



## **Isolation, Identification and Characterization of Flavonoids from *Basella alba* L.**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

*Basella alba* is a perennial plant of the Basellaceae and is known by various common names including Malabar spinach. Various parts of the plant are used for treatment of the diseases as well as for different healing activities of human beings as well as animals across the globe especially in India and China. There are several phytochemical present in plants, viz. flavonoids, tannins, phytosterols, alkaloids and triterpenes, etc. Flavonoids are an unusually large group of naturally occurring phenolic compounds ubiquitously distributed in plant kingdom. *Basella alba* L. used as medicinal herbs for rubefacient and catarrhal infections. In the present study, focus has been made to identify the flavonoid in different samples of *Basella alba* L. by TLC and IR. Further, the isolation of the same compound was confirmed by GC-MS analysis. Total amount of flavonoids were found in *Basella alba* (0.45 mg/gdw in stem and 0.36 mg/gdw in seeds). Forty nine compounds found in GC-MS analysis. 1-Methyl-4-isopropylcyclohexyl 2-hydroperfluoro butanoate (Area of % 24.50) was found in highest amount.

**Keywords:** *Basella alba* L.; flavonoids; TLC; IR and GC-MS.

## 1. INTRODUCTION

*Basella alba* L. (Synonym: *Basella rubra* Roxb.) is an extremely heat tolerant [1], fast growing perennial vine which belongs to family Basellaceae [2]. It is commonly known as Malabar spinach, Indian spinach, Ceylon spinach, vine spinach [3], climbing spinach [4], East-Indian spinach, Chinese spinach [5] and cyclone spinach [6]. *Basella alba* L. is native to tropical Southern Asia, probably originated from India or Indonesia [7]. *Basella alba* L. is considered one of the best tropical spinach throughout the tropical world. *Basella alba* L. is one of the wild leafy vegetables, which is rare in its natural habitat but now a days it is an important leafy vegetable grown for its nutritive value [8], *Basella alba* L. is grown as a pot-herb almost in every part of India [9]. Flora is regarded as molecular factory synthesizing enormous diversity of by-products called as "Bioactive compounds". Plants are the richest source of many compounds like flavonoids, polyphenols, polysaccharides, alkaloids, vitamins, tannins and lignin etc., represented as phytochemicals and these compounds played a vital role in scavenging the free radicals and shielding against ailments caused by the ionizing radiations. A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs [10]. Flavonoids are one of the most promising metabolites which have drawn attention by several workers. More than 5000 flavonoids have been identified [11]. Flavonoids are a group of polyphenolic compounds possessing low molecular weight that exhibit a common benzo- $\gamma$ -pyrone structure. They are categorized into various subclasses including flavones, flavonols, flavanones, isoflavanones, isoflavanoids, anthocyanidins, and catechins [12]. They play a major role in the successful medical treatments in ancient times and their use has preseeded till date [13] Besides this, these are willingly consumed by humans and they possess crucial anti-allergic, anti-inflammatory and anti-cancer activities.

## 2. MATERIALS AND METHODS

### 2.1 Collection, Identification and Extraction of Plant material

The experimental plant sample was collected from Narayan vihar at Jaipur. The plant materials

were taxonomically identified and authenticated by Department of Botany university of Rajasthan (RUBL no. 211570) Jaipur. Stem and seeds of selected plant were cleaned, shade dried and grinded to powdered and kept for further use. Each of these extracted separately with 80% methanol on water bath [14] for 24 h. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (FrI), diethyl ether (FrII) and ethyl acetate (FrIII) separately. Each step was repeated thrice for complete extraction, fraction I was discarded in each case because it contained fatty substance, where as fraction II and fraction III were concentrated and used for determining flavonoids. Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid (10mLg<sup>-1</sup> plant material for 2 h), filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings and concentrated *in vacuo*. Both fraction II and III were taken up in small volume of ethanol (2-5mL) before chromatographic examination.

### 2.2 Qualitative Analysis

#### 2.2.1 Thin layer chromatography (TLC)

Thin glass plates (20x20 cm) were coated with Silica gel G (250 $\mu$  thick). The freshly prepared plates were air dried at room temperature; thereafter these were kept at 100 °C for 30 minutes to activate and then cooled at room temperature. The freshly prepared and activated plates were used for analysis. Each of the extract was co- chromatographed with authentic flavonoid as a marker (kaempferol, quercetin and Luteolin). These plates were developed in an air tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water:: 125:72:3). The developed plates were air dried and visualized under UV light by exposure to ammonia fumes. The mouth of a 100 mL containing concentrated NH<sub>4</sub>OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard markers were marked. The developed plates were also sprayed with 5% FeCl<sub>3</sub>, 0.1% alcoholic AlCl<sub>3</sub> and kept in I<sub>2</sub> chamber separately. The coloured spots thus developed were noted and the R<sub>f</sub> value of each spot was calculated. Several others solvent systems such as n-butanol, acetic acid, water (4:1:5), tertiary butanol, acetic acid, water (3:1:1) were also

tested, but the solvent system containing benzene, acetic acid, water (125:72:3) gave better results.

