishna[1], S. Usha[2], R. Bharathi[3], V. K. Murthy[4*]

[1]Department of P.G. Studies & Research in Biotechnology & Bioinformatics, Kuvempu University, Shankaraghatta-577451, Karnataka, India; [2] Department of Biotechnology, Dayananda Sagar College of Biological Sciences, Bangalore 560078, Karnataka, India; [3]Department of Computer Science Engineering, BMS Institute of Technology, Bangalore-560 064; Karnataka, India; [4*]Department of Biotechnology, PES University, Bangalore-560 085, Karnataka; India

Manuscript received 08June, 2017, revised 30th June 2017, accepted 1st July 2017

Abstract

Objective: To identify the bioactive components present in the stem and root bark extracts of *Kirganelia reticulata.* **Methods:** The soxhlet extracted crude methanolic extracts of stem and root bark of *Kirganelia reticulata* were analysed by GC-MS. **Results:** GC-MS analysis revealed the presence of 27 and 24 potential bioactive components in stem and root bark extract of *Kirganelia reticulata* respectively. These components were mostly esters, phenols, flavonoids, aldehydes, alkaloids, sterols and terpenoids with biological activity. **Conclusion:** Many of the bioactive components identified in the study are multifunctional in nature with potent antioxidant, antimicrobial, antifungal, antiviral, candidicide, hypocholesterolemic, anti-inflammatory, anti-cancer, anti-androgenic, antiasthma, diuretic and hepatoprotective in nature justifying the use of this plant in traditional medicine for treating various ailments and also providing an opportunity for identification of potential drug candidates.

Keywords: *Kirganelia reticulata*, Root, Stem, Bark, Methanol extract, GC-MS analysis, Bioactive components, Anticancer.

1. Introduction

Plant based biologically active molecules have been found applications in pharmaceuticals, nutritional supplements, cosmetics, agrochemicals and fine chemicals. Plants are the major source of novel drugs with potential biomedical activity. The plant based drugs are derived either from the whole plant or individual organs such as leaves, stem, bark, root etc. and are safer and environment friendly. Many plant derived drugs have been used for prevention as well as for treatment of many serious diseases.

Gas chromatography coupled with Mass spectrometry (GC–MS) is a powerful and a valuable tool due its simplicity, sensitivity and effectiveness in separating components of mixtures. Hence, it is being used extensively for the analysis of non-polar components and volatile essential oils, fatty acids, lipids and alkaloids in plant extracts (1). In addition, it is also used for quality control and standardization of phyto therapeuticals (2).

Kirganelia reticulata, a monoecious scandent shrub belonging to the family euphorbiaceae grows throughout the tropical areas of India, China, Bangladesh as well as Malay islands (3, 4). The leaf juice of this plant is diuretic, cooling and antidiarrheal in nature. The stems are used to treat sore eyes while bark is used to treat rheumatism, dysentery, venereal diseases, small pox, syphilis, asthma, diarrhoea and bleeding gums (5,6,7). This plant is also known to possess antimicrobial, antiprotozoal, antiviral and antioxidant activities (8, 9, 10). The chemical analysis of this plant has revealed the presence of octacosanol, texerol acetate, berlin, sitosterol, tannins, flavanoids and glycosides etc (11, 12, 9). GC-MS analysis of aerial part of this plant revealed the presence of many bioactive components (13). Although, our previous study has indicated the potential cytotoxic and antitumor nature of methanolic extracts of *Kirganelia reticulata*, the potential secondary metabolites responsible for such an activity is not known (14). In addition, in-depth analysis for the presence of

***Corresponding author**
Full Address :
Department of Biotechnology, PES University, Bangalore-560 085, Karnataka; India
Phone no. +91-9449867806
E-mail: v.krishnamurthy@pes.edu

other bioactive components in stem and root bark has not been studied. Hence, the present study was conducted to analyze the methanolic extracts of stem and root bark of *Kirganelia reticulata* for the presence of bioactive components by GC-MS.

