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Allelopathically active flavonoid Quercetin from *Garcinia gummi - gutta* (L.) Robs.Var. *gummi-gutta* – A Potential natural bioherbicide

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allelopathy, *Garcinia gummi- gutta*, allelopathic property, quercetin, seed germination bioassay, chromatography, activity-guided fractionation, qualitative chemical analysis, characterization by spectral analysis.

Abstract

Garcinia gummi- gutta is a common plant present throughout Kerala and other tropical areas. Its fruits are used as a food addictive in many parts of Kerala. Methanol-soluble seed germination inhibitor present in the leaves of this plant was purified. The active principle present in the plant was isolated and purified by activity guided fractionation. Chemical nature of the purified compound determined through qualitative chemical analysis. The purified compound exhibited a strong allelopathic activity against a variety of test plants. Spectral analysis revealed the presence of a novel flavonoid compound with structure similar to the flavonoid, Quercetin.

1. Introduction

Allelopathy, which involves a plant secondary metabolite (PSM) mediated plant-to-plant interactions can be exploited for the protection of crops. Plant products are usually ecofriendly, cost-effective, pose reduced damages and health hazards to animals and also helps to maintain the quality and quantity of crops. One of the directions taken by allelopathy research is the discovery of new donor plants for allelopathic substances (Dorota, 2005).

Allelopathic property of the methanolic extract of the plant *Garcinia gummi-gutta* have been established. A variety of secondary metabolites like hydroxycitric acid (HCA), flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garcinol, xanthochymol and guttiferone isoforms, benzophenones, xanthones, biflavonoids, coumarins, alkaloids, tannins, phenols and saponins having ecological benefits have been isolated from the plant *Garcinia gummi-gutta* (Okwu DE, 2005, Farombi et al.,2002).To explore the nature of the compound and for developing natural herbicides it is necessory to isolate, purify and characterize the bioactive compound/s.

Natural products may be obtained from dried materials by extracting with solvents of different polarity. The solvent to sample dry weight ratio of 10:1 (v/w) used is ideal (Das et al., 2010). One common method is serial exhaustive extraction, which

involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol). This is done to ensure that a wide polarity range of compounds could be extracted (Green RJ, 2004).

Column chromatography and TLC techniques are most suitable methods for purification of samples, qualitative assay and preliminary estimate of the compound in plant extracts (Handa et al., 2008). Thin layer chromatography is useful for the determination of suitable solvent system for Column chromatography and also to estimate the purity of the extract (Wagner and Bladt, 1996). Purification followed by activity testing is known as activity-guided fractionation. The purified fractions obtained were then evaluated for bioactivity (Rebecca Clare Guza, 2004).

Natural products are easily degraded in nature and possess high cost of delivery. Hence, direct use of allelochemicals as natural pesticides is difficult in the field (Khanh et al., 2007). These drawbacks can be resolved by structural modification and chemical synthesis of the compound. For these purposes, identification of the exact chemical nature is necessary. Spectral characters like infrared (IR), nuclear magnetic resonance (NMR), Liquid chromatography-mass spectral (LC-MS) measurements as well as microanalysis can be utilized for elucidating the chemistry of the compound (Harborne JB, 1999). In the present study, the active principle responsible for the allelopathic activity in *Garcinia gummi-gutta* had been isolated, purified and characterized.

2. Materials and Methods

Preparation of crude extract: Leaves of *Garcinia gummi-gutta* were collected, washed, dripped dry, shade-dried, pulverized and were kept in a refrigerator at 4˚C. 100g of the powdered leaves were subjected to extraction in 500 ml of 95% methanol (AR grade) using Soxhlet extractor for 24 hrs.

2.1. TLC plate preparation : Silica gel plates were made by uniformly spreading an aqueous slurry of silica powder over a clean glass plate of 10cm x 5cm size. The dried plates were activated in a hot air oven at 120˚C for 3 hrs. The extract was loaded using a capillary tube. A mixture of chloroform and methanol in the ratio 30:1 is used as the solvent system. The developed chromatograms were visualized by keeping in Iodine chamber for 10 minutes.

2.2. Coloumn chromatography: For preparing a chromatographic column, a column of 45 cm x 3 cm size was used. Prior to the procedure, silica gel (60-120 mesh size) was activated in a hot air oven at 120˚C for about 3hrs. Activated silica, after cooling was made into slurry using chloroform. The slurry was then being carefully packed in the column up to a height of 30cm. A 25 ml of methanolic leaf extract containing 5 gram dried mass was then added gently on the top of the column without disturbing the column. It was then subjected to gradient elution using a mixture of chloroform and increasing concentration of methanol in a step-wise manner. The component fractions (30 ml) were collected separately. About 1 ml of the collected fractions were evaporated to dryness, redissolved in distilled water and used for testing the allelopathic activity. Fractions that exhibited the desired activity were tested for purity by TLC. Identical fractions were pooled together and were then again subjected to column chromatography. The procedure had been repeated several times until a single spot in TLC was obtained.