### 2.2.2 Identification

The identity of the isolated flavonoids were confirmed by mp, mmp performed in capillaries (Toshniwal Melting Point Apparatus), IR (Infra-red spectrophotometer; Perkin, Elmer 337, Grating Infra-red spectrophotometer), UV (Ultraviolet and visible spectrophotometer; Carl Zeiss, Jena, DDR, VSU-2P spectrophotometer) analysis along with their respective authentic samples.

### 2.3 Quantitative Analysis

The isolated flavonoids were estimated by spectrophotometer following the method [15]. Stock solution ( $1\text{mgL}^{-1}$ ) of kaempferol, quercetin and Luteolin were prepared separately by dissolving authentic compounds in methanol. Different concentrations ranging from  $20\mu\text{g}$  to  $160\mu\text{g}$  of each of the compounds spotted separately on silica gel G plates. For each concentration of reference authentic standards separate plates were used and developed in the same manner as described earlier. These developed plates were air dried and visualized under UV light. The fluorescent spots were marked and collected along with the absorbance in separate test tubes. Spectroscopic grade methanol (5mL) was added to each test tube, shaken vigorously, centrifuged and supernatants were collected separately. The volume of each of the eluate was made up to 10mL by adding methanol. To each of these samples, 3mL of 0.1 M  $\text{AlCl}_3$  solution was added again shaken vigorously and kept at room temperature for 20 min. Five such replicates were run in each case and their optical densities were measured using spectrophotometer at 426nm for kaempferol and luteolin and at 440nm for quercetin against blank (10ml of spectroscopic grade methanol and 3mL of 0.1 M  $\text{AlCl}_3$ ). The standard curves were plotted between concentration and their respective average optical density of each of the compound. The regression curve so achieved followed Beer's law.

Each of the plant extract sample (petroleum ether, diethyl ether and ethyl acetate) was dissolved in 5 mL of spectroscopic grade methanol and 0.1mL was applied on silica gel G coated plates along with standard markers, separately. The plates were developed as above and the spots coinciding with that of standard

markers were marked on each plate under UV. Each spot was collected along with the silica gel, eluted in methanol and test samples were prepared in the same way as described above. The optical density in each case was recorded and concentration of each sample was computed using the regression curve of authentic flavonoids samples. The concentrations were calculated on mg/g dry weight basis.

### 2.4 Gas Chromatography and Mass Spectroscopy (GC-MS)

The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane,  $25\text{ m}\times 0.25\text{ mm}$  i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was  $100^\circ\text{C}$  (3 min) to  $280^\circ\text{C}$  at 1 to  $40^\circ\text{C}/\text{min}$ ; detector temperature, 250 to  $280^\circ\text{C}$ ; carrier gas, He ( $0.9\text{ mL}/\text{min}$ ). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

## 3. RESULTS

### 3.1 Qualitative Evaluation

Three spots which were (yellowish brown in colour after keeping plates in iodine chamber) of flavonoids were observed in different plant parts (stem and seed) of *Basella alba* L. on thin layer chromatography plates developed and sprayed with 5%  $\text{FeCl}_3$ . The  $R_f$  values of these spots matched with their respective authentic standards and were identified as Kaempferol, Quercetin and Luteolin. Solvent system Benzene: Acetic Acid: Water (125:72:3) gave best results with  $R_f$  values viz., kaempferol, 0.86; quercetin, 0.78, and luteolin., 0.56 (Table 1). The isolated flavonoids viz., kaempferol, quercetin, and luteolin were also characterized and confirmed by super imposable IR peaks (Figs. 2-7), mp (kaempferol,  $276\text{-}278^\circ\text{C}$  quercetin  $315\text{-}320^\circ\text{C}$  and luteolin  $326\text{-}329^\circ\text{C}$ ) which were comparable to the respective authentic standards.