2. Materials and methods

2.1 Plant Material

Kirganelia reticulata plants were collected from Savanadurga forest, one of the most important medicinal plant conservation areas of Karnataka, India. The bark from the stem and root of *Kirganelia reticulata* plant were separated and washed with deionized water. Further, the bark was shade dried at room temperature for ten days and ground to coarse powder using a mechanical blender.

2.2 Extraction of Plant Material

The secondary metabolites were extracted from separated bark of *Kirganelia reticulata* using methanol as a solvent as reported previously (14). Briefly, 10 g of powder from stem and root bark was packed separately with Whatman filter paper No1 and extracted with 150 ml of 70% methanol in a soxhlet extractor at 70˚C for 4 hours. After filtration, the solvent was removed by evaporation using a rotary evaporator under reduced pressure at temperature below 50^{0}C. The dried methanolic bark extracts were stored in a refrigerator and used for GC-MS analysis.

2.3 GC-MS Analysis

The chemical composition of the methanolic extracts of stem and root bark was analyzed by GC-MS. The analysis was carried out on Shimadzu GCMS QP2010S comprising a AOC-20i auto sampler and chromatograph interfaced to a mass spectrometer with a column (5% Diphenyl, 95% Dimethyl Poly Siloxane) length of 30 m with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. An injection volume of 1µl plant extract was injected in split ratio of 10:1 with an injection temperature of 300ºC. The column oven temperature was 100 ºC and gradually increased to 320 ºC at the rate of 10 ºC/min. The linear velocity was 37.2 cm/sec with a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200ºC and 325ºC respectively with solvent cut time of 2.00 min. The MS program starting time was 2.00 min which ended at 30.00 min with interval time of 0.50 sec with a scan speed of 1000. The relative percentage of the extract was expressed as percentage with peak area normalization.

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST). The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library. The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total area. The name and molecular weight of the components of the test materials were ascertained. The biological activities described are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3. Results and discussion

GC-MS analysis of methanolic extract of stem bark of *Kirganelia reticulata* revealed the presence of 27 components (Fig. I) while that of root bark extract 24 components (Fig. II). The molecular weight, molecular formula, retention time and concentration (%) of active components from stem and root bark is presented in Table I and Table II respectively. Thirteen compounds were found to be common for both stem and root bark. The major phytochemicals found in stem bark extracts are 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (CAS) 3,5-DIHYDROXY-2-METHYL-5,6-DIHYDROPYRAN (17.04%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)-(15.08%), Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester (11.28%), n-Hexadecanoic acid (10.45%) and .beta.-Sitosterol (4.39%). In the root bark extract, Lupeol (10.70%) was present at the highest level followed by 2-AMINO-9-(3,4 -DIHYDROXY-5-HYDROXYMETHYL-TETRAHYDRO-FURAN-2-YL)-3,9-DIHYDRO PURI (9.99%), Cholest-5-en-3-ol (3.beta.)-, tetradecanoate (9.77%), Stigmasterol (9.16%) and Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-(9.13%). Many of the identified compounds tend to possess multiple biological functions.

In the previous study, presence of 21 compounds from the ethanolic extract of aerial parts of *Kirganelia reticulata* has been reported (13). But the present study revealed the presence of 27 compounds from stem bark extract alone. However, a few of the components reported to be present in the earlier study such as vitamin E, phytol , squaline, octacosane, nonadecane etc are missing in our study. This anomaly could be due to use of different solvents as well as plant parts for extraction. In the previous study, combined ethanolic extract of stem and leaves was used while in the present study only the methanolic extract of stem bark was used for analysis.