2.3.Qualitative chemical analysis : Chemical tests were carried out on the purified compound using standard procedure to identify the constituents (Sofowora A,1993, Tiwari et al.,2011).

2.3.1.Carbohydrates: Benedict's test: Take 1ml of the filtrate and add 5ml Benedict's reagent. Boil for 5 minutes. A bluish green color indicates the presence of carbohydrates.

2.3.2.Protein: Millon's Test: To 1ml of filtrate add 5 to 6 drops of Millon's reagent. If a white precipitate develops which turns red on heating, then the test is positive for proteins.

2.3.3.Steroids: To 1ml of the filtrate, add 10ml of chloroform and 10 ml of H_2SO_4 slowly through the sides of the test tube. Positive indication is like the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.

2.3.4.Alkaloids: To 1ml of the filtrate, add 2ml of Drangendroff's reagent. Positive test shows turbid orange color.

2.3.5.Anthraquinones: To 1ml of the filtrate, add 10 ml benzene and filter. To the filtrate, add 5ml of 10% {v/v} ammonia and shake well. Development of pinkish colored solution indicates the presence of anthraquinones.

2.3.6.Terpenoids: Salkowski test: To 1ml of the filtrate, add 2ml Chloroform ($CHCl_3$) and then add a few drops of Concentrated Sulphuric acid (H_2SO_4) carefully. An interface with a reddish brown coloration is formed showing the presence of terpenoids.

2.3.7.Saponins: Frothing test: A drop of sodium bicarbonate was added in a test tube containing about 50 ml of aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth will be formed which shows the presence of saponins.

2.3.8.Test for phenolic compounds:

Ferric chloride test: On addition of ferric chloride solution (5%) to 1mlof filtrate, a brown color appeared which shows the presence of phenolic compound.

Lead acetate test: Few drops of lead acetate solution (5%) is added to1ml of the filtrate. Appearance of a white precipitate confirms the presence of phenolic compounds.

2.3.8.a. Flavonoids: To 1 ml of filtrate, add 2ml of dilute sodium hydroxide (NaOH). Positive test shows the development of a golden yellow color.

2.3.8.a.(1). Tannins: To 1ml of the filtrate, add 2ml of Ferric chloride. A blue or black precipitate indicates the presence of tannins.

2.3.8.a.(2). Pew test (Confirmatory test for flavonoids): In a test tube containing 0.5ml of extract, 5 to 10 drops of diluted Hydrochloric acid and small amount of Zinc or Magnesium were added and the solution were boiled for few minutes. Appearance of reddish pink or dirty brown color indicated the presence of flavonoids.

2.4.Characterization of the active principle from *Garcinia gummi-gutta (l.) Robs. Var. Gummi-gutta*

To elucidate the chemistry of the purified compound, the techniques adopted are FT-IR Spectroscopy, LC-MS, Micro analysis & NMR Spectroscopy.

2.5.Allelopathic effect of Flavonoids isolated from the leaf extracts of *Garcinia Gummi-Gutta (L.)* Robs. Var.*Gummi-Gutta*

Preparation of extract: 15 mg of the flavonoid compound isolated and purified from the plant extract was dissolved in 100 ml distilled water and was used for testing the allelopathic activity. A series of four concentrations had been tested against the seeds *Cicer arietnum* Acc_1(Desi chana), *Pisum sativum*, *Cicer arietnum* Acc_2 (Kabul Chana), *Arachis hypogea* and *Vigna radiata*.

2.5.a. Allelopathic Activity Assay using petri-dish method-Germination test

Healthy test seeds of uniform size were surface sterilized with 1% sodium hypochlorate for 2 minutes (to prevent fungal infection) followed by three washes with sterile distilled water. After surface sterilization, seeds were blotted dry before being sowed. Layer of sterile cotton were placed in 15 cm diameter glass Petri dishes. Control plate and Test plate were set. Fifteen seeds of each seed species evenly layered on sterile cotton soaked with 20 ml of extracts on a petri-plate served as Test. Another set of plates with same amount of water instead of extract served as Control. Both the plates were kept for incubation at 28˚ C in dark and were allowed to germinate for six days. Equal volume of distilled water was added in the dishes when moisture content of the cotton declined. Each treatment had six replicates. Those seeds with visible radicle were considered germinated (Turkey HB,1969). The monitoring of the number of seeds germinated were done daily from the second day onwards. Termination of the experiment was done after three consecutive recordings had shown no further variation. The percentage inhibition, root length, shoot length, fresh weight as well as dry weight of the treated seedlings were determined. Six replicates were performed. The values were expressed as mean ± SD and the results were compared statistically using One-Way Analysis of Variance. Percentage inhibition of germination had been calculated using the equation

