IJPSR (2013), Vol. 4, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 November, 2012; received in revised form, 22 January, 2013; accepted, 29 January, 2013

PHYTOCHEMICAL ANALYSIS AND ANTICANCER ACTIVITY OF LEAF EXTRACT OF *MANGIFERA INDICA* (*KOTTUKONAM VARIKA*)

P.A. Mary Helen*, M.R. Aswathy, K.G. Deepthi, R. Rathi Mol, J. Jaison Joseph and S. Jaya Sree

Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai – 629153, Kanyakumari District, Tamil Nadu, India

Keywords:

Mangifera indica, antimicrobial screening, GC/MS analysis, Histochemistry, Anticancer, Larvicide

Correspondence to Author:

P.A. Mary Helen

Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai – 629153, Kanyakumari District, Tamil Nadu, India

E-mail: helen_CD@yahoo.co.in

ABSTRACT

About 6 extract (methanol, acetone, hexane, ethyl acetate, hexane-ethyl acetate, aqueous extraction) from the leaves of *Mangifera indica* was extracted. Hexane-ethyl acetate was characterized by Gas Chromatography-Mass Spectroscopy. Ten constituents from 10 peaks were identified. Terpinyl acetate (5.80%) and phytol isomer (5.12%) are as the major constituents and the minor constituents like oxirane (3.57%), sabinene (3.24%), beta-pinen (3.34%), beta-myrcene (3.23%) , cymene (3.68%), alpha-limonene (2.82%), eucalyptol (1,8-cineo (4.71%), 1,3-benzodioxole, 5-(2-, (3.68%) were identified. The antimicrobial activity of different extract was tested against human and plant pathogenic bacteria. Hexane-ethyl acetate extract showed significant role on inhibiting almost all tested pathogenic organisms. Using histochemical reagents such as Phloroglucinol-HCl, Dragendorff reagent was used for the localization of lignin and alkaloids respectively. *Mangifera indica* showed 100% larvicide activity. The addition of various concentrations of hexane-ethyl acetate extract of *Mangifera indica* on the L929 cell lines showed cytotoxic activity.

INTRODUCTION: The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community [1]. Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations [2]. Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives [3]. Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology. Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity [4].

Mango (*Mangifera indica* L.) is one of the most important tropical plants in the world [5]. It grows in the tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies [6]. Many phenolic compounds have been detected in mango peels [7], mango bark [8], mango puree concentrate [9], mango pulps and seed kernels [10]. Several pharmacological activities of mango extracts have been reported including anti-inflammatory [6], antioxidant [11], antiallergic and antihelmintic [12] and antiamoebic [13].

In the present study, certain works such as phyto-chemical characterization, antimicrobial activity of extract, histochemical studies, cytotoxic effects of hexane-ethyl acetate extract against L-929 cell lines.

MATERIALS AND METHODS:

Plant Material: The leaves of *Mangifera indica* (Kottukonam varikka) was collected from tropical area of Kerala (**Figure 1, 2**).

FIGURE 1: MANGO LEAF

FIGURE 2: *MANGIFERA INDICA TREE*

Leaf extract: An amount of 5gm of fresh leaves was weighed and grind using mortar and pestle with 5ml of solvent. Then the solution was kept for centrifugation at 5000rpm for 15 minutes and the supernatant was collected and filtered through Whatmann number 1 filter paper and kept it under UV for 1 hour to prevent contamination and then stored at 5^{o}C for further use.

Analysis of Hexane-ethyl acetate extract: Mass spectrometry analysis was performed on Shimadzu GC 17A QP 5000MS coupled with a mass detector, fitted non- polar DB-5 (DiPhenyl Dimethyl Siloxane). Capillary column of length 25m-0.25mm Id. GC-MS operation conditions are initial temperature 60°C, programmed from 60°C-300°C with the injection temperature at 260°C and detector temperature at 300°C. The injection volume was 0.1µl with helium gas as carrier at flow rate of 0.6 ml per minute. Relative retention times (RRts) of constituents were determined using C5-C30 straight chain alkanes as standards. Individual constituents of the extract were identified by WILEY and NIST database matching by comparison of their RRts.