The flavanoid compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (CAS) 3,5-DIHYDROXY-2-METHYL-5,6-DIHYDROPYRAN is a major component of stem bark with as much as four times higher than that of root bark. Its presence has been reported previously from *Mussaenda frondosa* and *Aegle marmelos* albeit at lower levels (15, 16). It has potential antimicrobial, anti-inflammatory and antiproliferative activity. Similarly, the concentration of 2-Furancarboxaldehyde, 5-

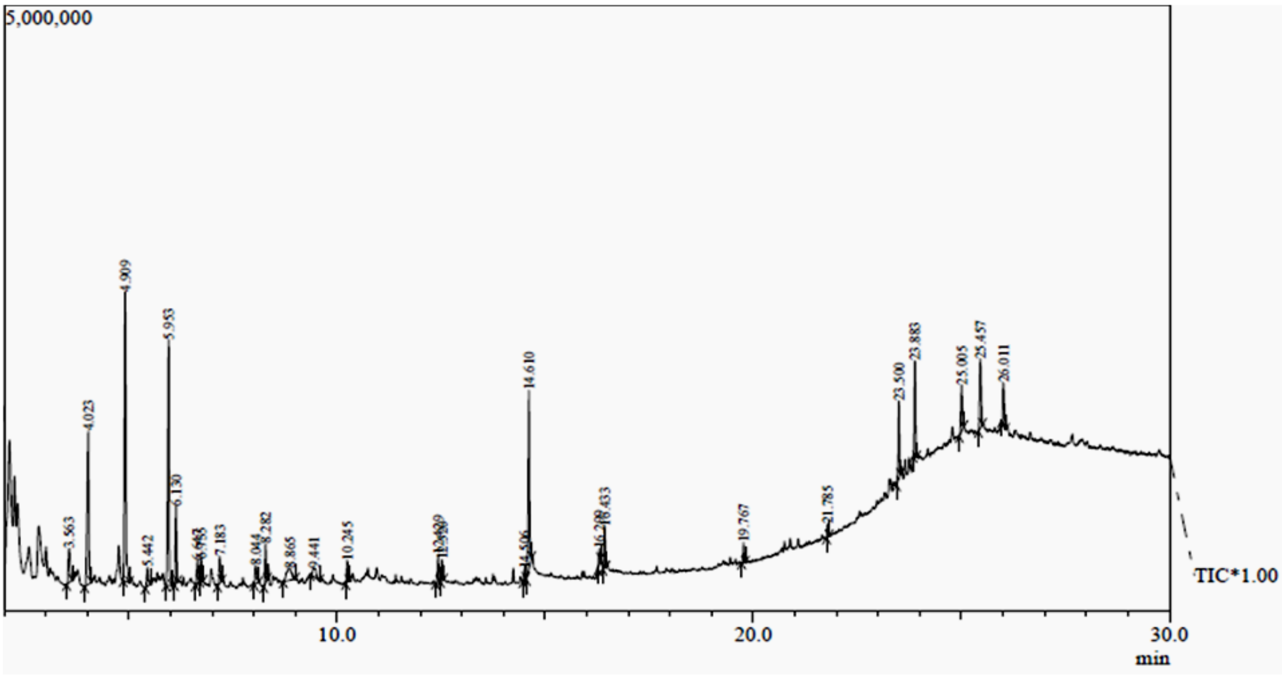

Figure I: GC-MS Chromatogram of the methanolic stem bark extract of *Kirganelia reticulata*