$$I=100-\left(\frac{E_2 X\ 100}{E_1}\right)$$

where, E_1 represents Percentage inhibition, E_1represents response of Control plant and E_2 represents response of Treated plant (Surendra MP and Pota KB, 1978).

3. Results and Discussion

Plants contain an enormous number of secondary metabolites that varies in polarities. Previous studies conducted with aqueous and methanolic extracts of the plant, *Garcinia gummi-gutta* revealed the presence of an effective allelopathic compound in the methanolic extracts. Polyphenolic compounds such as flavonols

and most other reported bioactive compounds are generally soluble in polar solvents such as methanol (Houghton & Raman ,1998). Thus, it was expected that the active principle present in plant may be a polar or a semi-polar compound.

3.1.Isolation and purification

Isolation and purification of the active component present in the leaves of *Garcinia gummi-gutta* were performed through column chromatography in which chloroform-methanol mixture was used. Thirty fractions were collected and were tested for allelopathic activity using seed germination test. Pulses were selected for seed germination assay due to its easy availability, ease of handling and easy germination ability. Results showed that the fractions, from 15 to 20, exhibited allelopathic activity and hence those fractions were pooled together and subjected to TLC for testing the purity. Four cycles had been performed for complete purification. Identical fractions purified were pooled together and evaporated to dryness. The quantity of the material used and the yield of the compound obtained after each fractionation were noted. About 1.43 g of purified compound possessing the allelopathic property were obtained and were utilized for further studies. Chemistry of the purified compound was determined by color tests Results are given in Table.1.

Table 1.Purification Procedure of the components of *Garcinia gummi-gutta*

Sl. No.	Purification steps	Quantity of material used	Yield of active fraction	Recovery Percentage	No. of component as observed in TLC plate
1	Soxhlet Extraction	500g	26.15g	5.23%	7
2	1stColoumn	26.15g	5.49g	1.098%	5
3	2nd Column	5.49g	3.5g	0.7%	2
4	3rd Column	3.50g	1.43g	0.29%	1

3.2.Qualitative chemical analysis

Preliminary phytochemical analysis of the secondary metabolites in the leaves of *Garcinia gummi-gutta* showed that the plant possesses high contents of alkaloids, tannins, phenolic flavonoids, carbohydrates and proteins (Madappa & Bopaiah , 2012). The observations from the present study gave data that the active principle possessing allelopathic potential in the leaves of the plant *Garcinia gummi-gutta* was Flavonoids. Results are given in Table.2.

Table 2.Qualitative chemical analysis of the purified compound isolated from the leaf extracts of *Garcinia gummi-gutta*

Sl.No	Name of Test/ Reagents	Observation	Inference
1	Dragendroff's Test	No orange turbidity	Absence of alkaloids
2	Lead acetate test	White precipitate	Presence of phenolics
3	Alkaline reagent test	Golden yellow colour	Presence of flavonoids
4	Ferric chloride test	No dark green colour	Absence of tannin
5	Benzene + Ammonia	No pink colour	Absence of anthraquinone
6	Filtrate+ Distilled water	Foam not formed	Absence of saponin
7	$CHCl_{3+}$ Con.H_2SO_4	No reddish brown color	Absence of terpenoid
8	Benedict's Test	No bluish green color	Absence of carbohydrates
9	Millon's Test	No white precipitate	Absence of protein
10	$CHCl_{3+}$ Con.H_2SO_4	No red color	Absence of steroid
	Confirmatory Test for Flavonoids		
	Pew Test	A Brown color	Presence of flavonoids

3.3.Allelopathic effect of Flavonoids isolated from the leaf extracts of *Garcinia Gummi-Gutta (L.)* Robs. Var.*Gummi-Gutta*

The effect of flavonoids isolated from the leaves of the plant *Garcinia - gummi gutta* on seed germination of the five receptor plants are shown in Table. 3, 4, 5, 6, 7. A varying concentration of the compound had been tested. The percentage inhibition, root length, shoot length, fresh weight as well as dry weight of the treated seedlings were determined. Six replicates were performed. The values were expressed as mean ± SD and the results were compared statistically using One-Way Analysis of Variance. The results from the present study showed that all the five parameters that were monitored decreased concomitantly when compared with that of the control. Maximum inhibition was observed with a 100% concentration. A minimum concentration of 10% showed results similar to that of control. Concentration above 10% showed an inhibitory action. Here, the allelopathic effect increased with an increase in the concentration of the compound. There are reports suggesting that the phenolic compounds like coumarins and flavonoids can serve as allelopathic agents in plants (Razavi SM. 2012). Results are given in Table 3.

