ejpmr, 2017,4(1), 541-545

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INVITRO ANALYSIS OF *KALANCHOE PINNATA* LEAVES TO ASSESS BIOACTIVE COMPOUNDS BY USING GC-MS

***[1]A. Rajesh and [2]Dr. Mohamed Shamsudin**

[1]*Department of Biotechnology, Thanthai Hans Roever College-Perambalur.
[2]Dean & Head, Department of Zoology, Jamal Mohamed College-Thruchirapalli.

***Corresponding Author: A. Rajesh**

Department of Biotechnology, Thanthai Hans Roever College-Perambalur.

Article Received on 22/11/2016 **Article Revised on 12/12/2016** **Article Accepted on 01/01/2017**

ABSTRACT

Kalanchoe pinnata (lam) per belonging to the family of Crassulaceae (stone crop family), which is a commonly known as air and succulent plant and native to Madagascar. In India it is distributed throughout the plains, rain forest and other places. *Kalanchoe pinnata* considered to be as a significant folk medicine for the various ailments and insect bite. A very less scientific study has been conducted on its biological control activity, medicinal, pharmacological and ethano botanical aspects of this plant. The current study was carried out to analyze the active phytoconstituents present in the Ethyl acetate solvent extract of plant leaves. Totally forty-four constituents were identified in the gas chromatography with mass spectroscopic analysis of Ethyl acetate extract of plant leaves of *Kalanchoe pinnata.*

KEYWORDS: *Kalanchoe pinnata*, Bisphthalate, 1-2 Benzene-di-carboxylic acid, 2-3 Dihydroxypropyl acetate, Pesticidal activity, vector control agent.

INTRODUCTION

Medicinal plants are used in traditional treatments to cure variety of diseases and act as biological control agents. In the last few decades there has been an exponential growth in the field of herbal medicine. Natural products have been a source of drugs for centuries. GC-MS based metabolites analysis has profound applications in discovering the mode of action of drugs, biopesticides or herbicides and helps unravel the effect of altered gene expression on metabolism and organism performance in biotechnological applications.[4] Fresh leaf juice or infusion is used for leishmaniasis and applied externally and taken internally for various bacterial, viral and fungal infections.[13]

Description

Kalanchoe pinnata (syn: Bryophyllum pinnatum, B. calycinum; Tamil name: Ranakalli; English name: Air plant; Family: Crassulaceae) is an herb found ubiquitously. *Kalanchoe* is succulent perennial that grows 3-5-feet tall, hallow stem, fleshy dark green leaves that are distinctly scalloped and trimmed in reddish purple and bell-like pendulous flowers. It is commonly known as Air plant, love plant, miracle leaf, life plant, Zakham-e-hyat, panfutti, Ghayamari has been accepted as a herbal remedy in almost all parts of the world.[2,5,10] The leaves of the plant often produce, on their crenature at the extremities of the lateral nerves, buds furnished with root, stems and leaves, which drop off and at one become new plants.[6]

BOTANICAL DESCRIPTION

Kingdom	:	Plantae (plants)
Subkingdom	:	Tracheobionta (vascular plants)
Super division	:	Spermatophyta (seed plant)
Division	:	Magnoliophyta (Flowering plant)
Class	:	Magnoliopsida (Dicotyledonous)
Sub class	:	Rosidae
Order	:	Saxifragales
Family	:	Crassulaceae (stone crop family)
Genus	:	*Kalanchoe*
Species	:	*Kalanchoe pinnata* (Lam) pers

DISTRIBUTION

Kalanchoe pinnata has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Micronesia, Macaronis, Galapagos, Melanesia, Polynesia and Hawaii. In many of these, such as Hawaii, it is regarded as an invasive species. In French Polynesia, *Kalanchoe pinnata* has been declared a threat of biodiversity. It is also widely distributed in the Philippines and it is known as katakataka which is also an adjective meaning astonishing or remarkable. In India it is cultivated in gardens and wild on the hills of north-western India and Bengal.[11]

Kalanchoe is a medicinal plant largely used in folk medicine for various treatments. It is distributed throughout India and cultivated in gardens and wild on the hills of north-western India, Deccan and Bengal. It grows widely and used as folk medicine in tropical Africa, India, China, Australia and tropical America, Madagascar, Asia and Hawaii.[7,18]

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties.[1]

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.[12]

MATERIALS AND METHODS

Collection and authentication of plant material

Fully developed leaves of the *Kalanchoe pinnata* were collected from Perambalur district and Western hills (Nilgiris, Kerala), India. The plant was identified, authenticated by the voucher specimen (Herbarium) have been deposited in the ABS botanical garden in Karipatty, Salem District and Tamil Nadu. Mr.A. Balsubramnian (Consultant central siddha research) Executive Director ABS botanical garden, Salem, authenticated the plant as ***Kalanchoe pinnata (LAM.)PERS.*** (Family-Crassulaceae). The leaves of Plant *Kalanchoe pinnata* were dried under shade and pulverized.

