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Isolation and identification of flavonoids from *Alhagi maurorum*

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Abstract
There are several photochemical in plants, viz. Tannins, flavonoids, sterols, alkaloids and triterpenes, etc., which has vital role in nutrition, physiology and cure of diseases. Flavonoids are one of the most important classes of phenol compounds in higher plants. The primary significant role in this pattern is the screening of these photochemical in the plants. The chromatographic study of the compounds serves to be a very important and dependable basis in the process of bioactive compounds screening in plant *Alhagi maurorum* used as medicinal herbs for its gestroprotective, diaphoretic, expectorant, antidiarrhoeal and antiseptic properties, and in the treatment of rheumatism and hemorrhoids. It is also been used as a sweeteners. Hence in the present study, focus has been made to identify the flavonoid in different samples of the medicinal plant (*Alhagi maurorum*) by TLC and PTLC. Further, the isolation of the same compound was confirmed by TLC, IR and GC- MS analysis.

Keywords: *Alhagi maurorum*, flavonoids; IR, TLC, GC-MS

Introduction
Bioactive photochemical that occur naturally in floras are accountable for the color and organoleptic properties such as deep purple color of blue berries and smell of garlic. These compounds are called as the secondary metabolites. The term, photochemical, is normally used to indicate to those chemicals which possess therapeutic ventures. These are used in drugs since many decades. Most of natural product has been characterized for secondary metabolite screening and their possible use in the harmful diseases like cancer. Present research is engaged in the identification and isolation of new therapeutic compounds of medicinal importance from the plants for first time [1].

Flavonoids are a phenolic substituent's synthesized by plants which bear similar structure. They can be easily identified as flower pigments in angiosperms. Though, their amount is not restricted to flowers only, as they are present in every plant part. They are also known to play a vital role in providing resistance to the plant species, like rotenone, which is flavonoid, acting as a potent insecticide. The probable effect of these compounds on human health is also widely researched especially in the curing of cancer particular breast cancer [2]. Further, utilization of soy foods rich in is flavones has been unnoticeably linked with decrease colon cancer.

Alhagi maurorum locally called camelthron belongs to the family, fabaceae and found globally, *Alhagi maurorum* has been used locally in folk medicine as a treatment for glandular tumors, nasal polyps, and ailments related to the ducts Retrieved [3]. During photosynthesis primary metabolites are synthesized and these bioactive compounds are necessary for plant life, growth and development [4]. Many primary metabolites used as pharmacologically active metabolites in pharmaceutical compounds. Many types of metabolic reactions create diverse types of 'oxidant' and 'antioxidant' species in human bodies. Super oxide anion, hydrogen peroxide, hydroxyl radicals are work as Reactive Oxygen Species (ROS) [5].When oxidants increase in human body, oxidative stress is developed. Due to this oxidative stress many diverse diseases and complexities including aging, cataract cancer, autoimmune disorders, arthritis, cardiovascular and neurodegenerative diseases developed in body [6].

In the present research estimation of flavonoids was done along in *Alhagi maurorum in* to find out their therapeutic ventures.

Material and Methods
Extraction
The experimental plant sample was collected from Jaipur- Ajmer highway Rajasthan (India). The plant samples were cleaned, shade dried and grinded to powder and kept for further use. These samples were finally rinsed with 80% methanol for 24 h.

The samples which were soluble in methanol were filtered, concentrated *in vacuo* and were separated by sequential extraction with petroleum ether (Fr1), diethyl ether (FrII) and ethyl acetate (FrIII) separately. Every method was done three times to isolate the compound completely, fraction 1 was discarded in each case as it had fatty substance, while fraction II and III were resolute and used for identifying flavonoids.
Fraction III was more reacted by refluxing with 7% H_2SO_4, filtered and filtrate was isolated three times with ethyl acetate. All ethyl acetate layers were collected separately; pH was maintained by distilled water with continuous washings and dried *in vacuo.* Both fraction II and III were added in ethanol (2-5ml) for TLC.

Qualitative

Thin Layer Chromatography (TLC)

Glass plates of size (20x20 cm) were encrusted with Silica gel G (250mμ thick). The new plates were evaporated at room temperature; and further heated at 100 ^{0}C for 30 min to activate and collected for further analysis. Every sample was co- chromatographed with standards (quercetin, luteolin, kaempferol).These plates were kept in an saturated chamber with solvent system containing (Benzene: Acetic Acid: Water: 125:72:3 [7]. The developed plates were evaporated and seen under UV light with little exposure to ammonia fumes. The developed plates were sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and fumigated withI_2 chamber. The colored spots developed were recorded and the RF value of each spot was noted down. Other solvent mixtures having tertiary butanol, acetic acid, water (3:1:1), n- butanol, acetic acid, water (4:1:5), were also screened, however solvent system of benzene, acetic acid, water (125:72:3) was found to be best among all the samples tested.