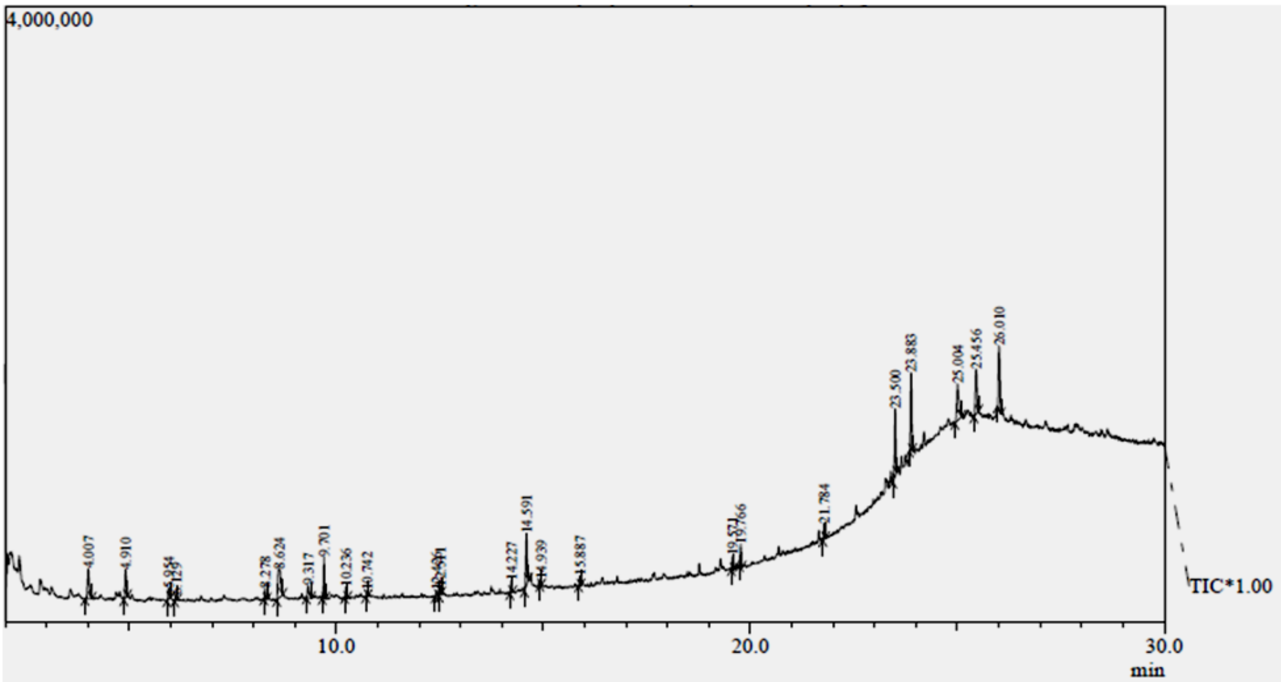

Figure II: GC-MS Chromatogram of the methanolic root extract of *Kirganelia reticulata*

(hydroxymethyl)- is almost 8 times higher in stem than in root. This compound exhibits antimicrobial activity and its presence has been reported previously from *Mussaenda frondosa and Emblica officinalis* (15, 17). n-Hexadecanoic acid and tetradecanoic acid are found both in stem and root bark extracts. The former is antioxidant, hypocholesterolemic, nematicide, antiandrogenic and has hemolytic 5-Alpha reductase inhibitor activity while the latter is a nematicide and antibacterial.

The level of beta.-Sitosterol and Cholest-5-en-3-ol (3.beta.)-tetradecanoate is two to three times higher in root bark than in stem bark. beta.-Sitosterol is antimicrobial, anti-inflammatory, anticancer, antiasthma, diuretic and hepatoprotective and its presence has been reported earlier in *Atalantia wightii* and *Evolvulus alsinoides* (18, 19). Similarly, the level of stigmasterol in root bark is almost thrice that of stem bark. This compound exhibits antimicrobial, anti-inflammatory, anticancer, antiasthma and hepatoprotective activity. In addition, Stigmasterol also possesses anti-osteoarthritis and cholesterol lowering activity (20).

Lupeol (10.70%) was found to be the major bioactive component in the bark extract and exhibits broad-spectrum of biological activities such as antimalarial, antiflu, antiviral, antioxidant, anti-inflammatory, antiperoxidant, antitumor and antimalarial. Previously,

Table I: Components detected in the methanolic stem bark extract of *Kirganelia reticulata*

