

Original Research Article

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Preliminary Screening and Phytochemical Profile of *Mangifera indica* Leave's Extracts, Cultivated in Iraq

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ABSTRACT

Keywords

Phytochemical screening, Secondary metabolites, *Mangifera indica*, Anacardiaceae, Preparative high performance liquid chromatography, etc.

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The main objective of this study was to evaluate the Mango leaves that recently cultivated in Iraq, depends on preliminary investigation and phytochemical profiles of *Mangifera indica* leaves (F.Anacardiaceae) extracted with different polar solvents. Then we chose ethyl acetate, the most appropriate solvent to obtain maximum amount of phenolic compounds from mango leaves so that it can be used as nutritional supplement. The preliminary phytochemical screening was performed by the standard methods as described by Harborne. These tests indicate the presence of alkaloids, Tannins, flavonoids, steroids and absence of saponin glycosides. In addition Four different leaf extracts (methanol, ethanol, n-butanol and ethyl acetate) were analyzed by Preparative high performance liquid chromatography (PHPLC) to identify bioactive compounds. The results confirmed that *Mangifera indica* leaves accumulate large number of secondary metabolites in different percentages, but the higher percentage related to flavonoid glycosides. So that we analyzed the ethyl acetate extract of fresh mango leaves by GC-MS and the result show high percentage of quercetin. These findings will be helpful to the phytochemists and pharmacologists for identification of new active principles in future.

Introduction

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.

Plants have limitless ability to synthesize aromatic secondary metabolites, most of

which are phenols or their oxygen-substituted derivatives (Rajendra Prasad *et al.*, 2013).

Extraction involves separation of medicinally active constituents in plant tissue from inactive/inert components by using selective solvents and the most appropriate extraction technologies. Solvents diffuse into the solid plant tissues

and solubilize compounds of similar polarity (Joonak, Sowmia *et al.*, 2013). Our plant is *Mangifera indica* Linn., it is a large evergreen tree, belongs to the family Anacardiaceae. It is commonly known as 'Maram' in Tamil, 'Mango' in English, 'Aam' in Hindi and 'Aamra' in Sanskrit. Different varieties of mango have been cultivated throughout the world and recently cultivated in Iraq (Rajan *et al.*, 2011). It consists of about sixty genera and six hundred species, which are mainly tropical trees and shrubs. Its parts are commonly used in folk medicine for a wide variety of remedies. Many phenolic compounds have been detected in Mango peels, bark, Pulp and seed kernels. Several pharmacological activities of mango extracts have been reported including anti-inflammatory, antioxidant, anti-allergic, anthelmintic and antiamoebic. Herbal drinks are very popular, as they contain natural constituents especially phenolic compounds (Abdelnaser Abdelghany *et al.*, 2010).

The mango is a rich source of various polyphenolic compounds. The major polyphenols in the mango are: mangiferin, catechins, rutin, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid. The amounts of the different polyphenolic compounds in the mango vary from part to part (pulp, peel, seed, bark, leaf, and flower) (Talba *et al.*, 2014). Polyphenols are secondary metabolites of plants and are widely distributed in beverages and plant-derived foods. Human consumption studies indicate 1 g of total polyphenols is frequently consumed per day and it is not anticipated that any acute or lethal toxicity would be observed through the oral intake route (Augustin Scalbert *et al.*, 2000). Phenolic compounds have the capacities to quench lipid peroxidation, prevent DNA oxidative damage, scavenge

free radicals and prevent inhibition of cell communication, all of which are precursors to degenerative diseases.

Free radicals cause depletion of the immune system antioxidants, change in gene expression, and induce abnormal proteins resulting in degenerative diseases and aging. Use of pure isolated compounds has been found to be less effective than the use of crude mixtures from the particular mango part suggesting that synergism of the various mango polyphenols is important for maximum antioxidative activity (Martin Masibo *et al.*, 2008).