Preparative thin layer chromatography (PTLC)

PTLC of above extracts were done using silica gel G coated plates having 500mμ thickness by spotting the extract along with standards. These plates were saturated in the solvent mixture of benzene, acetic acid, and water (125:72:3), evaporated and screened under UV light. Each of spots resembling with the standard were noted, eluted from 200 plates, and isolated with 50% CH_3OH. The eluted fractions were filtered, evaporated and again spotted along with standard to confirm their purity which were subjected to crystallization separately and UV (Ultraviolet and visible spectrophotometer; Carl Zeiss, Jena, DDR, VSU-2P spectrophotometer) and IR (Perkin, Elmer 337,Grating Infra-red spectrophotometer), melting point, mixed melting point (Toshniwal Melting Point Apparatus) was determined.

Quantification

The isolated flavonoids were estimated by spectrophotometer using established protocol [8].
Stock solution (1mgL^{-1}) of all standards was prepared by dissolving markers in methanol. Different stocks from 20μg to 160μg of every sample were marked separately on silica gel G plates. These developed plates were evaporated and seen under UV light. The fluorescent spots were marked and eluted along with the absorbance in separate test tubes. Methanol of higher grade (5ml) was added to each test tube, stunned robustly, centrifuged and supernatants were eluted separately. The volume of each of the sample was rinsed to 10ml by adding methanol. To each of these samples, 3ml of 0.1 M $AlCl_3$ solution was added again stunned robustly and kept at room temperature for 20 min. 5 replicates done in each case and their O.D. were measured at 426nm for kaempferol and luteolin and at 440nm for quercetin against blank (10ml of \methanol and 3ml of 0.1 M $AlCl_3$). The standard curves were drawn between concentration and their respective average optical density of each of the compound.
Every extract (Fr. II and III) was dissolved in 5 ml of methanol and 0.1ml was spotted on silica gel G coated plates along with standard, separately. The plates were developed as above and the spots resembling with that of standard were marked on each plate under UV. Each spot was isolated with the silica gel, in methanol and plant extracts were prepared in the same way as described above. The O.D. in each case was recorded and concentration of each sample was drawn using the regression curve of standards flavonoids. The concentrations were quantified on mg/g dry weight basis.

GC-MS analysis

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 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40 °C/min; detector temperature, 250 to 280 °C; carrier gas, He (0.9 ml/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The component of the standard and plant sample was 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.

Results

Three spots of flavonoids were observed in plant part (leaf, stem) of both selected samples on thin layer chromatography plates developed and sprayed with 5% $FeCl_3$.The R_f values of these spots matched with their respective authentic standards and were identified as kaempferol, quercetin, luteolin.Solvent system Benzene: Acetic Acid: Water (125:72:3) gave best results with R_F values viz., kaempferol, 0.17; luteolin, 0.11; quercetin, 0.6 (Table-1). When other solvents viz n-Butanol: Acetic acid: Water (4:1:5) and conc. HCl: Acetic acid : Water (3:30:10) the R_f value of kaempferol was found to be 1.13 and 1.39 that of quercetin was found to be 0.75 while that of luteolin was found to be 0.11 and 0.6, respectively.
The isolated flavonoids viz., kaempferol, quercetin, luteolin were also identified and characterized by super imposable IR peaks (Fig. 1, 2, 3.), mp (kaempferol, 271-273 ^{o}C; luteolin327-328 ^{o}C; quercetin 309-311 ^{o}C and UV maxima (nm) in methanol (kaempferol, 253sh, 266, 394sh, 322sh, 368; quercetin 255, 269sh, 301sh 374; luteolin 242sh, 253, 267, 291sh, 349 which were comparable to the respective authentic standards.