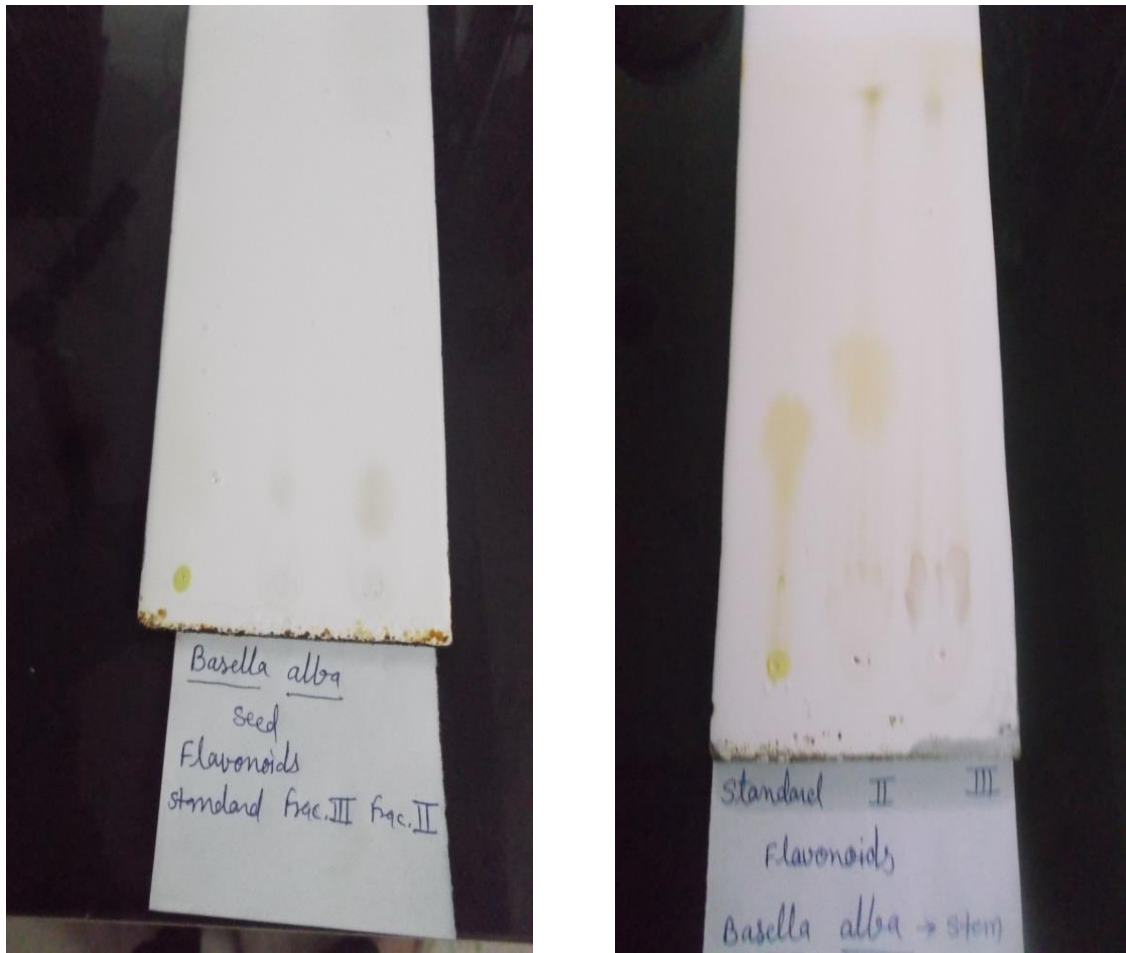


Fig. 1. TLC plates of different parts from *Basella alba* L

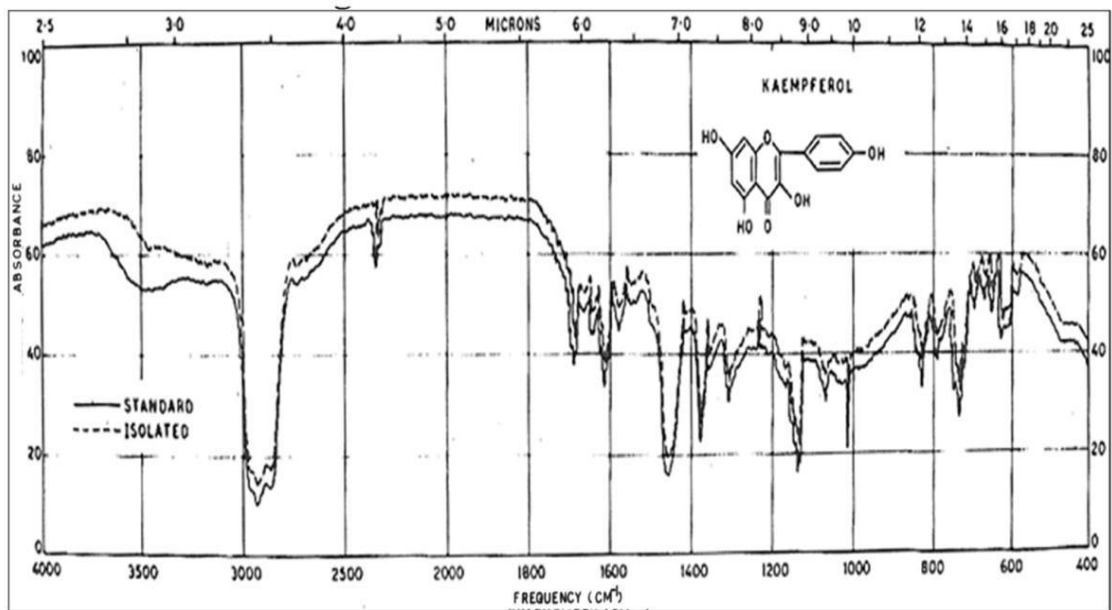


Fig. 2. IR spectra of Kaempferol from *Basella alba* L.