Materials and Methods

Identification of mango leaves

The mango species used in this study were identified by their leaves by the taxonomist Dr. Alialmosawi.

Collection of the mango leaves

Mangifera indica leaves were collected from mango trees in the garden. The leaves were washed by tap water and dried at room temperature, then made as powder using mortar and pestle. The powder was preserved in an airtight container and kept in a cool dry place.

Preparation of methanol extract of mango leaves

50g of the powdered leaves was extracted exhaustively over a period of 10 hours using continuous hot extraction method with 350ml of methanol in a soxhlet apparatus. The extract was concentrated to a solid form using rotary evaporator at 40°C.

Preparation of ethanol extract of mango leaves

50g of the powdered leaves was extracted

with 200ml of ethanol by maceration over night at room temperature. The extract was filtered then concentrated and divided into small portions, one for identification and the other part was partitioning with n-butanol and ethyl acetate respectively, then each fraction was concentrated to small volume for further study.

Preparation of ethyl acetate extract of mango leaves

50g of fresh mango leaves was extracted with ethyl acetate by maceration at room temperature over night using magnetic stirrer, then filtered and concentrated by rotary evaporator to small volume for further study.

Phytochemical tests

10g of dried mango leaves powder was extracted by boiling with water for 15min. Filtered and was evaluated for the presence of phytochemical compounds using standard methods (Harborn, 1998).

Detection of Steroids by Lieberman-Burchard test

Take 1ml of the aqueous extract in a test tube then add 5ml of anhydrous acetic acid and shake well. Take 4 drops of the above mixture and place in a porcelain dish, and then add one drop of conc. H_2SO_4 . A change of color from rose, through red, violet and blue to green.

The colors are slightly different from compound to compound.

Detection of Cardiac Glycosides (Keller-Killians Test)

To 1ml of aqueous extract add 1ml of glacial acetic acid containing one drop of ferric chloride solution. Then add 1ml of

concentrated sulphoric acid to the above mixture in drops to make two layers. A brown ring obtained at the interface indicated the presence of ade-oxy sugar characteristic of cardenolides.

Detection of Saponins (Froth Test)

1ml of aqueous extract diluted with distilled water to 20ml which was shaken in a graduated cylinder for 15 Seconds. Formation of foam layer after 15 minutes indicated the presence of saponins.

Detection of Tannins

To 1ml of aqueous extract add few drops of alcoholic solution of 0.1% $FeCl_3$ dark blue, greenish black soluble compounds indicate the presence of tannins (Aiyegoro *et al.*, 2010).

Detection of Flavonoids

2ml of aqueous extract added few drops of 10% sodium hydroxide solution. This produces a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid is an indication for the presence of flavonoids (Syamsudin Abdillah *et al.*, 2015).

Detection of Alkaloids

Add few drops of mayer's reagent to 1ml of the queous extract. White precipitate indicate the presence of alkaloids. Also few drops of dragendorf's reagent give orange precipitate with alkaloids (Javier *et al.*, 2014).

Preparative High performance liquid chromatography (PHPLC)

Fractionation of the *Mangifera indica* extract was performed by PHPLC to identify active compounds. An isocratic HPLC,

variable wavelength UV-Visible Detector, and reverse phase C18, Phenomenex column (250 X 4.60mm) was used. The mobile phase components acetonitrile: water: acetic acid (60:40:0.5) were filtered through 0.2 micron membrane filter before use, and pumped from the solvent reservoir at a flow rate of 10ml/min, which yielded column backup, max.pressure 25pk. The column was maintained at room temperature. 2ml of different Mango leaves extracts were injected (Javier *et al.*, 2014).

Results and Discussion

Phytochemical analysis

The results of qualitative phytochemical

analysis of the crude extract of *M. indica* leaves are shown in Table 1.

Preparative High performance liquid chromatography (PHPLC)

The following chromatograms confirmed the presence of some phenolic compounds in all Mango leaves extracts.