Table. 3. Multiple Comparisons versus Control Group (Holm- Sidak method). One Way Analysis of Variance statistics for the comparison of alleopathic effect of varying concentration of flavonoids isolated from the leaf extracts of *Garcinia gummi-gutta on Cicer arietnum* Acc_1 (S_1)

Receptor seed	Concentration of the compound	Alleopathic effect of varying concentration of flavonoids isolated				
		Parameters Tested				
		% Inhibition	Root length(cm)	Shoot length(cm)	Fresh Weight(mg)	Dry Weight(mg)
Cicer arietnum Acc_1 (S_1)	Control	-	5.36±1.11	12.49±2.55	2.18±0.56	0.99±0.82
	10%	2.75±2.22	4.68±0.54	9.92±2.04	2.11±0.13	0.98±0.68
	25%	12.84±2.5	3.95±1.63	6.67±2.22	1.88±0.45	0.78±0.71
	50%	16±3.37	2.45±0.61	4.76±1.22	1.66±0.35	0.56±0.19
	75%	60.5±4.12	1.25±0.42	2.93±0.16	1.12±0.34	0.34±0.22
	100%	96.25±3.5	0.21±0.21	0.74±0.43	0.83±0.64	0.12±0.02

Statistical Analysis

Comparison df=10	t value					p				
	% Inhibition	RL	SL	FW	DW	% Inhibition	RL	SL	FW	DW
C vs10%	1.622	1.321	2.622	0.273	0.032	0.115	0.197	0.014	0.786	0.974
C vs25%	7.572	2.738	5.938	1.171	0.679	<0.001	0.020	<0.001	0.439	0.753
C vs50%	9.435	5.651	7.887	2.030	1.390	<0.001	<0.001	<0.001	0.146	0.438
C vs75%	33.678	7.981	9.754	4.139	2.100	<0.001	<0.001	<0.001	0.001	0.165
C vs100%	56.760	10.001	11.750	5.271	2.811	<0.001	<0.001	<0.001	<0.001	0.042

C represents Control, RL represents Root length, SL represents Shoot length, FW represents Fresh weight and DW represents Dry weight. Values are expressed as mean ± SD. $P < 0.001$ is significant.

Table.4. Multiple Comparisons versus Control Group (Holm- Sidak method). One Way Analysis of Variance statistics for the comparison of alleopathic effect of varying concentration of Flavonoids isolated from the leaf extracts of *Garcinia gummi-gutta* on *Pisum sativum* (S_2).

Receptor seed	Concentration of the compound	Alleopathic effect of varying concentration of flavonoids isolated				
		Parameters Tested				
		% Inhibition	Root length(cm)	Shoot length(cm)	Fresh Weight(mg)	Dry Weight(mg)
Pisum sativum (S_2)	Control	-	6.78±1.71	12.23±2.78	1.49±0.32	0.84±0.47
	10%	12.28±2.04	6.18±0.36	11.3±2.01	1.37±0.62	0.83±0.73
	25%	10.17±0.62	5.12±0.79	8.18±3.5	1.34±0.71	0.82±0.64
	50%	30.88±2.09	2.88±1.02	4.54±1.23	1.10±0.43	0.54±0.42
	75%	44.7±0.57	0.86±0.13	1.85±0.66	0.82±0.09	0.24±0.13
	100%	97.45±2.04	0.35±0.12	0.77±0.32	0.53±0.42	0.10±0.09

Statistical Analysis

Comparison df=10	t value					p				
	% Inhibition	RL	SL	FW	DW	% Inhibition	RL	SL	FW	DW
C vs10%	14.232	1.168	0.776	0.437	0.036	<0.001	0.252	0.446	0.666	0.971
C vs25%	11.787	3.232	3.365	0.546	0.072	<0.001	0.006	0.004	0.831	0.997
C vs50%	35.789	7.592	6.390	1.419	1.089	<0.001	<0.001	<0.001	0.421	0.634
C vs75%	51.806	11.525	8.625	2.437	2.179	<0.001	<0.001	<0.001	0.081	0.141
C vs100%	112.942	12.518	9.523	3.492	2.687	<0.001	<0.001	<0.001	0.008	0.057

C represents Control, RL represents Root length, SL represents Shoot length, FW represents Fresh weight and DW represents Dry weight. Values are expressed as mean ± SD. $P < 0.001$ is significant.