Table 1. Chromatographic behavior and physico-chemical characteristics of isolated flavonoids in *Basella alba* L

Isolated Compounds	Rf Value			Physical appearance			Color after spray				Melting point (°C)	IR Spectral Peaks (KBr) cm <sup>-1</sup>
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	Daylight	UV ammonia	Iodine vapor	R1		R2			
							Visible	UV	Visible	UV		
Kaempferol	0.86	0.83	0.55	GN-YW	BT-YW	YW-BN	BN	BK	YW	YW-GN	276-278	(O-H) 3410 cm <sup>-1</sup> (270,295,344,1690)
Quercetin	0.78	0.83	0.77	GN-YW	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN	315-320	3423,1739,1655(O-H) , 1608, 1508, 1305,1203 (C=C),1088
Luteolin	0.56	0.64	0.41	GN-YW	YW	YW-BN	TN	BK	DL-YW	YW-GN	326-329	3421,2065,1736,Lactone),1510,(Fauran),1461,1388,1360,1274,1242,1187,1136,1028,903,850 cm <sup>-1</sup>

Abbreviations: S 1 - Benzene: acetic acid: water (125: 72: 3), S 2 - n-butanol: acetic acid: water (4: 1: 5), S 3 - Conc. Hydrochloric acid : acetic acid : water (3: 30: 10), R 1 - 5% FeCl<sub>3</sub> solution, R 2 – 0.1% Alc. AlCl<sub>3</sub> solution, YW- Yellow, BK- Black, BN, Brown, BT-Bright, DL-Dull, GN- Green, GY- Gray and TN-Tan

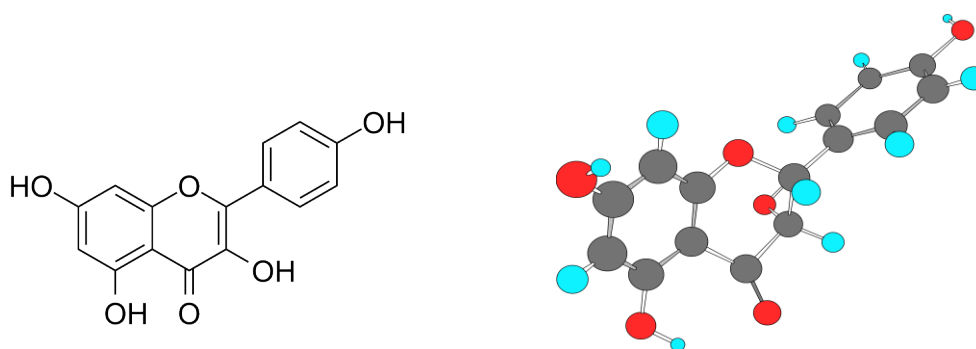


Fig. 3. (A) Structure of Kaempferol (B) 3-Dimensional model of Kaempferol

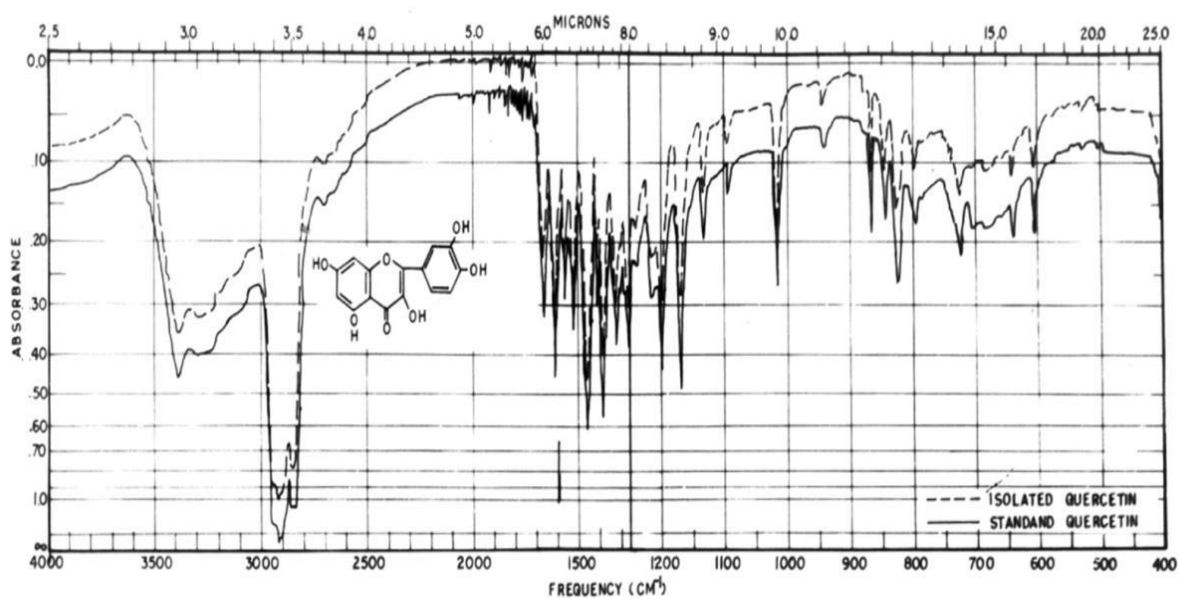


Fig. 4. IR spectra of Quercetin from *Basella alba* L.