Preliminary phytochemical investigation confirmed that mango leaves are rich with several chemical constituents as shown in table (1) by using specific tests for each group of compounds. Mango leaves are good source for Alkaloids, Flavonoids, Tannins, Steroids and Cardiac glycosides while saponin glycosides are absent.

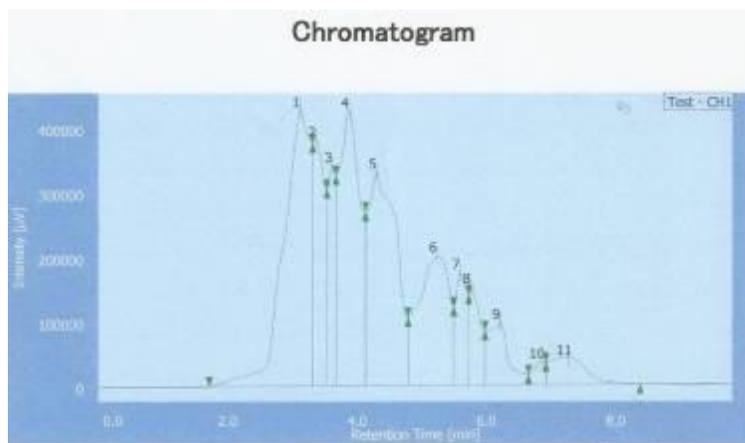
Table.1 Qualitative phytochemical analysis of *M. indica* leaves

Phytochemicals	Test	Crude extract
Alkaloids	Dragendroff's test	+
	Mayer's test	+
Flavonoids	Alkaline reagent	+
Tannins	FeCl3 test	+
Steroids	Liebermann-Burchard test	+
Saponins	Frothing test	-
Cardiac glycosides	Keller- Killians Test	+

Fig.1 Mango tree

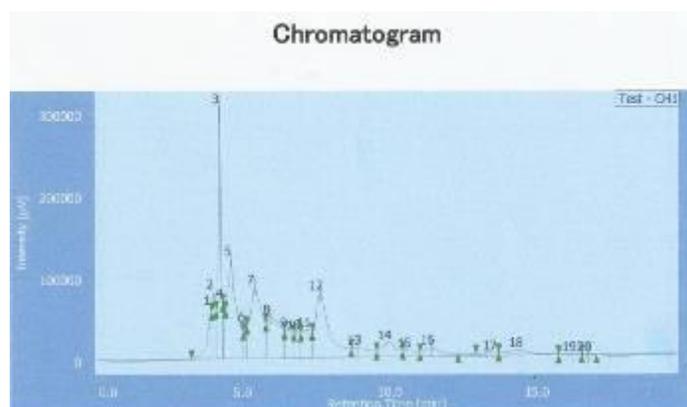


Fig.2 Methanolic Mango leaf extract



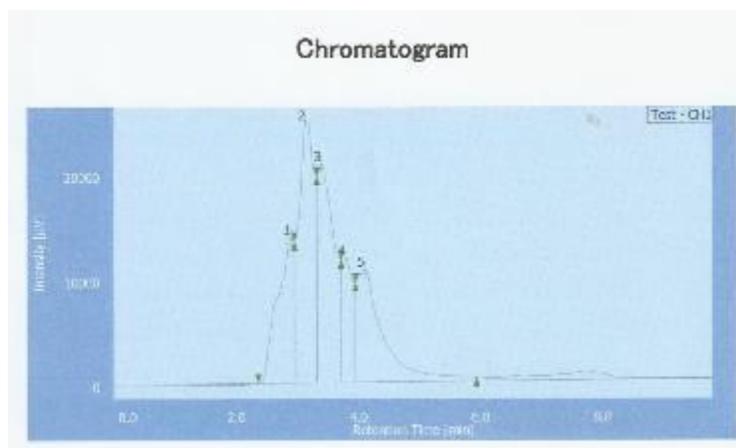
CH	tR [min]	Area [$\mu\text{V}\cdot\text{sec}$]	Height [μV]	Area%	Height%
1	3.117	12351524	430644	22.744	16.489
1	3.367	4571572	381079	8.418	14.591
1	3.617	2772721	341060	5.106	13.059
1	3.875	9602864	424866	17.683	16.268
1	4.300	10038327	330871	18.485	12.669
1	5.233	6716438	200990	12.368	7.696
1	5.592	2126882	174863	3.916	6.695
1	5.758	1800219	152949	3.315	5.856
1	6.208	2206906	96984	4.064	3.713
1	6.842	452299	36137	0.833	1.384
1	7.267	1666183	41262	3.068	1.580