Table.5. Multiple Comparisons versus Control Group (Holm-Sidak method). One Way Analysis of Variance statistics for the comparison of alleopathic effect of varying concentration of flavonoids isolated from the leaf extracts of *Garcinia gummi-gutta on Cicer arietnum* Acc_2 (S_3).

Receptor seed	Concentration of the compound	Alleopathic effect of varying concentration of flavonoids isolated				
		Parameters Tested				
		% Inhibition	Root length(cm)	Shoot length(cm)	Fresh Weight(mg)	Dry Weight(mg)
Cicer arietnum Acc_2 (S_3)	Control	-	4.68±1.11	8.22±2.13	2.01±0.25	1.52±0.63
	10%	20.08±3.45	4.43±0.21	7.86±1.11	1.98±0.7	1.51±1.2
	25%	18.75±4.73	3.13±0.32	5.39±0.86	1.71±0.82	0.92±0.24
	50%	32.67±2.5	2.21±1.04	3.87±1.02	1.43±1.01	0.43±0.14
	75%	48.35±3.35	1.53±1.63	2.12±0.21	1.12±0.82	0.34±0.06
	100%	95.74±4.02	0.75±0.31	1.25±0.02	0.64±0.43	0.19±0.11

Statistical Analysis

Comparison df=10	t value					p				
	% Inhibition	RL	SL	FW	DW	% Inhibition	RL	SL	FW	DW
C vs10%	10.338	0.465	0.554	0.0723	0.030	<0.001	0.646	0.584	0.943	0.976
C vs25%	9.653	2.880	4.357	0.723	1.832	<0.001	0.014	<0.001	0.725	0.148
C vs50%	16.820	4.590	6.697	1.397	3.329	<0.001	<0.001	<0.001	0.434	0.007
C vs75%	24.893	5.853	9.392	2.144	3.604	<0.001	<0.001	<0.001	0.152	0.004
C vs100%	49.291	7.303	10.731	3.300	4.062	<0.001	<0.001	<0.001	0.012	0.002

C represents Control, RL represents Root length, SL represents Shoot length, FW represents Fresh weight and DW represents Dry weight. Values are expressed as mean ± SD. $P < 0.001$ is significant.

Table.6. Multiple Comparisons versus Control Group (Holm-Sidak method. One Way Analysis of Variance statistics for the comparison of Alleopathic effect of varying concentration of Flavonoids isolated and purified from the leaf extracts of *Garcinia gummi-gutta on Arachis hypogea* (S_4).

Receptor seed	Concentration of the compound	Alleopathic effect of varying concentration of flavonoids isolated				
		Parameters Tested				
		% Inhibition	Root length(cm)	Shoot length(cm)	Fresh Weight(mg)	Dry Weight(mg)
Arachis hypogea (S_4)	Control	-	6.5±0.92	7.97±3.32	2.14±0.22	1.69±0.23
	10%	34.55±3.4	4.87±0.21	6.34±3.12	2.13±0.77	1.65±0.44
	25%	32.34±2.5	4.23±0.12	5.86±2.15	1.93±0.83	0.81±0.21
	50%	36.17±0.58	2.92±0.31	4.53±3.21	1.32±0.41	0.77±0.18
	75%	54.59±3.37	0.89±0.04	0.77±0.34	0.95±0.24	0.72±0.09
	100%	96.45±3.11	0.5±0.2	0.47±.04	0.71±0.11	0.48±0.16

Statistical Analysis

Comparison df=10	t value					p				
	% Inhibition	RL	SL	FW	DW	% Inhibition	RL	SL	FW	DW
C vs10%	23.419	6.773	1.156	0.0339	0.284	<0.001	<0.001	0.257	0.973	0.778
C vs25%	21.921	9.432	1.496	0.712	6.251	<0.001	<0.001	0.269	0.732	<0.001
C vs50%	24.518	14.875	2.439	2.778	6.535	<0.001	<0.001	0.061	0.028	<0.001
C vs75%	37.003	23.310	5.105	4.032	6.891	<0.001	<0.001	<0.001	0.001	<0.001
C vs100%	67.358	24.930	5.318	4.845	8.595	<0.001	<0.001	<0.001	<0.001	<0.001

C represents Control, RL represents Root length, SL represents Shoot length, FW represents Fresh weight and DW represents Dry weight.Values are expressed as mean ± SD. P < 0.001 is significant.