Antimicrobial Activity: The different leaf extracts were subjected to the antimicrobial assay followed by Kirby Bauer method [14, 15, 16, 17, 18]. The filter paper discs of 5mm size were prepared and the extracts of *Mangifera indica* was applied over the filter paper discs. The extract was evaporated after each addition and allowed to dry for 30 minutes. Nine bacterial culture (Microbial Type Culture Collection- MTCC Collection) were maintained as pure cultures in nutrient agar slants with periodic sub-culturing was done every 4–5 days. The plates were incubated at room temperature for three days. After three days inhibition zones including the diameter of the disc were measured using digital vernier caliper.

Histochemical Studies: For Fluorescence microscopy and histochemical works, fresh plant materials are collected and serial hand sections were produced. To this thin hand sections specific fluorescent and histochemical reagents were added and observed under the fluorescent microscope.

By the appearance of specific colour change of storage and cellular chemicals, the presence of various histochemicals will be identified. The histochemical reagents such as Phloroglucinol-HCl, Dragendorff reagent was used for the localization of lignin and alkaloids respectively.

Larvicidal activity: The mosquito larvae were collected in a sterile, disposable plastic container from a tank. The 5 larvae were transferred to a china dish and extracts was added at different concentration (0.5, 1, 1.5, 2, and 2.5). The activity of different extract of *Mangifera indica* against the larvae was noted. Another 5 larvae were transferred into a vial and different solvent were added and kept as control.

Anticancer activity: L929 cell line procured from NCCS Pune was maintained in DMEM and 10% heat inactivated FBS. The cells were trypsinised using 0.025% trypsin (cell culture grade HIMEDIA) upon reaching confluency, subcultured on to micro culture plates and used for further studies. Anti cancer effect of Hexane-ethyl acetate extracts was determined on L929 cell lines. Increasing concentrations of sample (100-400µg/ml) was added and incubated for 24 hours. The anti proliferative effect was determined by standard MTT cell viability assay.

RESULTS AND DISCUSSION: In the present study, an amount of 500gm of *Mangifera indica* leaf and solvents such as methanol, acetone, hexane, ethyl acetate, hexane-ethyl acetate and water were used for the extraction. From each sample, ~10ml extracts were collected. In this 20 % was the yield of methanol, acetone, hexane, ethyl acetate, hexane-ethyl acetate and aqueous extract of leaf.

GC-MS analysis of Hexane-ethyl acetate extract: GC–MS analysis indicated that the hexane-ethyl acetate extract contained about 10 peaks. The composition of hexane-ethyl acetate extract and its relative percentages are given in **Table 1**. Terpinyl acetate (5.80%) and phytol isomer (5.12%) are as the major constituents and this leaf extract contains the minor constituents like oxirane (3.57%), sabinene (3.24%), beta-pinen (3.34%), beta-myrcene (3.23%), cymene (3.68%), alpha-limonene (2.82%), eucalyptol (1,8-cineo (4.71%), 1,3-benzodioxole, 5-(2-, (3.68%). The phenolic profile of ethyl acetate fraction of mango leaves and a total of 8 compounds including benzoic acid, pyrogallol, p-hydroxybenzoic acid, vanillic acid, syringic acid ferulic acid, ethyl gallate and gallic acid were tentatively identified on the basis of spectral data and standard chemicals [19]. The composition of *Mangifera indica* hexane-ethyl acetate and its relative percentages are given in **Table 1 and Figure 3**.