Fig.3 Ethanolic Mango leaf extract



CH	tR [min]	Area [$\mu\text{V}\cdot\text{sec}$]	Height [μV]	Area%	Height%
1	3.917	609129	62362	3.589	5.057
1	4.033	625251	81171	3.663	7.624
1	4.217	1884001	310493	11.038	29.161
1	4.358	397023	70598	2.326	6.631
1	4.625	2485233	122323	14.561	11.488
1	5.100	290342	41304	1.701	3.879
1	5.408	2388824	87063	13.996	8.179
1	5.950	1639033	50564	9.603	4.749
1	6.525	602642	33819	3.531	3.176
1	6.850	461902	31174	2.706	2.926
1	7.208	745101	33869	4.366	3.181
1	7.633	2573025	79006	15.075	7.420
1	8.925	500445	12029	2.932	1.130
1	9.950	813966	17892	3.597	1.680
1	10.625	233317	7740	1.367	0.727
1	11.408	457042	11197	2.678	1.052
1	13.525	129649	3402	0.760	0.320
1	14.417	367686	6517	2.154	0.612
1	16.225	41413	1198	0.243	0.113
1	16.733	22844	1011	0.134	0.095

Fig.4 n-butanol Mango leaf extract



CH	tR [min]	Area [$\mu\text{V}\cdot\text{sec}$]	Height [μV]	Area%	Height%
1	2.892	255709	13796	16.216	16.816
1	3.142	443263	24917	28.111	30.373
1	3.400	398297	20781	25.259	25.332
1	3.800	152203	11804	9.652	14.389
1	4.100	327373	10739	20.761	13.091