Table.7 Multiple Comparisons versus Control Group (Holm-Sidak method). One Way Analysis of Variance statistics for the comparison of Alleopathic effect of varying concentration of flavonoids isolated from the leaf extracts of *Garcinia gummi-gutta on Vigna radiata* (S_5).

Receptor seed	Concentration of the compound	Alleopathic effect of varying concentration of flavonoids isolated				
		Parameters Tested				
		% Inhibition	Root length(cm)	Shoot length(cm)	Fresh Weight(mg)	Dry Weight(mg)
Vigna radiata (S5)	Control	-	13.56±2.2	18.58±2.33	0.49±0.18	0.38±0.14
	10%	3.43±1.23	13.85±3.5	17.92±0.43	0.48±0.05	0.35±0.07
	25%	8.78±4.55	9.56±0.14	13.44±1.63	0.35±0.02	0.28±0.09
	50%	28.87±4.12	7.67±0.22	6.53±1.66	0.21±0.01	0.06±0.02
	75%	58.09±3.35	3.79±0.98	2.76±0.96	0.18±0.03	0.12±0.02
	100%	98.34±1.22	2.18±0.33	1.84±0.71	0.14±0.07	0.07±0.03

Statistical Analysis

Comparison df=10	t value					p				
	% Inhibition	RL	SL	FW	DW	% Inhibition	RL	SL	FW	DW
C vs10%	2.020	0.288	0.794	0.209	0.687	0.052	0.775	0.434	0.836	0.497
C vs25%	5.171	3.975	6.180	2.926	2.291	<0.001	<0.001	<0.001	0.013	0.057
C vs50%	17.002	5.853	14.488	5.853	5.956	<0.001	<0.001	<0.001	<0.001	<0.001
C vs75%	34.210	9.709	19.021	6.480	7.102	<0.001	<0.001	<0.001	<0.001	<0.001
C vs100%	57.914	11.309	20.127	7.316	7.331	<0.001	<0.001	<0.001	<0.001	<0.001

C represents Control, RL represents Root length, SL represents Shoot length, FW represents Fresh weight and DW represents Dry weight. Values are expressed as mean ± SD. P < 0.001 is significant.

3.4.Structure Elucidation of Compound:

3.4.1.FT-IR spectrum of the purified compound: Infrared spectroscopy is a spectroscopic technique that has been systematically used to study hydroxyl and carbonyl groups present in the molecule. FT-IR spectrum of the compound is shown in figure. 1.

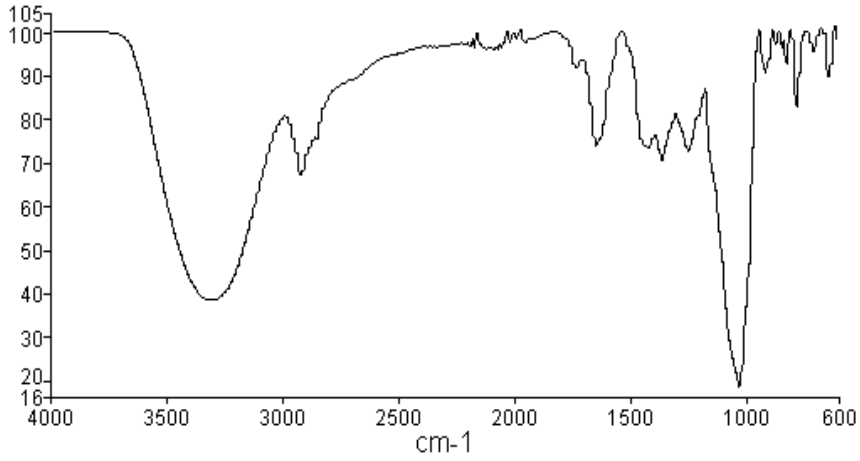

Figure.1.FT-IR spectrum of the purified compound

3.4.2.Liquid Chromatography-Mass Spectrometry (LC-MS) : LC-MS coupling is routinely used for the overall structure elucidation of flavonoids (Fossen T & Andersen OM, 2005). MS gives data about molecular and ion/fragment masses.