TABLE 1: PERCENTAGE, COMPOSITION OF *MANGIFERA INDICA* HEXANE-ETHYL ACETATE EXTRACT

Number of Peaks	Retention Time (minutes)	Compounds	Abundance (%)
1	8.907	Oxirane	3.57
2	9.022	Sabinene	3.24
3	9.514	Beta-pinen	3.34
4	10.758	Beta-myrcene	3.23
5	10.896	Cymene	3.68
6	11.037	Alpha-limonene	2.82
7	20.222	Eucalyptol (1,8-cineo	4.71
8	22.433	1,3-Benzodioxole, 5-(2-	3.68
9	49.350	Terpinyl acetate	5.80
10	51.647	Phytol isomer	5.12

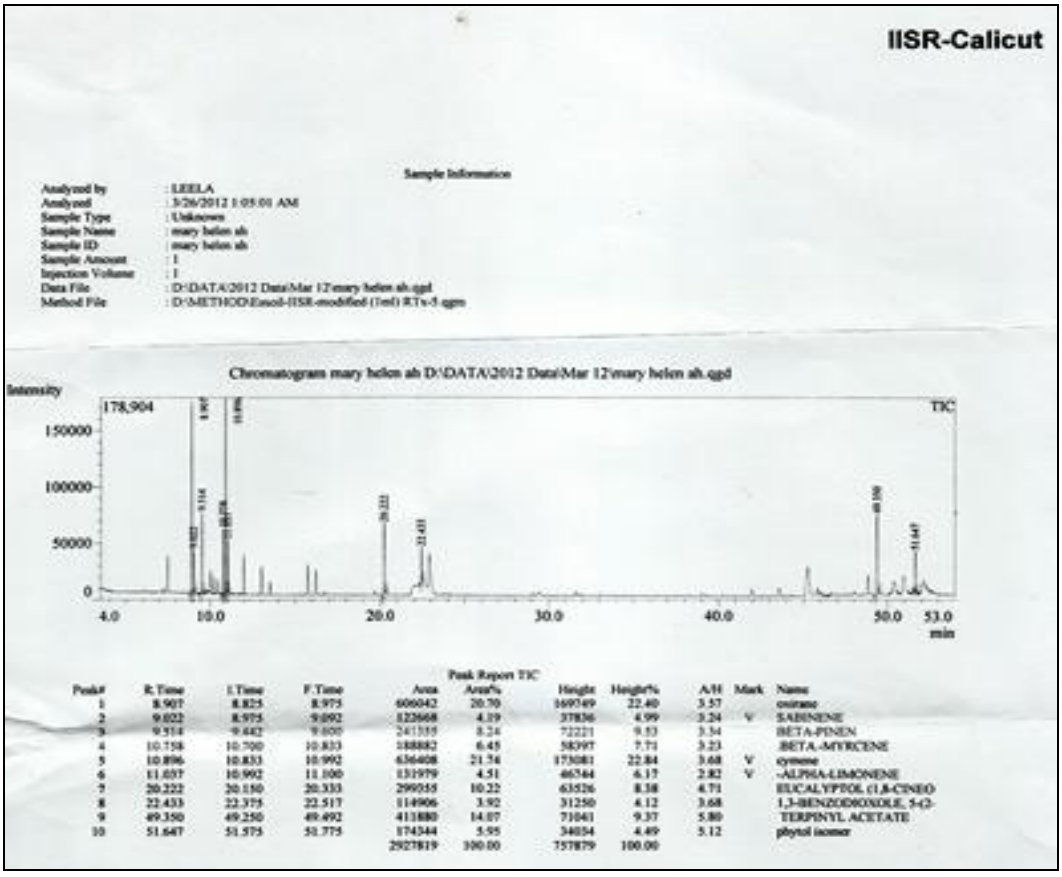

FIGURE 3: CHROMATOGRAM OF *MANGIFERA INDICA* HEXANE-ETHYL ACETATE EXTRACT

Antimicrobial activity of leaf extract of *Mangifera indica*: In the present study, the antimicrobial activity of different extract of *Mangifera indica* was tested against nine bacteria (*Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogens, Mycobacterium tuberculosis, Streptococcus pyrogens, Pseudomonas aeuroginosa, Proteus vulgaris, Escherichia coli, Staphylococcus aureus*). In antimicrobial activity, the methanol extract showed maximum zone of inhibition against *Entrobacter aerogens* (1.3 cm). The acetone extract showed a maximum zone of inhibition against *Salmonella typhi* (3.0cm).