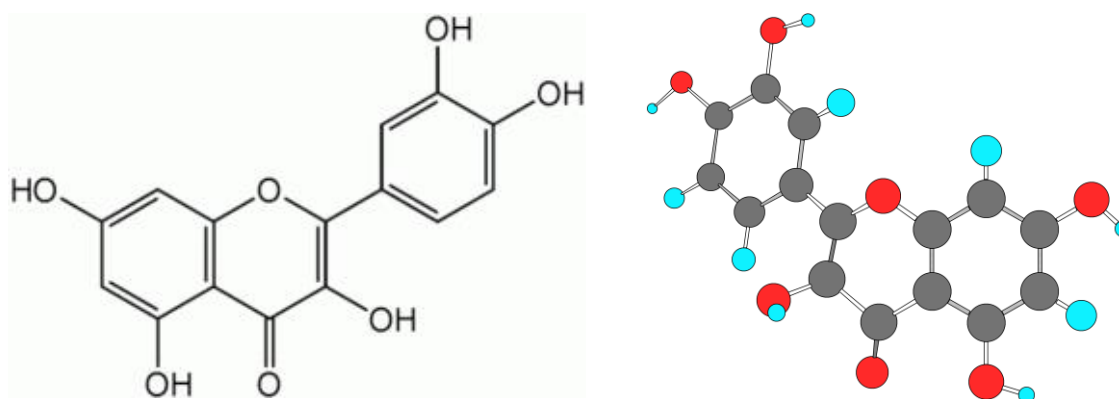


Fig. 5. (A) Structure of Quercetin (B) 3-Dimensional model of Quercetin

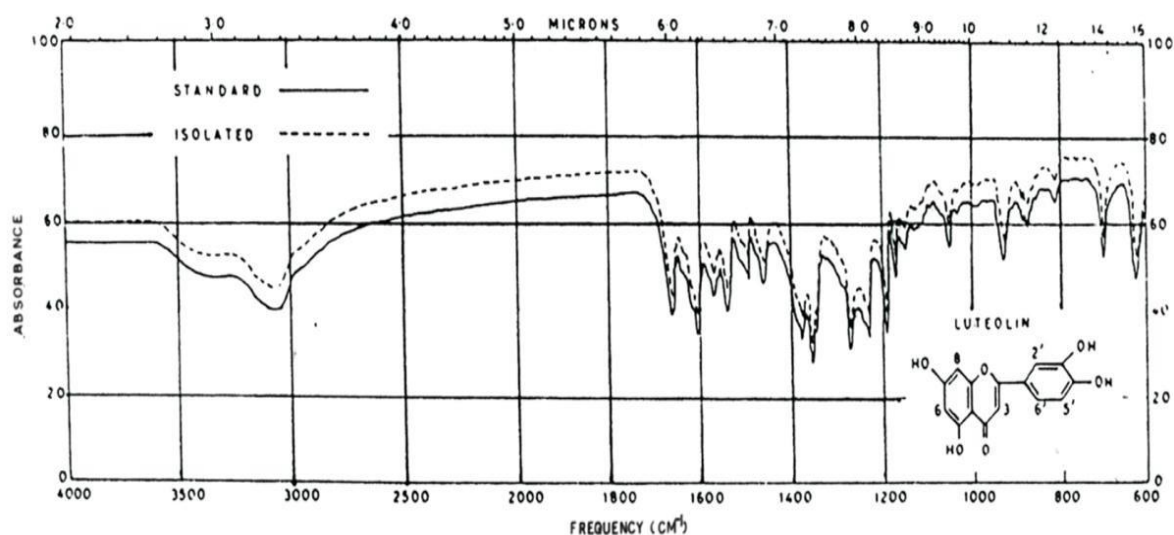


Fig. 6. IR spectra of Luteolin from *Basella alba* L .