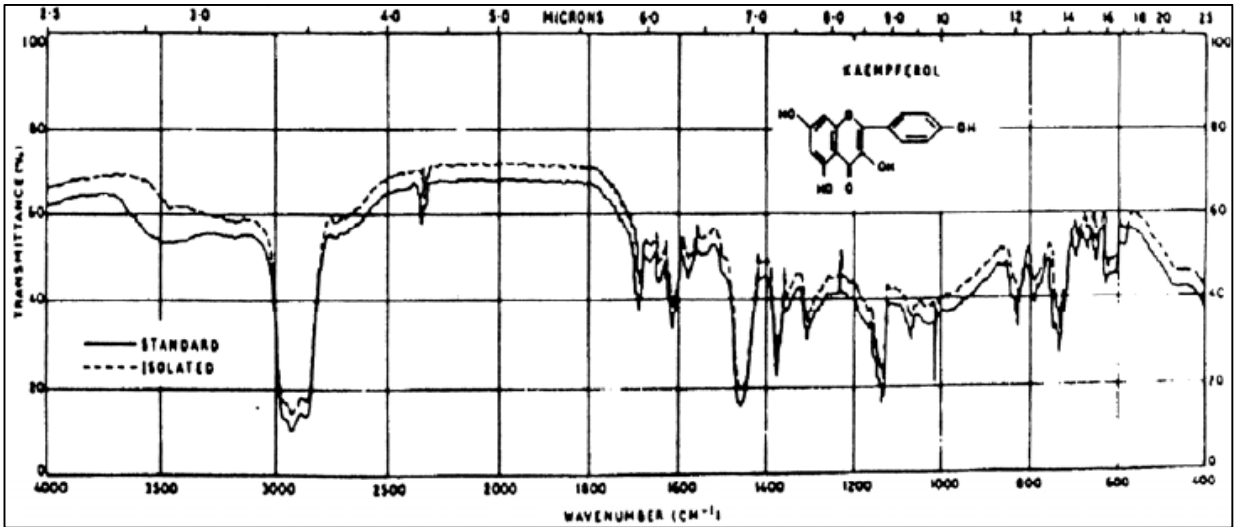

Fig 1

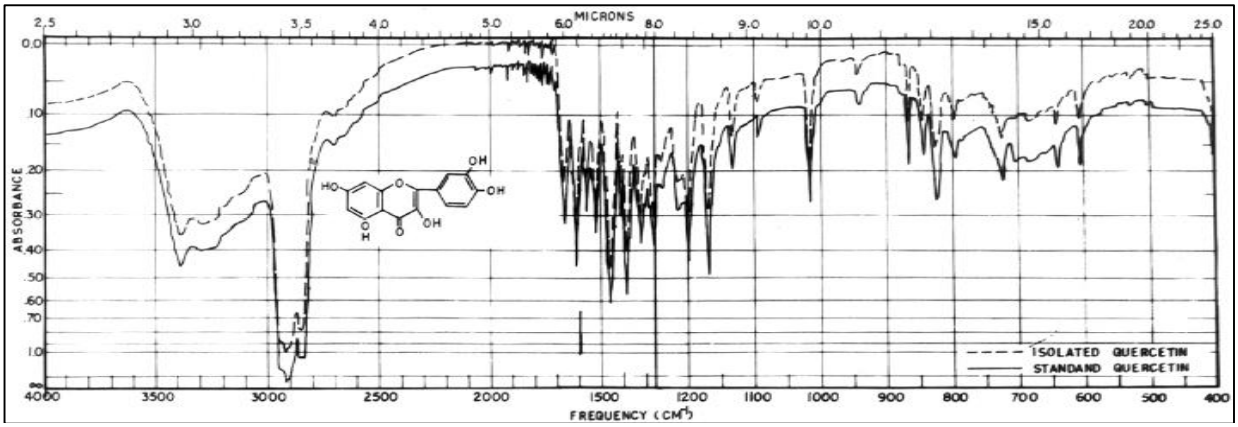

Fig 2

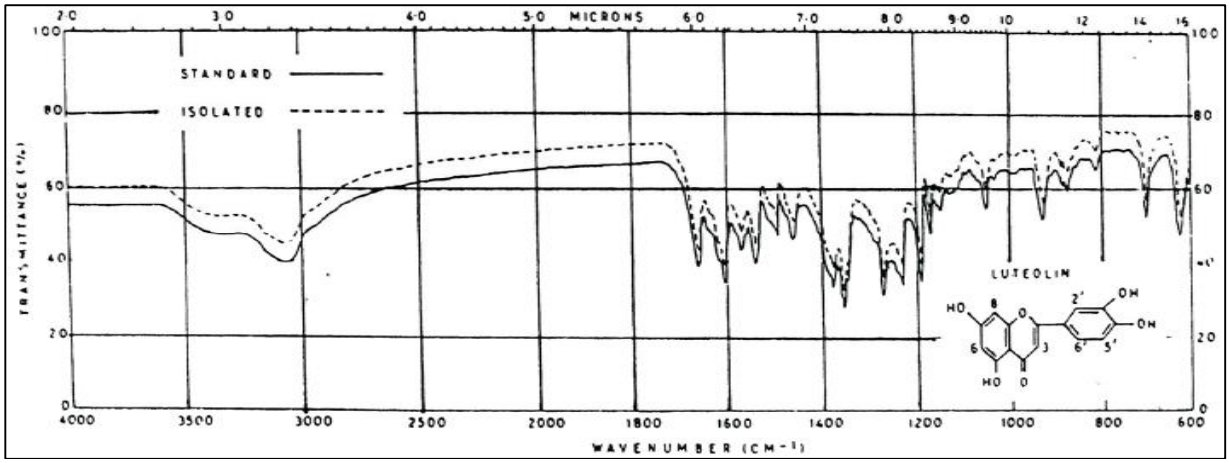

Fig 3

Quantitative analysis

Alhagi maurorum

Among the plant parts total flavonoid content (free & bound) was maximum in leaf (6.65mg/gdw), and minimum (5.55mg/gdw) in stem. Flavonoid content in its free form was more as compared to the bound form in plant parts.

Table 1: Flavonoids content (mg/gdw) in plant parts of *Alhagi maurorum.*

S. No.	Plant Parts	Free flavonoids (mg/gdw)				Bound flavonoids (mg/gdw)				Total flavonoids (free+ bound) (mg/gdw)
		K	Q	L	T	K	Q	L	T	
1.	Stem	0.17	0.6	0.11	0.88	1.28	2.25	1.33	4.86	5.74
2.	leaf	1.13	1.39	0.75	3.27	0.89	1.59	0.69	3.17	6.44

GC-MS analysis

In GC-MS analysis of flavonoids from leaf of *Alhagi maurorum* total 32 compounds were found. Maximum area was observed in 1Cyclohexyl1, 3bistrifluoroacetyl3 (2chloroethyl) urea (5.91%).