The hexane extract showed maximum zone of inhibition against *Mycobacterium tuberculosis* (0.5 cm). The ethyl acetate extract showed maximum zone of inhibition against *Enterobacter aerogens* (1.9 cm). The hexane-ethyl acetate extract showed maximum zone of inhibition against *Streptococcus pyrogens* (2.6 cm) and also against *Salmonella typhi* (2.5 cm). It was clear from the present results, that hexane- ethyl acetate extract exhibited pronounced activity against all the tested bacteria. The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents [20].

The difference in activities among the solvents recorded in this study may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported to be capable of blocking the active ingredients in the plant extract , thus, preventing the plant extract from accessing the bacterial cell wall [21]. The result was represented in **table 2**.

Table 2: Antimicrobial activity of *Mangifera indica* leaf extract against 9 bacterial strains by Kirby Bauer method

Microorganisms	Zone of inhibition in 10 µl sample (cm)					
	Methanol extract	Acetone extract	Hexane extract	Ethyl acetate extract	Hexane ethyl acetate extract	Aqueous extract
Salmonella typhi	0.9	3.0	0.1	1.6	2.5	0.2
Klebsiella pneumoniae	1.0	1.2	0.3	1.4	1.4	0.3
Enterobacter aerogens	1.3	1.2	0.1	1.9	1.5	0.2
Mycobacterium tuberculosis	0.3	1.3	0.5	1.2	1.0	0.1
Streptococcus pyrogens	1.0	2.2	0.5	1.1	2.6	0.3
Pseudomonas aeuroginsa	0.2	1.1	0.2	1.4	1.4	0
Proteus vulgaris	0.8	1.0	0.1	1.3	1.6	0.2
Escherichia coli	0.6	1.4	0.1	1.0	1.7	0.3
Staphylococcus aureus	0.6	1.9	0	1.3	0.6	0.6

Histochemical analysis: The stains localize specific histochemicals. Localizations include lignin content and alkaloids (**Figure 4, 5**). In general, parenchymatous cells showed less amount of lignin because infection with any pathogenic agent might have delayed the process of lignification in cortical and pericycle region [22].

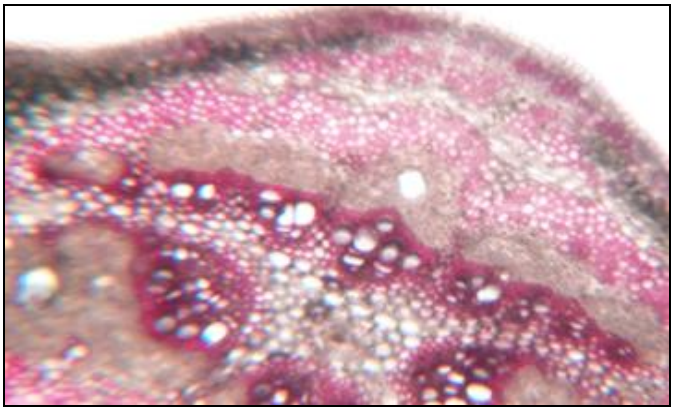

FIGURE 4: LOCALIZATION OF LIGNIN CONTENT

FIGURE 5: LOCALIZATION OF ALKALOIDS

Larvicidal activity: Hexane-ethyl acetate extract showed best antilarvicidal activity. Acetone extract and ethyl acetate also showed best antilarvicidal activity. Aqueous extract and methanol extract showed comparitively low activity. Respective solvents were taken as control and the result obtained are showed in **table 3**.