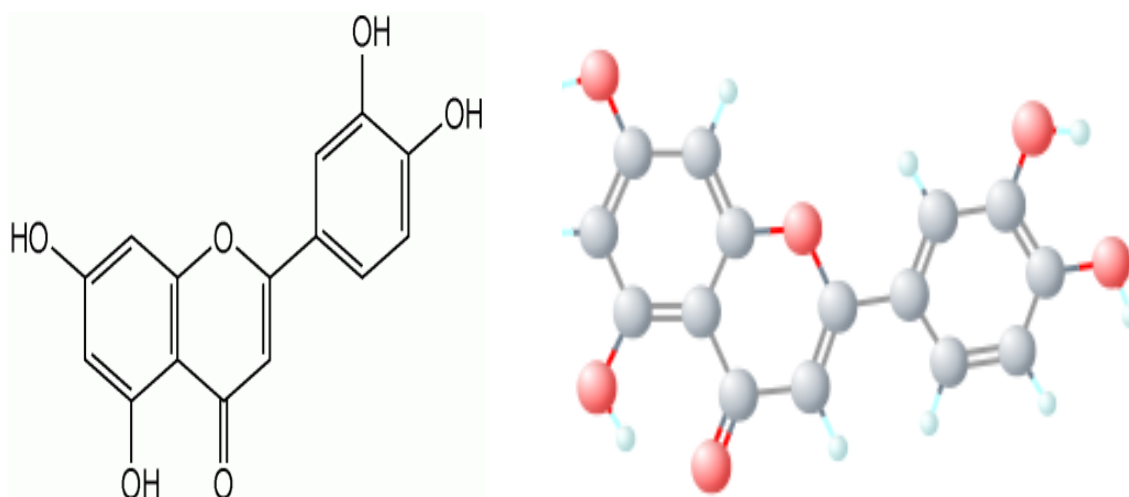


Fig. 7. (A) Structure of Luteolin (B) 3-Dimensional model of Luteolin

Table 2. Flavonoids content (mg/gdw) in different plant parts of *Basella alba* L.

S No.	Plant Parts	Free flavonoids (mg/gdw)				Bound flavonoids (mg/gdw)				Total flavonoids (Free+bounds) (mg/gdw)
		K	Q	L	T	K	Q	L	T	
1.	Stem	0.13±	0.12±	0.10±	0.35±	0.05±	0.03±	0.02±	0.10±	0.45± 0.005
		0.002	0.002	0.001	0.003	0.001	0.001	0.001	0.001	
2.	Seeds	0.04±	0.07±	0.05±	0.16±	0.07±	0.08±	0.05±	0.20±	0.36± 0.004
		0.001	0.002	0.001	0.002	0.002	0.002	0.001	0.003	

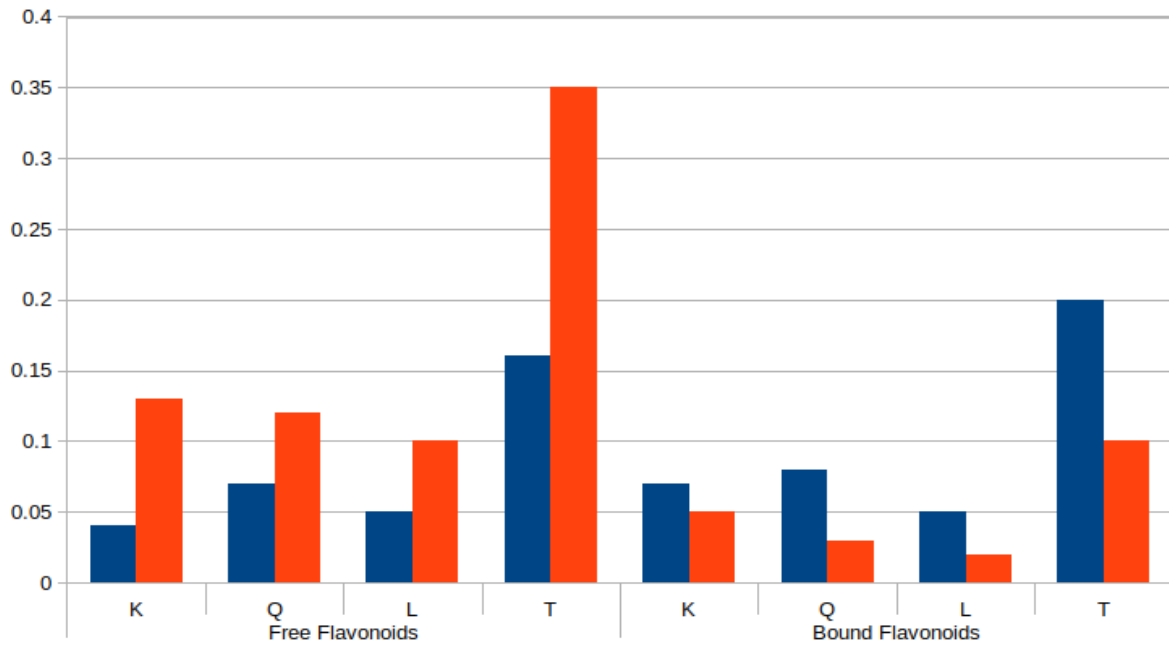
Abbreviation : K-Kaempferol, Q-Quercetin and L-Luteolin

Value are the mean =SEM (n = 3 variable in each group ) mg/gdw : milligram /gram dry weight