Fig.5 Ethyl acetate Mango leaf extract

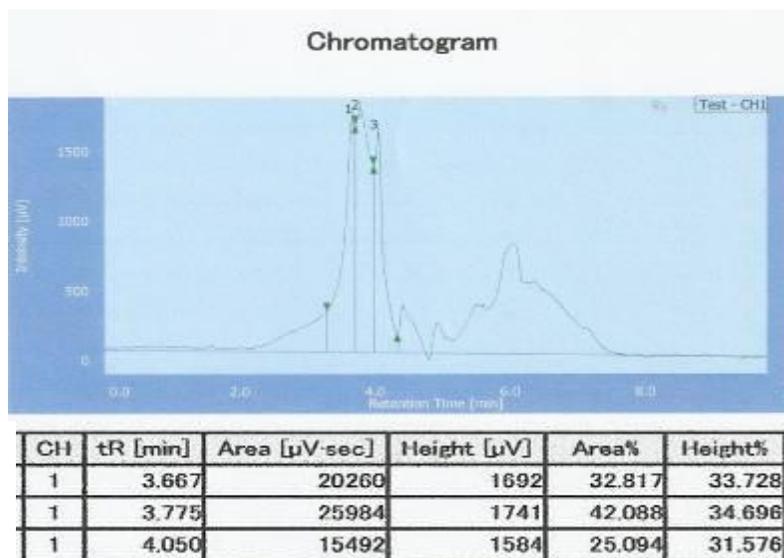


Fig.6 Std.Caffeic acid

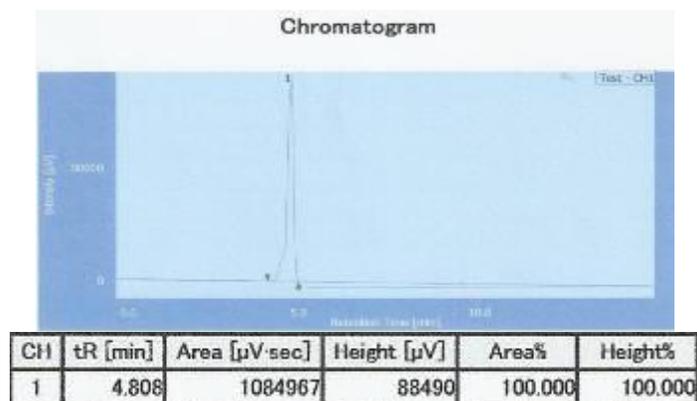


Fig.7 Std.Chlorogenic acid

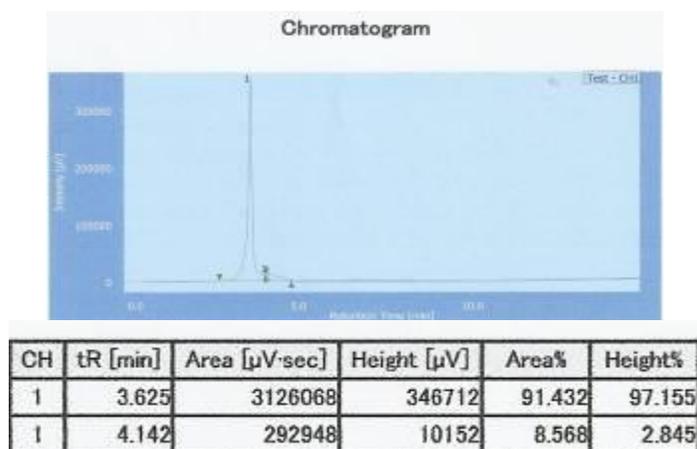


Fig.8 Std.Rutin

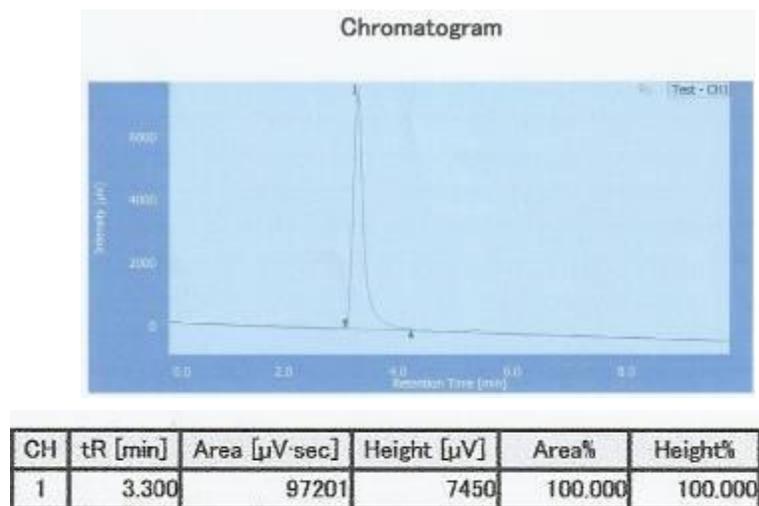


Fig.9 Std.Quercetin

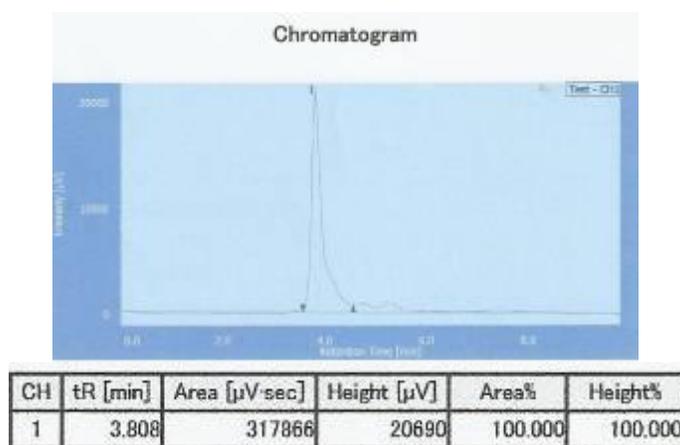


Fig.10 Std.Kambferol

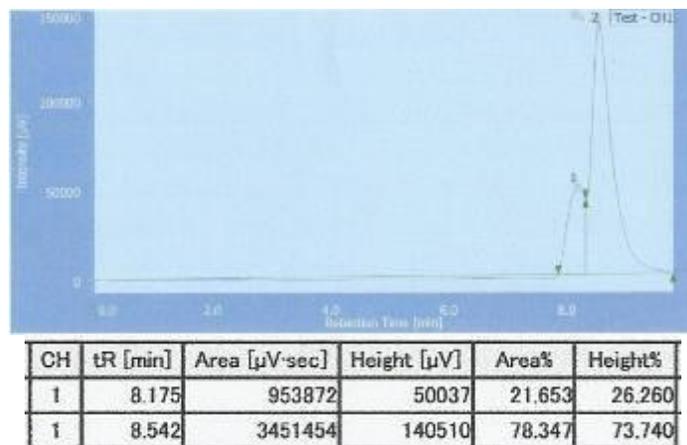