TABLE 3: LARVICIDAL ACTIVITY OF DIFFERENT LEAF EXTRACT OF *MANGIFERA INDICA*

Sample	Concentration (µl)	Time taken for death (min)	
		Control	Sample
Methanol extract	0.5	45	15
	1.0	37	12
	1.5	30	10
	2.0	25	07
	2.5	20	05
Acetone extract	0.5	50	13
	1.0	45	11
	1.5	30	10
	2.0	27	08
	2.5	22	05
Hexane extract	0.5	58	18
	1.0	55	17
	1.5	50	14
	2.0	48	10
	2.5	45	08
Ethyl acetate extract	0.5	30	12
	1.0	26	11
	1.5	24	09
	0.2	22	05
	2.5	19	03
Hexane-ethyl acetate extract	0.5	45	10
	1.0	40	08
	1.5	35	06
	2.0	30	03
	2.5	25	01
Aqueous extract	0.5	0	22
	1.0	0	20
	1.5	0	16
	2.0	0	15
	2.5	0	11

Anticancer activity: The addition of various concentrations of hexane-ethyl acetate extract of *Mangifera indica* in the L-929 cell lines showed catatonic activity. Result was showed in **table 4** and **figure 6**. The significant cytotoxic activities has been demonstrated by the stem bark extract of mango against the breast cancer cell lines MCF 7, MDA-MB-435 and MDA-N, as well as against a colon cancer cell line (SW-620) and a renal cancer cell line (786-0) [23].

TABLE 4: ANTICANCER ACTIVITY OF SAMPLE EXTRACT ON L-929 CELL LINES

Sample	Viability
ADH1	43.75
ADH2	29.56
ADH3	13.70

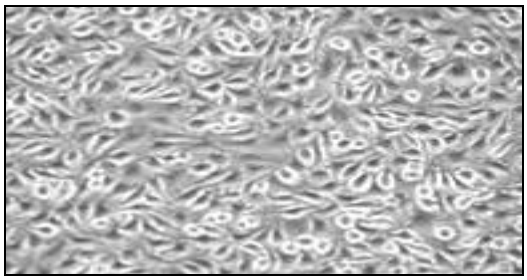

FIGURE 6.1: ANTICANCER ACTIVITY OF CONTROL (DMSO) ON L-929 CELLS

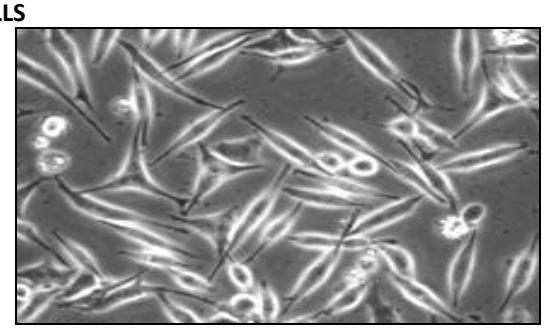

FIGURE 6.2: ANTICANCER ACTIVITY OF CRUDE EXTRACT ON L-929 CELLS

FIGURE 6: ANTICANCER ACTIVITY OF LEAF EXTRACT ON L-929 CELL LINES

CONCLUSION: From this study it can be concluded that the hexane-ethyl acetate leaf extract of *Mangifera indica* exhibited pronounced activity against all the tested bacteria. The presence of phytoconstituents in the leaf extracts may be responsible for the antimicrobial activity. The use of medicinal plants to cure diseases has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for the therapeutic treatment and have not been completely investigated. Additional studies would be needed further to evaluate the potential of this leaf extract as antimicrobial agents.

ACKNOWLEDGEMENT: We are grateful to staff members of Biotechnology Department, Malankara Catholic College for their encouragement throughout this work. We are also thankful to Dr.B.Raja Gopal, Head, Department of Biotechnology, Malankara Catholic College, Mariagiri for his immense help throughout this work. We are also thankful to Rev. Fr. Prem Kumar, Correspondent and Secretary, Malankara Catholic College, Mariagiri for his constant encouragement and support. We express our grateful to Indian Institute of Spices Research (IISR), Kozhikode (Calicut) for GC/MS analysis.