Preparation of plant extracts

The leaves of the plant were washed with tap water, cut into small pieces, shade-dried for two months and finely pulverized by a mechanical grinder and passed through a mesh sieve. The powder was successively extracted with solvent as ethyl acetate (40°-60°C). The extracts were concentrated under reduced pressure in a rotary evaporator (Buchi, USA). The solvent extracts of the plant was used for GC-MS analysis.

Gas chromatography – Mass Spectrum analysis

The GC-MS analysis of the plant extract was made in a (QP 2010 Ultra) instrument under computer control at 70 eV. About 1µL of the ethyl acetate extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT). While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using Gas Chromatography and Mass Spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane).

Identification of components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures NIST.LIB[14,15], WILEY.LIB.[8,9] Library sources were used for matching the identified components from the plant material. Interpretation mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Techniques which consist of more than 8,000,000 patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULT

GC-MS chromatogram of the ethyl acetate extract of plant leaves of *Kalanchoe pinnata (lam.)pers.* **(fig.1)** clearly shows 44 peaks indicating the presence of 44 phytochemical compounds. The identification of the phytochemical compounds were based on the peak area, retention time and compound name. The **table 1** shows the compound name with its, molecular weight, run time and % area. The results reveal the presence of first and second compounds identified with less retention time (6.632 min) was 1, 2, 3-Propanetriol- 1-Acetate, Third compound identified 2, 3-Dihydroxypropyl acetate, Fourth Compound 1, 2, 3 Propanetriol, Triacetate, Fifth compound Phenol, 2, 4 –Bis (1, 1-Dimethylethyl, Sixth compound 2 (4H) –Benzofuranone, 5, 6, 7, 7a, tetra hydro -4, 4, 7a-trimetyl, Seventh compound Dodecanoic

acid, Eighth compound 1- Hexadecanone, Ninth compound Sulfurous acid, 2-ethylhexyl ester, Tenth compound 8-Pentadecanone.

Eleventh compound 2- Bromododecane Tetradecanoic acid, Twelfth compound Benzene, Ethylphenoxy, Thirteen compound Tetradecanoic acid, Fourteenth compound 1-Decan –one, Fifteenth compound n-Nonadecanol-1, Sixteenth compound Erythro-9,10 diromopentacosane, Seventeenth compound 2,3- Bis (1-methylallyl) pyrolidine, Eighteenth compound 2, 6, 10-Trimethyl, 14-ethylene-14-pentadecane, Nineteenth compound 9-Octadecan-1-ol,(z), Twentieth compound 1,2, Benzene dicarboxylic acid, Bis (2-Methylpropyl) Ester. Twenty first compound Phthalic acid, butyl undecyl ester, Twenty second compound Pentadcanoic acid, Twenty third compound 1- Nonadecane, Twenty fourth compound Hexadecanoic acid, trim ethyl silyl ester, Twenty fifth compound 10-Nondecanone, Twenty sixth compound 2-Hexadecan -1-ol, 3,7, 11, 15 tetra methyl, Twenty seventh compound 9, 12, Octadecadienoic acid, Twenty eighth compound N-teracosenol-1, Twenty ninth 1, 3, 3, -Trimethyl-1-(2hydroxy phenyl) indan-6-ol, Thirtieth compound N-Tetracosanol-1.

Thirty first compound Phenol, 2, 4, bis (1-phenylethyl), Thirty second compound Phenol, 2, 4, bis (1-phenylethyl), Thirty third compound 1, 2, Benzene dicarboxylic acid, Thirty-fourth compound Phenol, 2, 4 –bis (1-phenylethyl), Thirty fifth compound Bis(2-Ethylhexyl) phthalate, Thirty sixth compound 2-menthyloctacoscane, Thirty-seventh compound IsoQuinoline,1-(3, 4-Dimethoxy phenyl) methyl, 6, 7-Dimethoxy, Thirty-eighth compound 2, 8-Dimethyl-2-(4,8,12, Trim ethyl Tridecyl)-6-Chromanol, Thirty-ninth compound Octacosyl acetate, Fortieth compound Octadecanal. Forty-first compound 4-5-2H- Oxazole-5-one, 4-(3,5-di-t-butyl-4-methoxyphenyl), Methylene-2-phenyl, Forty-second compound 2-Methyloctacosane, Forty-third compound Octadecanal, Forty-fourth compound Tetracontane was the last compound which took longest retention time (37.851 min) to identify. Finally the GC-MS analysis reveal 44 bioactive phytochemical compounds were identified in the Ethyl acetate leaf extract of *K.pinnata.*