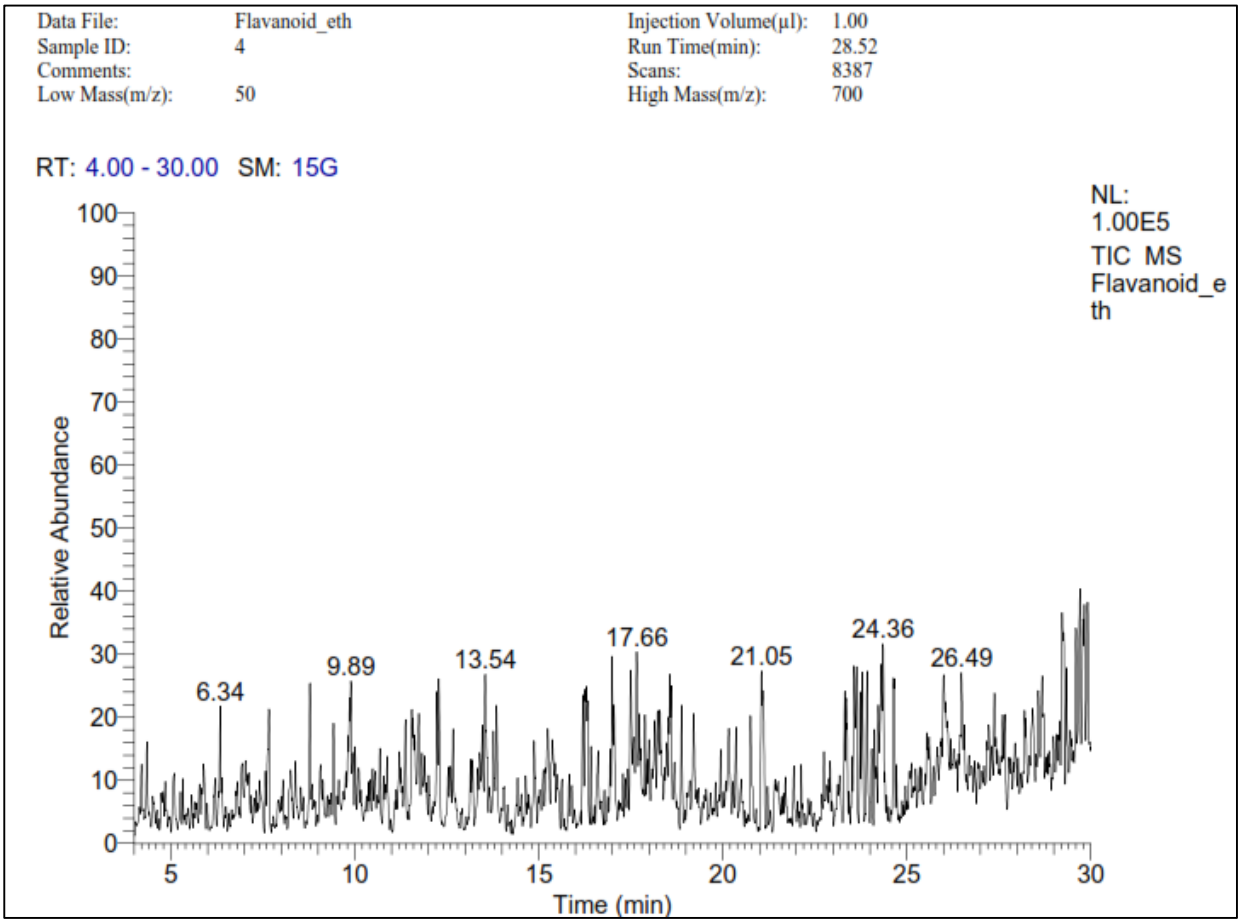

Table 2: GC MS analysis of flavonoids from Leaf of *Alhagi maurorum*

RT	Compound Name	Area	Area %
6.34	4Pyridinamine, Nnitro	22941	1.21
7.67	Benzene, 1bromo4(methylsulfonyl)	50622	2.67
8.78	Octatriene,	31610	1.67
9.42	2Cyclohexen1one,5hydroxy2,3,5,6,6pentamethyl4methylene	19275	1.02
9.89	1'Acetoxy4' methoxy2,2'binaphthalene1,4dione	39342	2.08
11.38	N(2,2,3,3Tetrafluoroaziridino) difluoromethyleneimine	24179	1.27
11.55	Trans3Methoxybmethylbnirostyrene	32507	1.71
12.23	2Chloro3(2chloroethyl)6phenyl3,4dihydro2H1,3,2oxazaphosphinine 2oxide	22568	1.19
12.28	1,3Oxathiolane, 5methyl	40103	2.11
12.67	Chloro(methylthio)methylenimino]sulfur pentafluoride	15284	0.81
13.54	1HImidazole, 2(diethoxymethyl)1methyl	40551	3.31
13.85	3Methyl2,3dihydro1,5benzothiazepin4(5H)one 1oxide	29279	1.54
16.21	1,4Diamino2(hydroxymethyl)anthraquinone	40845	2.15
16.26	Chlorodifluoroamine	65730	3.47
16.33 4	[N(2Methylphenyl) Nmethylamino] 2methoxy2 4ch lorophenyl)1,2,2a, 3tetrahydroazeto(1,2a)(1,5)benzodiaze pin1one 16.42 Butanoic acid, 3methyl3nitroso,	26353	1.39
16.42	Butanoic acid, 3methyl3nitroso,methyl ester	12124	0.64
16.99	Isoquinolin7ol6one, 2,3,4,6tetrahydro1[4hydroxybenzyl]	31599	1.67
17.05	Benzene, 1,2dibromo3,4,5,6tetrafluoro	25926	1.37
17.51	2,2Diphenyl1,3,6trioxa2silacyclooctane	45977	2.42
17.66	1H1,3,4Benzotriazepin2amine, 7chloroN, 1dimethyl5phenyl	49092	2.59
17.89	Mercaptoacetone	17356	0.91