No.	RT (Min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	3.563	1,4-Dioxin, 2,3-dihydro-5,6-dimethyl- (CAS) 5,6-DIMETHYL-2,3-DIHYDRO-1,4-DIOXIN	C6 H10 O2	114	2.83
2	4.023	Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester	C6H8N4O2	168	**11.28**
3	4.909	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (CAS) 3,5-DIHYDROXY-2-METHYL-5,6-DIHYDROPYRAN	C6 H8 O4	144	**17.04**
4	5.442	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	C6H6O4	142	1.33
5	5.953	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C6H6O3	126	**15.08**
6	6.130	1,2,3-Propanetriol, diacetate	C7H12O5	176	4.04
7	6.647	1,3-Dioxolane, 2-ethenyl-2,4-dimethyl-, trans- (CAS) TRANS-2-VINYL-2,4-DIMETHYL-1,3-DIOXOLANE	C7 H12 O2	128	1.57
8	6.735	n-Propyl acetate	C5H10O2	102	1.25
9	7.183	Cyclopentanone, 2-methyl- (CAS) 2-Methylcyclopentanone	C6 H10 O	98	1.59
10	8.044	1,2,3-Benzenetriol (CAS) 1,2,3-Trihydroxybenzene	C6 H6 O3	126	1.54
11	8.282	1-Methyl-1-(3-methylbutyl)oxy-1-silacyclobutane	C9H20OSi	172	2.15
12	8.865	2-AMINO-9-(3,4-DIHYDROXY-5-HYDROXYMETHYL-TETRAHYDRO-FURAN-2-YL)-3,9-DIHYDRO-PURI	C10 H13 N5 O5	283	2.75
13	9.441	D-Allose	C6H12O6	180	1.39
14	10.245	Dodecanoic acid (CAS) Lauric acid	C12 H24 O2	200	0.95
15	12.429	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C10H12O3	180	1.54
16	12.520	Tetradecanoic acid	C14H28O2	228	0.92
17	14.506	Hexadecenoic acid, Z-11-	C16H30O2	254	0.47
18	14.610	n-Hexadecanoic acid	C16H32O2	256	**10.45**
19	16.299	9-Octadecenoic acid (Z)- (CAS) Oleic acid	C18 H34 O2	282	0.68
20	16.433	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]- (CAS) Dowanol TBAT	C10 H22 O4	206	1.65
21	19.767	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate	C24 H38 O4	390	0.83
22	21.785	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	C30 H50	410	0.54
23	23.500	Cholesta-6,22,24-triene, 4,4-dimethyl-	C29H46	394	3.89
24	23.883	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate	C41H72O2	596	4.34
25	25.005	Stigmasterol	C29H48O	412	3.15
26	25.457	.beta.-Sitosterol	C29H50O	414	**4.39**
27	26.011	Oct-5-en-2-ol, 8-(1,4,4a,5,6,7,8,8a-octahydro-2, 5, 5, 8a -tetramethylnaphth-1-yl)-6-methyl-	C23H40O	332	2.35

its presence has been reported in bark extracts of *Pterocarpus marsupium* Roxb. (21). Stigmasta-5,22-dien -3-ol, acetate, (3.beta.)- (9.13%) was found only in root extracts and its presence has been previously reported from *Nymphaea mexicana* and *Lawsonia inermis* albeit at lower levels (22, 23). 3, 5-di-t-butyl phenol (4.50%) found only in root bark is a potent antioxidant, antimicrobial, antifungal and anti-inflammatory. Similarly, 1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate (2.36%) present only in root bark extract has antifouling and antimicrobial activity.

Dodecanoic acid (CAS) Lauric acid, a minor component found both in stem and root bark is antibacterial, antioxidant, antiviral, COX-1 & COX-2 inhibitor, hypercholesterolemic and candidicide (24).