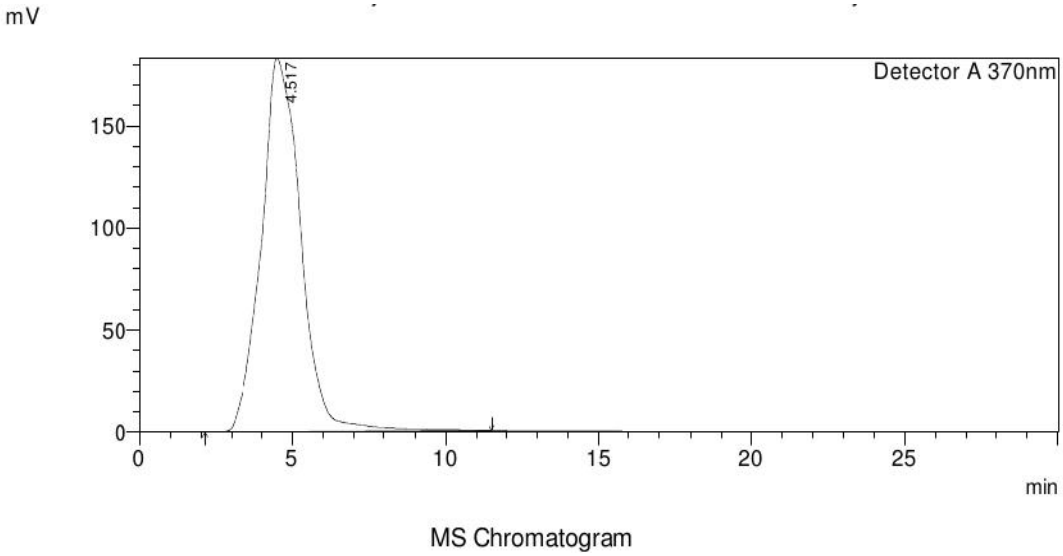

Fig.2.MS Chromatogram of flavonoids isolated from *Garcinia gummi-gutta*

3.4.3.NMR Spectroscopy: This spectroscopy gives information about atoms and bonding between them. The spectrum gives strong evidences regarding the structure of flavonoids.

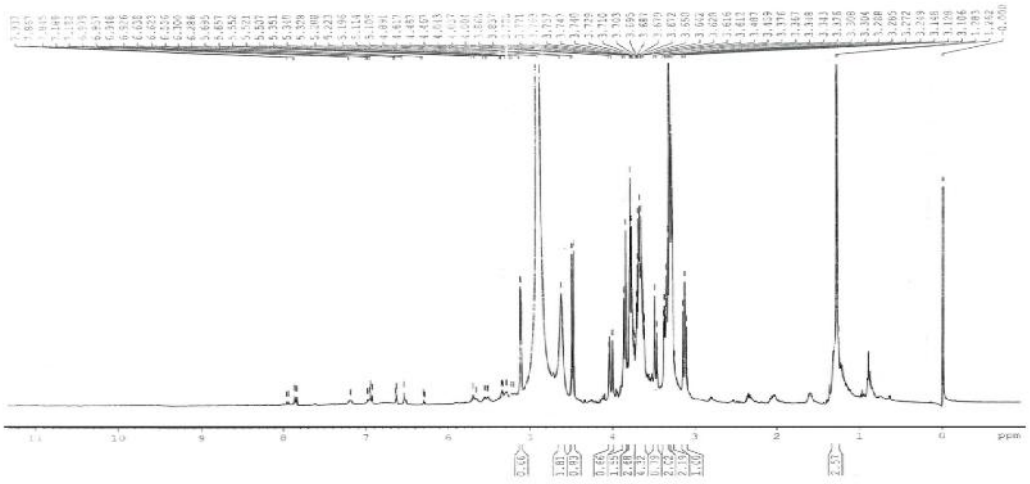

Fig.3.[1]HNMR Spectrum of flavonoids isolated from *Garcinia gummi-gutta*

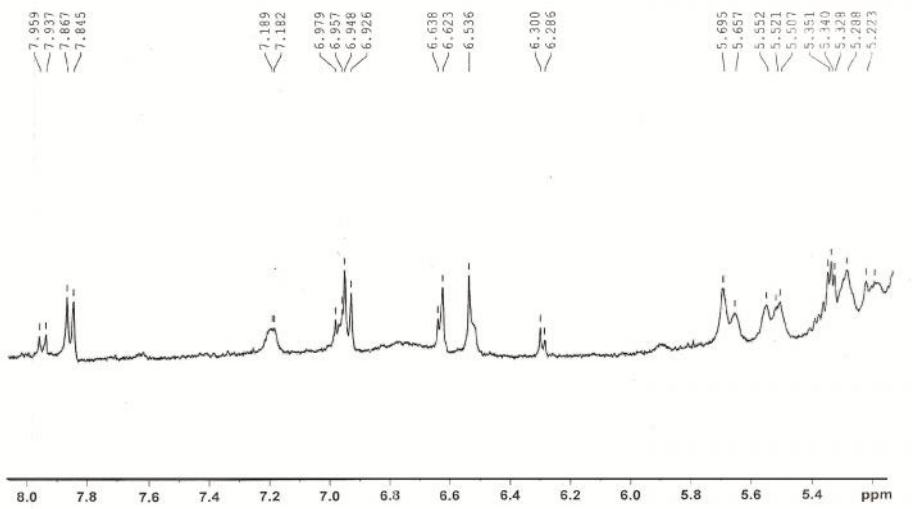

Fig.4.a .[13]C NMR Spectrum of the flavonoids isolated from *Garcinia gummi-gutta*