REFERENCES:

1. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA: Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afri J Biotechnol 2007; 6: 90- 1696.
2. Taylor JLS, Rabe T, McGaw LJ, Jager AK, van Staden J: Towards the scientific validation of traditional medicinal plants. Plant Growth Regul 2001; 34: 23-37.
3. Geissman TA: Flavonoid Compounds,Tannins, Lignins and Related Compounds, Elsevier, New York, Vol. 9, 1963: 265.
4. Green RJ: Antioxidant Activity of Peanut Plant Tissues. Masters Thesis, North Carolina State University, USA, 2004.
5. Severi JA, Lima ZP, Kushima H, Brito ARMS, Dos Santos LC, Vilegas W, Hiruma-Lima CA: Polyphenols with antiulcerogenic action from aqueous decoction of mango leaves (*Mangifera indica* L.). Molecules 2009; 14: 1098-1110.
6. Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero G, Delporte C, Nunez Selles AJ and Delgado R: Invivo and in vitro anti-inflammatory activity of *Mangifera indica* L. extract (Vimang). Pharmacol Res 2004; 50: 143-149.
7. Schieber A, Beaardini N and Carle R: Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L.) peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Agric Food Chem 2003; 51: 5006-5011.
8. Nong C, He W, Fleming D, Pan L and Huang H: Capillary electrophoresis analysis of mangiferin extracted from *Mangifera indica* L. bark and *Mangifera persiciformis.* J Chromatogr B 2005; 826: 226-231.
9. Schieber A, Ullrich W and Carle R: Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innov Food Sci Emerg 2000; 1: 161-166.
10. Ribeiro SMR, Barbosa LCA, Queiroz JH, Knodler M and Schieber A: Phenolic compounds amd antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chem 2008; 110: 620-626.
11. Maisuthisakul P and Gordan MH: Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. Food Chem 2009; 117: 332-341.
12. Garcia D, Escalante M, Delgado R, Ubeira FM, Leiro J: Anthelminthic and antiallergic activities of *Mangifera indica* L. stem bark components Vimang and mangiferin. Phytother Res 2003; 17: 1203-1208.
13. Tona L, Kambu K, Ngimbi N, Cimanga K and Vlietinck AJ: Antiamoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacol 1998; 61: 57-65.
14. Morris JA, Khettry A and Seitz EW: Antimicrobial activity of aroma chemicals and essential oils. Journal of the American oil Chemist's Society 1979; 56: 595-603.
15. Ross SA, El-Keltawi NE, Megalla SE: Antimicrobial activity of Egyptian aromatic plants. Fitoterapia 1980; 51: 201-205.
16. Yousef RT, Tawil GG: Antimicrobial activity of volatile oils. Pharmazie 1980; 35 (11): 698-701.
17. Deans SG and Ritchie G: Antibacterial properties of plant essential oils. International Journal of Food Microbiology 1987; 5: 165-180.
18. Hili P, Evans CS, Veness RG: Antimicrobial action of essential oils: The effect of dimethylsulfoxide on the activity of cinnamon oil. Letters in Applied Microbiology 1997; 24: 269-275.
19. Abdelnaser Abdelghany Elzaawely and Shinkichi Tawata; Preliminary phytochemical investigation on Mango (*Mangifera indica* L.) leaves. World Journal of Agricultural Sciences 2010; 6: 735-739.
20. Marjorie MC: Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12: 564-582.
21. Jigna P, Nehal K, Sumitra C: Evaluation of antibacterial and phytochemical analysis of *Bauhinia variegate* L. bark. Afri J Biomed Res 2006; 9: 53-56.
22. Darling HM, Faulknes IR and Wallendal: Culturing the potato root nematode. Phytopath 1957; 47: 70.
23. Muanza DN, Euler KL, Williams L, Newman DJ: Screening for antitumor and anti-HIV activities of nine medicinal plants from Zaire. Int J Pharmacol 1995; 33: 98.

How to cite this article:
Mary Helen PR, Aswathy MR, Deepthi MR, Rathi RM, Jaison Joseph J and Jaya Sree S: Phytochemical analysis and anticancer activity of leaf extract of *Mangifera indica* (*Kottukonam varika*). *Int J Pharm Sci Res*. 2013; 4(2); 823-828.