Many of the components found in the bark extracts of *Kirganelia reticulata* exhibits broad-spectrum biological activity indicating their importance has drug targets. This

Table II: Components detected in the methanolic root extract of *Kirganelia reticulata*

No.	RT (Min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	4.007	Acetic acid,1-(2-methyltetrazol-5-yl) ethenyl ester	$C_6H_8N_4O_2$	168	6.35
2	4.910	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl - (CAS) 3,5-DIHYDROXY-2-METHYL-5,6-DIHYDROPYRAN-	$C_6H_8O_4$	144	4.26
3	5.954	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	1.92
4	6.129	1,2,3-Propanetriol, diacetate (CAS) Diacetin	$C_7H_{12}O_5$	176	0.84
5	8.278	1-Allyl (dimethyl) silyloxypropane	$C_8H_{18}OSi$	158	1.35
6	8.624	2-AMINO-9-(3,4-DIHYDROXY-5-HYDROXYMETHYL-TETRAHYDRO-FURAN-2-YL)-3,9-DIHYDRO-PURI	$C_{10}H_{13}N_5O_5$	283	**9.99**
7	9.317	1-Dimethyl(ethenyl) siloxybutane	$C_8H_{18}OSi$	283	2.67
8	9.701	Phenol,3,5-bis(1,1-dimethylethyl)-(CAS) 3,5-Di-tert-butylphenol	$C_{14}H_{22}O$	206	4.50
9	10.236	Dodecanoic acid (CAS) Lauric acid	$C_{12}H_{24}O_2$	200	1.27
10	10.742	Phthalic acid, di-(1-hexen-5-yl)ester	$C_{20}H_{26}O_4$	330	0.66
11	12.426	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	0.97
12	12.511	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.01
13	14.227	Octadecanoic acid, methyl ester (CAS) Methyl stearate	$C_{19}H_{38}O_2$	298	1.51
14	14.591	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.75
15	14.939	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	0.54
16	15.887	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	1.15
17	19.571	Hexadecanal (CAS) PALMITIC ALDEHYDE	$C_{16}H_{32}O$	240	1.74
18	19.766	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate	$C_{24}H_{38}O_4$	390	2.36
19	21.784	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	$C_{30}H_{50}$	410	1.93
20	23.500	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	$C_{31}H_{50}O_2$	454	**9.13**
21	23.883	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate	$C_{41}H_{72}O_2$	597	**9.77**
22	25.004	Stigmasta-5,22-dien-3-ol, (3.beta.,22E)- (CAS) Stigmasterol	$C_{29}H_{48}O$	412	**9.16**
23	25.456	Beta.-Sitosterol	$C_{29}H_{50}O$	414	8.50
24	26.010	Lupeol	$C_{30}H_{50}O$	426	**10.70**

study also reveals the presence of many potential anticancer components which could be responsible for cytotoxic and antitumor ability reported for this plant by our group (14). However, further analysis of individual components is necessary for selection of these components as drug targets.

4. Conclusion

The present study has revealed the presence of many bioactive components in the methanolic extracts of stem and root bark wherein around 50% of the components were common to both stem and root. Many of the identified components found to exhibit broad-spectrum activity including anticancer activity. The presence of various bioactive compounds in *Kirganelia reticulata* not only proves its pharmaceutical importance but also reinforces its potential anticancer nature as previously reported by our group. Further investigation of the individual components which exhibited anticancer activity is necessary for exploiting them as a source of anticancer drugs in future.

Acknowledgement

We sincerely thank the management of PES Institute of Technology, Bangalore, India for providing encouragement and laboratory facilities and Vittal Mallya Research Foundation, Bangalore for GC-MS Analysis.

Conflict of interest

The author's declares none.