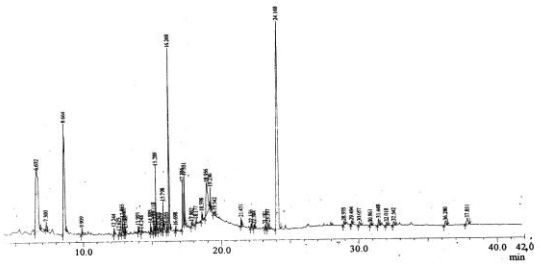

Figure 1: Screening of Phytocomponents in the Ethyl acetate leaf Extract of *Kalanchoe pinnata* by using GC-MS Peaks

Table1: Phytocomponents identified in the Ethyl acetate leaf Extract of *Kalanchoe pinnata* by peaks of GC-MS

Peak#	R.Time	Area	Area%	Name
1	6.632	13809041	16.45	1,2,3-PROPANETRIOL, 1-ACETATE
2	7.300	434636	0.52	1,2,3-Propanetriol, 1-acetate
3	8.664	12663119	15.08	2,3-DIHYDROXYPROPYL ACETATE
4	9.909	256297	0.31	1,2,3-PROPANETRIOL, TRIACETATE
5	12.244	179029	0.21	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-
6	12.625	106340	0.13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
7	12.855	612840	0.73	DODECANOIC ACID
8	13.007	351852	0.42	1-HEXADECENE
9	13.087	101533	0.12	Sulfurous acid, 2-ethylhexyl hexyl ester
10	13.993	175192	0.21	8-PENTADECANONE
11	14.248	107951	0.13	2-Bromo dodecane
12	14.885	285501	0.34	BENZENE, ETHYLPHENOXY-
13	15.118	568212	0.68	Tetradecanoic acid
14	15.220	134090	0.16	1-Decen-3-one
15	15.289	1740153	2.07	n-Nonadecanol-1
16	15.503	421224	0.50	erythro-9,10-Dibromopentacosane
17	15.679	160125	0.19	2,3-Bis(1-methylallyl)pyrrolidine
18	15.798	754515	0.90	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
19	16.055	143963	0.17	9-Octadecen-1-ol, (Z)-
20	16.268	8930844	10.64	1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLPROPYL) ESTER
21	16.698	164763	0.20	Phthalic acid, butyl undecyl ester

22	17.226	2467480	2.94	PENTADECANOIC ACID
23	17.351	1476313	1.76	1-NONADECENE
24	17.862	138103	0.16	Hexadecanoic acid, trimethylsilyl ester
25	18.177	223053	0.27	10-Nonadecanone
26	18.598	354134	0.42	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-,
27	18.956	4853862	5.78	9,12-OCTADECADIENOIC ACID (Z,Z)-
28	19.236	606046	0.72	n-Tetracosanol-1
29	19.542	352400	0.42	1,3,3-Trimethyl-1-(2'-hydroxyphenyl)indan-6-ol
30	21.431	309166	0.37	n-Tetracosanol-1
31	22.156	205370	0.24	Phenol, 2,4-bis(1-phenylethyl)-
32	22.384	182030	0.22	Phenol, 2,4-bis(1-phenylethyl)-
33	23.181	206916	0.25	1,2-BENZENEDICARBOXYLIC ACID
34	23.397	387748	0.46	Phenol, 2,4-bis(1-phenylethyl)-
35	24.168	26922643	32.07	BIS(2-ETHYLHEXYL) PHTHALATE
36	28.935	230929	0.28	2-methyloctacosane
37	29.494	208661	0.25	ISOQUINOLINE, 1-[(3,4-DIMETHOXYPHENYL)METHYL]-6,7-DIMETHOXY
38	30.057	289133	0.34	2,8-DIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-6-CHROMANOL
39	30.861	179844	0.21	Octacosyl acetate
40	31.448	512510	0.61	OCTADECANAL
41	32.018	249105	0.30	4,5-2H-Oxazole-5-one, 4-[3,5-di-t-butyl-4-methoxyphenyl]methylene-2-phenyl-
42	32.542	319027	0.38	2-methyloctacosanes
43	36.280	462027	0.55	OCTADECANAL
44	37.851	711723	0.85	TETRACONTANE
		83949443	100.00	