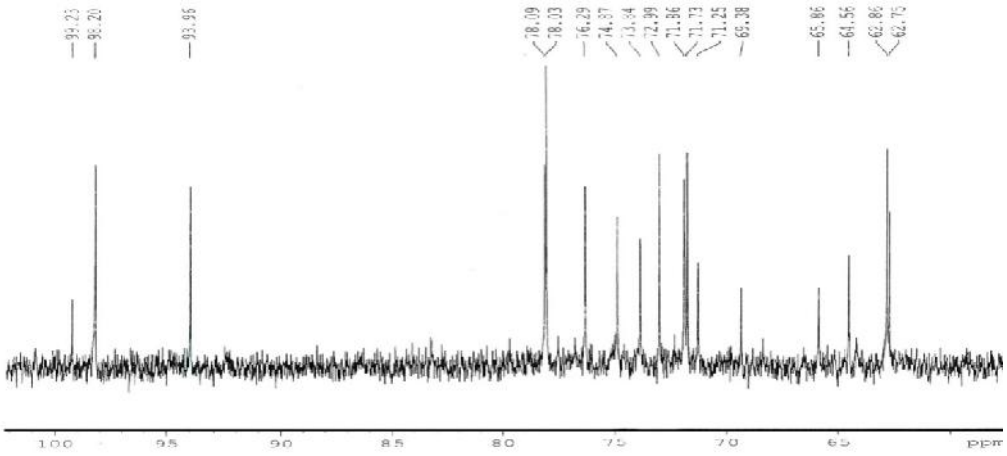

Fig.4.b .[13]C NMR Spectrum of the flavonoids isolated from *Garcinia gummi-gutta*

3.4.4.Micro analysis : Is usually carried out to determine the percentage of Carbon, Hydrogen, Nitrogen and Oxygen present in the compound.

Analysis showed that the compound contains 180% Carbon, 10.70 % Hydrogen and 112 % Oxygen. The percentage of Nitrogen and sulphur was obtained only in negligible quantities (≈0.22) and hence neglected. Thus the molecular formula obtained from the above data is $C_{15}H_{10}O_7$ with a molecular weight of 308.

The data from ^{1}H NMR and ^{13}C NMR when compared to the literature were found to be in similar with the data for the compound Quercetin, a flavonoid. The identity of the compound was further supported by elemental analysis, LC-MS, FT-IR data that were in agreement with the literature values for Quercetin. Thus, the structure of the compound elucidated is found to be as below

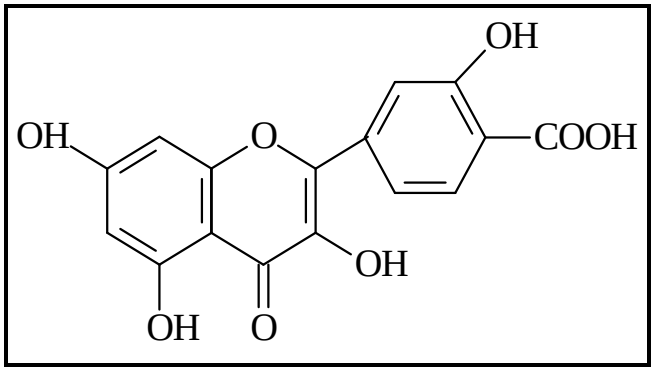

Fig.5.Structure of purified flavonoid isolated from *Garcinia gummi-gutta*
2-(5'-carboxy-6'- hydroxy phenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one ($C_{15}H_{10}O_7$)

The structural characterization of the flavonoids isolated from the leaves of *Garcinia gummi-gutta* showed that the active principle present in the plant is 2- (5'-carboxy-6'- hydroxy phenyl) - 3, 5, 7-trihydroxy-4H-chromen-4-one, which is having similarity to the structure of the flavonoid quercetin. Previous reports suggested seed germination inhibitory action of Quercetin on *Raphanus sativus* (Basile et al, 2000; Murphy et al, 2000).

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