References

1. Mythili K, Umamaheswara Reddy C, Chamundeeswari D and Manna PK. GC-MS analysis of phytocomponents and *in vitro* inhibitory effect of *Calantha triplicate*. J. Nat. Prod *2013;* 6:141-46.
2. Marston A. Role of Advances in Chromatographic Techniques in Phytochemistry. *Phytochemistry* 2007; 68: 2785-97.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants, vol 1. 2ndEd. Bishen Singh, Mahendra Pal Singh Publications. India; 1980. p. 345.
4. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses, 2ndEd. Dhaka. Asiatic Society of Bangladesh; 2003. p. 345.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, 2 ndEd. New Delhi, India; 1956.
6. Nadkarni KM. Indian Materia Medica. vol 2. City Publishers; 1982. p. 948.
7. Yoganarasimhan SN. Medicinal Plants of India. Vol I, Karnataka. Interline Publishing Private Limited. India; 1996. p. 275.
8. Dhar ML, Dhar MM, Dhawan BM, Mehrotra BK and Ray AC. 1968. Screening of Indian Plants for Biological Activity: Part I. Indian Journal of Experimental Biology. 6(4):232-47.
9. Shruthi SD, Ramachandra YL, Padmavathi Rai S, Veena Shetty A. Anti bacterial potential of leaf extracts from Kirganelia reticulata Baill. International journal of Pharma Research and Development 2010; 2(6): 1-7.
10. Shruthi SD, Rajeshwari A, Govardhan Raju K, Pavani A, Vedamurthy AB, Ramachandra YL. Phytochemical and antioxidant analysis of leaf extracts from *Kirganelia reticulata* Baill. Int J Pharm Pharm Sci 2012; 4(3):608-12.
11. Joshi KC, Singh P, Mehta A. Crystalline components of the roots of *Phyllanthus reticulatus*. J Ind Chem Soc 1991; 58: 102.
12. Rav MRR, Siddiqui HH. Screening of Indian plants for biological activity. Indian journal of Experimental Biology 1996; 2: 49.
13. Sudha T, Chidambarapillai S and Mohan VR. GC-MS analysis of bioactive components of aerial parts of *Kirganelia reticulata* Poir (Euphorbiaceae). J. Curr. Chem. Pharm 2013; *3(2):113-22.*
14. Ravikumar A, Madgaonkar, V, Venkatesha RT, Bharathi R and Krishnamurthy V. Potential cytotoxic drug effects of secondary metabolites derived from selected medicinal plants of Savanadurga forest in Karnataka. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6 (10): 38-24.
15. Gopalakrishnan S. GC-MS Analysis of some bioactive constituents of *Mussaenda frondosa Linn*. International Journal of Pharma and Bio Sciences 2011;2 (1): 313-20.
16. Mujeeb F, Bajpai P and Pathak N. Phytochemical Evaluation, Antimicrobial Activity and Determination of Bioactive Components from Leaves of *Aegle marmelos.* BioMed Research International 2014, 1-11.
17. Balasubramanian S, Ganesh D, Panchal P, Teimouri M and Surya Narayana VVS. GC-MS analysis of phytocomponents in the methanolic extract of *Emblica officinalis Gaertn* (Indian Gooseberry). Journal of Chemical and Pharmaceutical Research 2014; 6(6):843-45.
18. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K & Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L J Food Sci Technol 2013;52 (2):1212–17.
19. Das AK and Swamy S. Antioxidant activity and determination of bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia species* from south India. Journal of Applied Pharmaceutical Science 2016; 6 (02):130-34.
20. Chen W, Chong Y, Peng-Fei H, Jia-Peng B, Jing-Li T, Li-Dong W. Stigmasterol blocks cartilage degradation in rabbit model of osteoarthritis. Acta Biochim Pol 2012; 59(4):537-41.
21. Maruthupandian A, Mohan VR. GC-MS analysis of some bioactive constituents of *Pterocarpus marsupium* Roxb 2011; 3(3): 1652-57.
22. Shah U, Baba WN, Ahmad M, Shah A, Gani A, Masoodi FA, Gani A, Ashwar BA. *In vitro* antioxidant and anti-proliferative activities of seed extracts of *Nymphaea mexicana* in different solvents and GC-MS analysis 2014; 6 (4): 68-79.
23. Dev SNS, De K and Khan MW. GC-MS Analysis of phytochemicals of methanolic extract of leaves of *Lawsonia inermis* Lin. Indian Journal of Medical Research and Pharmaceutical Sciences 2016; 3(6): 77-82.
24. Mitova M, Taskova R, Popov S, Berger RG, Krings U, Handjieva N. GC/MS analysis of some bioactive constituents from *Carthamus lanatus* L. Z Naturforsch C 2003;58(9-10):697-703