DISCUSSION

Two insecticidal bufadienolides were isolated from methonalic extract of leaves of Kolanchoe pinnata and identified as bryophyllin- A and bryophyllin- C.[16] Five bufadienolides were isolated from plant responsible for anti-tumour activity which are bryophyollone, bryophyllin- A, bryophyllin- C, bersaldegenin-3-acetate, bersaldegenin-1,3,5-ortho acetate and diagremotianin.[17] *Kolanchoe's* bufadienolides have demonstrated in clinical research to posses antibacterial, antitumor, cancer preventive and insecticidal actions.[11] The physiological role of bioactive compounds present in plants has increased dramatically over the last decade, particularly in relation to human health. The pharmacological effects exerted by polyphenols on the human body are thought to be strongly related with their high antioxidant Capacity.[3]

The compounds identified by GC-MS in ethyl acetate extract are significant to control the *Culex quinquefasciatus* mosquito species and possess various pharmaceutical applications.

CONCLUSION

In the present study reveal that forty four chemical compounds have been identified from ethyl acetate extract of the plant leaves of *Kolanchoe pinnata by* Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the plant leaves for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Kolanchoe pinnata contains* various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance and vector control agent. However, further studies will need to be undertaken to ascertain fully its bioactivity like pesticidal activity, toxicity profile, effect on the ecosystem and agricultural products. The present study has been undertaken to investigate the bioactive compounds present in ethyl acetate extract of *Kolanchoe pinnata* leaves.

REFERENCES

1. De-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK, de-Carvalho JE: Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. Curr. Med. Chem. 2006; 13: 3371-3384.
2. Gupta R, Lohani M, Arora S. Anti-inflammatory activity of the leaf extracts/fractions of Bryophyllumpinnatum, 2010; 3(1): 003.
3. Hainal AR, Ignat I, Volf I, Popa VI. Transformation of polyphenols from biomass by some yeast species. Cell Chem Technol, 2011; 45(3-4): 211-219.
4. Hariprasad PS, Ramakrishnan N: GC-MS analysis of Rumex vesicarius L. Int. J Drug Dev & Res., 2011; 3(2): 272-279.
5. Igwe SA, Akunyili DN. Analgesic Effects of Aqueous extracts of the leaves of B. Pinnatum. Pharmaceutical Biology, 2005; 43(8): 658-661.
6. Jain VC, Shah DP and Patel NP. Microscopical and Preliminary phy tochemical studies on leaves of Bryophyllum pinnatum, Journal of pharmacy and Research, 2008; 1(2): 230-232.
7. Lans CA, Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus.

Journal of Ethnobiology and Ethnomedicine, 2006; 2: 45.
8. Mc Lafferty FW: Registry of mass spectral data, Edition. 5. Wiley New York; 1989.
9. McLafferty, F. W.; Hertel, R. H.; Villwock, R. D. "Probability based matching of mass spectra. Rapid identification of specific compounds in mixtures". Organic Mass Spectrometry, 1974; 9(7): 690–702.
10. Olajide OA. Analgesic, anti-inflammatory and antipyretic effects of Bryophyllum pinnatum. Fitoterapia, 1998; 69(3): 249-252.
11. Quazi Majaz, A., Tatiya, A. U., Molvi Khurshid, Sayyed Nazim, Shakh Siraj. The Miracle Plant (Kalanchoe Pinnata): A Phytochemical and pharmacological Review. IJRAP, 2011; 2(5): 1478-1482.
12. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997; 609-611.
13. Shashank Matthew, K.K. Khosla, Cathrin Matthew, Debjit Bhowmik. Preliminary Phytochemical Studies Of Kalanchoe pinnata (Lam.) Pers Journal of Medicinal Plants Studies: 2013; 1(2): 19-23.
14. Stein SE: National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA; 1990.
15. Stein, SE; Scott DR. "Optimization and testing of mass spectral library Research algorithms for compound identification". *J Am Soc Mass Spectrom*, 1994; 5(9): 859–866.
16. Unang Supratman, Tomoyuki Fujita, Kohki Akiyama, Hiedo Hayashi, Akira Murakami, Hgrofumi Sakari. Antitumour promoting activity of Bufadienolides from Kalanchoe pinnata and K.drgremontiana tubriflora. Journal of Bioscience, Biotechnology and Biochemistry. 2001; 65(4): 947-949.
17. Unang Supratman, Tomoyuki Fujita, Kohki Akiyama, Hiedo Hayashi. New Insecticidal Bufadienolide, Bryophyllin- C from Kalanchoe pinnata. Journal of Bioscience, Biotechnology and Biochemistry. 2000; 64(6): 1310-1312.
18. Yadav NP, Dixit VK, Hepatoprotective activity of leaves of Kalanchoe pinnata Pers. Journal of Ethnopharmacology, 2003; 86: 197–202.