Fig.11 GC-MS chromatogram of ethyl acetate extract of mango leaves

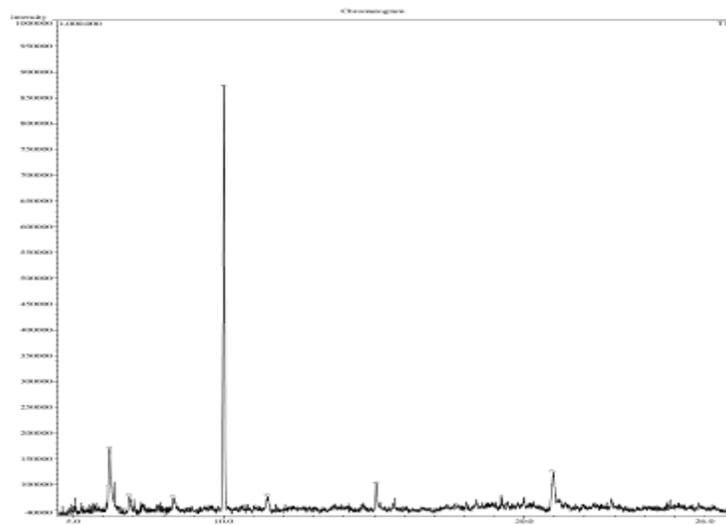


Fig.12 GC-MS chromatogram of phenolic standards

Peak	R.Time	Area	Area%	Height	Height%
1	6.21	652668	15.60	113011	10.00
2	6.86	161600	3.86	32012	2.83
3	8.33	78782	1.88	19910	1.76
4	10.01	2524326	60.33	821984	72.77
5	11.46	145204	3.47	26641	2.36
6	15.07	177047	4.23	47801	4.23
7	20.96	444644	10.63	68246	6.04
		4184271	100.00	1129605	100.00