SEM = SD/√3

SD = Standard Deviation

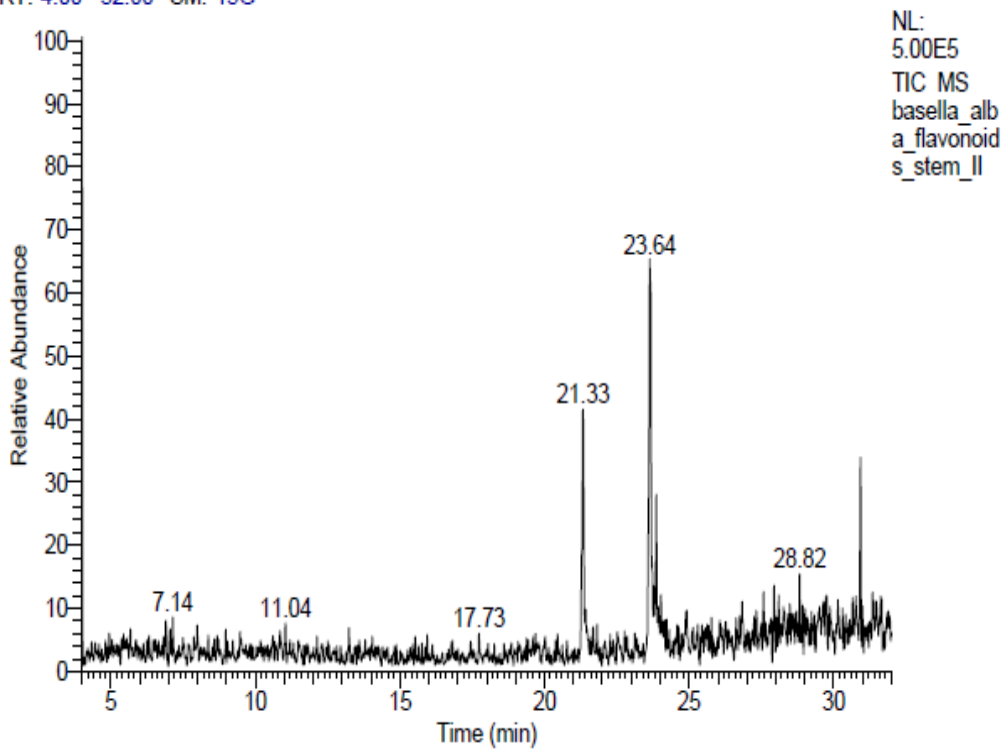
SEM = Standard Error Mean



**Fig. 8. Flavonoids content (mg/gdw) in different plant parts of *Basella alba* L.**  
Blue: Seeds and Orange: Stem

Data File:	basella_alba_flavonoids_stem_II	Injection Volume(μl):	1.00
Sample ID:	1	Run Time(min):	28.66
Comments:		Scans:	8428
Low Mass(m/z):	50	High Mass(m/z):	700