18.56	Rhenium, pentacarbonyliodo	45067	2.38
18.88	2,4(1H,3H)Pyrimidinedione,3,6dimethyl	24985	1.32
19.21	Methylphosphonic acid, 2,2dimethylcyclohexyl[(dimethyl)(tertbutyl)silyl] ester	21124	1.11
20.76	Trimethylsilyldimethylphosphinate	26198	1.38
21.05	1Cyclohexyl1,3bistrifluoroacetyl3(2chloroethyl) urea	112148	5.91
23.32	sTriazolo [4,3a] pyridine, 3,8dimethyl	26879	1.42

23.35	Trimethylthioborate	39799	2.10
23.58	2Methoxy3,4,4trimethylë1azetine	71504	3.77
23.64	2(Methylthio)4(4methylphenyl) 6phenylpyridine3carb oxamide	37775	1.99
29.34	LPhenylalanine, N[(5chloro3,4dihydro8hydroxy3methyl1oxo1 2b enzopyran7yl) carbonyl], methyl ester	21426	1.13
29.60	2Dimethyl(pentafluorophenyl)silyloxypentadecane	31223	1.65

Discussion

Flavonoids are natural product phenolic glycosides synthesized from aromatic amino acids, occur almost naturally in angiosperms. They provide color 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. The flavonoids usually present in their free form and at reactive sites in their bound form as glycosides. Therefore, the difference in content between free and bound forms shows their involvement at resting and active stages, thus giving higher or lower recovery of free and/or bound flavonoids (9).

Conclusion

The present investigation has been done to isolate and identify flavonoids in the experimental plant using 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.

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