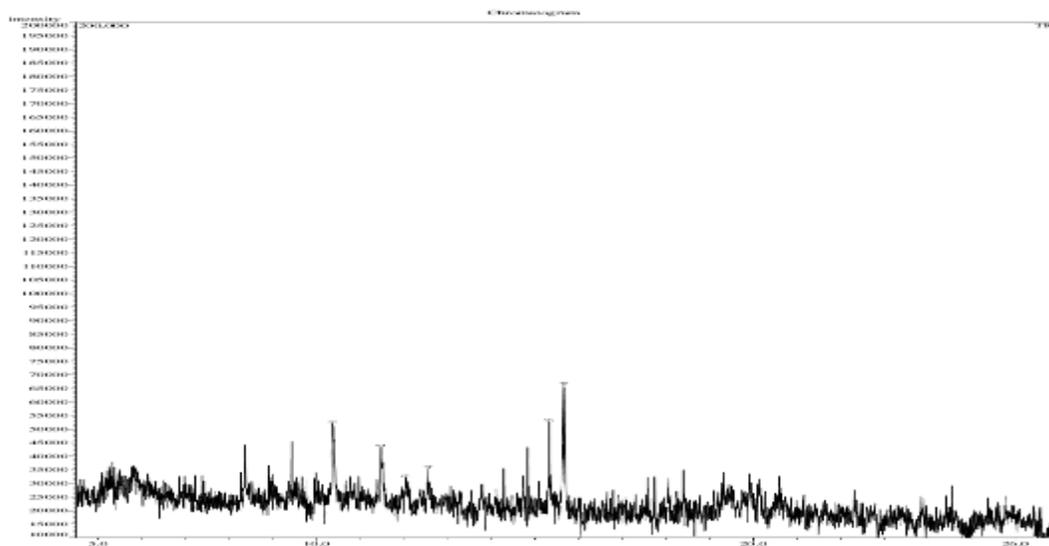
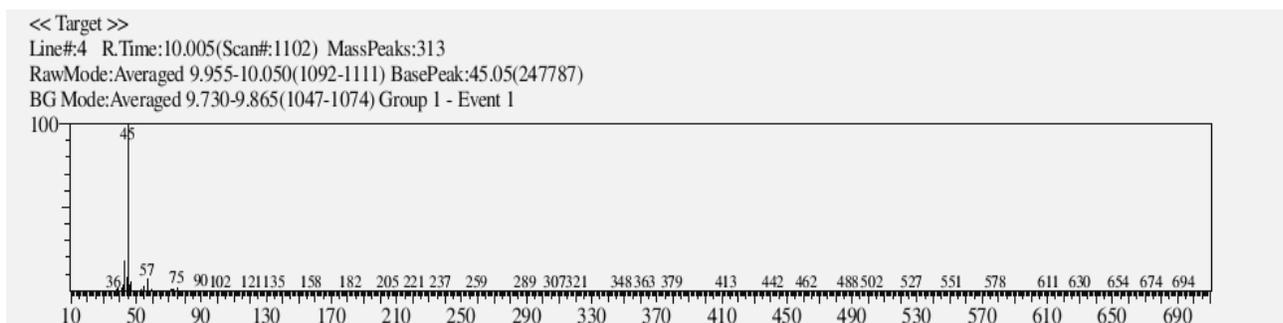


Fig.13 GC-MS chromatogram of Quercetin std.



Based on reversed phase PHPLC chromatograms for each fraction of Mango leaves extract together with most important phenol standards we confirmed that hot methanolic extract fraction contain high concentration of Quercetin, Rutin and Chlorogenic acid while ethanolic fraction contain high concentration of Quercetin and Caffeic acid, n-butanol fraction contain high concentration of Rutin and Quercetin, finally ethyl acetate fraction contain high concentration of Quercetin and chlorogenic acid. But the highest concentration of Quercetin found in ethyl acetate fraction of fresh leaves by using GC-MS.

In conclusion, the extensive survey of literature revealed that *Mangifera indica* is an important source of many pharmacologically and medicinally important chemicals. It is a potential source of polyphenolic compounds with high antioxidant activity that protect the body against damage linked to oxidative stress. The quantities of these compounds varies in different plant parts besides being affected by the geographic locations of the plants and method of extraction with suitable solvent. However, further researches are still required to elucidate the active ingredients and detail underlying mechanism that support its therapeutic use.

Acknowledgments

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