RT: 4.00 - 32.00 SM: 15G



**Fig. 9. Showing GC-MS of flavonoids isolated from stem of *Basella alba* L.**



**Table 3. GC-MS analysis of flavonoids from stem of *Basella alba L.***

S.No	R.Time	Area	Area%	Compound Name
1.	6.90	74985	1.21	2,5-Dichlorophenol,dimethylcyclohexylsilyl ether
2.	7.14	53230	0.86	(2S,6R,7S,8E)-(+)-2,7-Epoxy-4,8-megastigmadiene
3.	8.97	28428	0.46	Carbonic acid, 2-methoxyethyl 4-benzyloxyphenyl ester
4.	21.32	790543	12.75	1,1-Diisobutoxy-butane
5.	21.42	72091	1.16	Cis-3,5-Diethyl-1,2,4-trithiolane
6.	21.82	33815	0.55	2-Thiopheneaceticacid, hex-4-yn-3-yl ester
7.	23.64	1518960	24.50	1-Methyl-4-isopropylcyclohexyl 2-hydroperfluorobutanoate
8.	23.78	39228	0.63	Oxireno[4.5]cyclopenta[1,2c]Spyran2-(1bH)-one,1a,4,5,5a,6,6a-hexahydro-5a,6-dihydroxy-1a-methyl-,(1aà,1bà,5aà,6à,6aà)
9.	23.87	188332	3.0	3-Methylbut-2-enoic acid, 2-methylpentyl ester
10.	24.02	58625	0.95	Furan-2-carboxylic acid cyclohexylamide
11.	24.64	30020	0.48	L-Proline, N-pivaloyl-, isohexyl ester
12.	24.89	63007	1.02	2H-1,2-Oxazine, 6-(4-chlorophenyl) tetrahydro-2- methyl
13.	25.68	28196	0.45	Bicyclo[2.2.1]hept-5-ene-2-acetic acid, ethyl ester, endo
14.	25.77	54414	0.88	N-Methoxy-N-trifluoroacetyl-1,1-dimethyl-2-carbomethoxye thylamine
15.	26.63	34759	0.56	2-Methoxybenzoic acid, 3,4-dimethylphenyl Ester
16.	26.75	56745	0.92	Trimethyl(3,3-difluoro-2-propenyl) silane
17.	26.83	129970	2.10	N-Ethyl-3-methoxy-4-methylphenethylamine
18.	27.32	65753	1.06	Propanoicacid,2,2-dimethyl,2-(1,1-dimethylethyl)-4-methylphenyl Ester
19.	27.58	89523	1.44	Pyridine, 2-(bromomethyl)-
20.	27.84	83873	1.35	Benzenecarbothioic acid,S-propyl ester
21.	27.94	157520	2.54	Pyridinium, 1-amino-, chloride
22.	28.03	35577	0.57	2-Thiopheneacetic acid, benzyl ester
23.	28.10	85955	1.39	Silane, 2-butene-1,4-diylbis[ trimethyl
24.	28.26	85371	1.38	3,4-Hexanediol, 3,4-dimethyl
25.	28.42	50711	0.82	Benzoic acid, (2,3,4,5-tetrafluorophenyl) methyl ester
26.	28.48	51806	0.84	Cyclopentadienyl(4,5,6,7-tetrahydro-7-oxo-5-methoxycarbon ylindenyl)iron
27.	28.72	74082	1.19	Methylazoxymethanol acetate
28.	28.82	95305	1.54	5,10-Pentadecadienal, (E,E)-
29.	28.93	69960	1.13	Acetic acid, mercapto-, 3-methylbutyl ester
30.	29.01	102306	1.65	Acetamide, N-(3-methyl-2-oxobutyl)
31.	29.08	100284	1.62	2- Butenoic acid, 3-methyl-2-[( trimethylsilyl)oxy]-, trimethylsilyl ester
32.	29.24	42388	0.68	Penicillamine, tri-TMS
33.	29.33	45641	0.74	Cyclohexanol, 4-[(tert-butyl)dimethylsilyl) oxy]-
34.	29.51	188246	3.04	4-Cyclohexene-1,2-dicarboxylic acid, 4-chloro-, bis(trimethylsilyl) ester
35.	29.65	91364	1.47	1,3-Benzenediol, o'-(4-methylbenzoyl)-o'-( 2-methoxybenzoyl)-
36.	29.76	133496	2.15	3-Ethyl-3-octene
37.	29.83	33368	0.54	[1,4]Dioxino[2,3-b]-1,4-dioxin,Hexahydro
38.	29.87	98136	1.58	Methyl methanesulfonylacetate
39.	30.15	91713	1.48	Piperazine, 1,4-bis[( 4-methylphenyl)-sulfonyl]s
40.	30.31	43080	0.69	Á-l-Arabinopyranoside,methyl
41.	30.41	74384	1.20	5-Fluoro-2-trifluoromethylbenzoic acid, 4-benzyloxyphenyl ester
42.	30.65	87205	1.41	Sarcosine, N(4methoxybenzoyl), ethyl ester

S.No	R.Time	Area	Area%	Compound Name
43.	30.76	128271	2.07	Methyl trimethylsilyl carbonate
44.	30.93	337219	5.44	2,4Dimethyl5,6dithia2,7nonadienal
45.	31.00	55182	0.89	2,4-Pentadienoic acid, 1-cyclopenten-3-on 1-yl ester
46.	31.35	71729	1.16	Kurchessine
47.	31.39	50876	0.82	2-(-1-Methylcyclohexyloxy)- tetrahydropyran
48.	31.48	89587	1.44	1-Cyclohexyl-3-ethoxybutan2one
49	31.63	81702	1.32	2-(-1-Methyl1methoxyethyl)-2,3dihydrobenzo [b]thiophene-7-carboxylic acid, methyl ester

### 3.2 Quantitative Evaluation

Total flavonoids content (free and bound) was found to be slightly more in stem (0.45 mg/gdw) than in seeds (0.36 mg/gdw). Flavonoids content in its free form was more as compared to the bound form in plant part. Individually, all the isolated flavonoids were more in stem with the highest amount of kaempferol (0.13 mg/gdw) followed by quercetin (0.12 mg/gdw) (Table 2 and Fig. 8).

### 3.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Forty nine compounds were identified in GC-MS analysis of flavanoids from stem of *Basella alba* L. Compound 1-Methyl-4-isopropylcyclohexyl 2-hydroperfluorobutanoate was found in highest amount (area of % 24.50) at the retention time of 23.64 and compound Bicyclo[2.2.1]hept-5-ene-2-acetic acid, ethyl ester, endo was found in lowest amount (area of % 0.45) at the retention time of 25.68 (Fig. 9 and Table 3).

## 4. DISCUSSION

Flavonoids are natural product of phenolic glycosides synthesized from aromatic amino acids, occur almost naturally in angiosperms. They provide colour to flowers and fruits, which play a role in attraction of pollinating insects. Flavonoids have also been reported to have pathological significance in plants by providing resistance to the plants against pests and insects besides physiological importance for animals. (16). Total flavonoids content (free and bound) was found to be slightly more in stem (0.45 mg/gdw) than in seeds (0.36 mg/gdw). Flavonoids content in its free form was more as compared to the bound form in *Basella alba* L.

## 5. CONCLUSION

The present investigation has been done to isolate and identify flavonoids in the experimental plant using TLC, IR and GC-MS. The presence of

these bioactive compounds in selected plants lends credence to its use for welfare of mankind. It also accounts for the production of novel medicines with isolation of specific compounds.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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