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Qualitative and Quantitative Evaluation of Flavonoids from *Corchorus olitorius* L.

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ABSTRACT

Corchorus olitorius is fibre yielding plant with nutritive value so human used its various parts in their food. Flavonoids are secondary metabolites of plants and exist with variable phenolic structures.In the present study carried out the qualitative and quantitative analysis of flavonoids by using chromatographic and spectral studies.Total amount of flavonoids were found in *Corchorus olitorius* (0.55 mg/gdw in stem and 0.30 mg/gdw in fruit). Thirty three compound were found in GC-MS analysis, Ethyl Oleate found to be maximum from fruits of *Corchorus olitorius*.

Keywords: GC-MS,flavonoids , *Corchorus olitorius*.

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INTRODUCTION:

Jute plant C. *olitorus* known as tossa jute belongs to the family, Tiliaceae and has been used in different parts of the world, not only as spice for food but also for the treatment of chronic cystitis and dysuria[1]. In African countries the shoot tips and leaves of *Corchorus olitorius* are always eaten. In West Africa, their edible qualities are widely appreciated, where the shoots and leaves are combined in soups and stews. It contains high quantity of Vitamin A, protein, fiber, calcium, iron, carotene and folic acid.[2] Generally flavonoids give colour, taste in food and responsible for, prevention of fat oxidation, and protection of vitamins and enzymes. In 1930 scientists isolated a new compound from oranges and give name vitamin P .after some time it became clear that this Compound was a flavonoid (rutin)[3]. Flavonoids are included in plants secondary metabolites and characterised by C6-C8-C6 carbon-skeleton and flavan nucleus[4,5,6]. Flavonoids are play an important role in health-promoting properties by their high antioxidant capacity both *in vivo* and *in vitro* systems so flavonoids take as a dietary component in diet.[7,8] Flavonoids performs many biological activities like antiallergic, antibacterial, anti-inflammatory ,antiviral ,cardiovascular diseases, cancers, and other age-related diseases.[7,8,9,10]

MATERIAL AND METHODS

Collection and Identification of Plant Materials

Fresh stem and fruit of the selected plant *Corchorus olitorius* L. were collected from Jhunjhunu, India. The plant materials were taxonomically identified and authenticated by department of botany, University of Rajasthan (RUBL 211573), Jaipur. Whole plant were cleaned, shade dried and stem and fruit separately pulverized to powder in a mechanical grinder. The powdered materials were stored in air tight containers till use.

Extraction

Plant parts of *Corchorus olitorius* L.(stem and fruit). Each of these extracted separately with 80% methanol on water bath for 24 h[11]. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr1), diethyl ether (FrII) and ethyl acetate (FrIII) separately. Each step was repeated thrice for complete extraction, fraction 1 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^{-1} 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.

Qualitative analysis

Thin Layer Chromatography (TLC)

Thin glass plates (20x20 cm) were coated with Silica gel G (250mμ thick). The freshly prepared plates were air dried at room temperature; thereafter these were kept at 100 ^{0} 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. (quercetin, luteolin, kaempferol and rutin). These plates were developed in an air tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water:: 125:72:3; Wong & Francis, 1968)[12]. 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_4OH 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% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and kept in I_2 chamber separately. The coloured spots thus developed were noted and the Rf value of each spot was calculated. Several others solvent systems such as n- butanol, acetic acid, water (4:1:5), were also tested, but the solvent system- containing benzene, acetic acid, water (125:72:3) gave better results.

Preparative thin layer chromatography (PTLC)

PTLC of aforementioned flavonoid extracts was carried out using silica gel G coated plates (BDH ; 500mμ in thickness) by spotting the extract as well as standard markers (luteolin, kaempferol, quercetin and rutin). These plates were developed in the solvent mixture of benzene, acetic acid, and water (125:72:3), air dried and examined under UV light. Each of spots corresponding with the standard markers were marked, scraped from 200 plates, and eluted with 50% methanol. The eluted fractions were filtered, air dried and again co-chromatographed along with standard markers to test their purity. The eluted fractions were subjected to crystallization separately and melting point (mp), mixed melting point (mmp) was determined. The isolates were also subjected to ultraviolet and infrared spectral studies.

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.

Quantification

The isolated flavonoids were estimated by spectrophotometer following the method of Mabry *et al* (1970)[13].Stock solution (1mgL^{-1}) of kaempferol, luteolin, quercetin and rutin were prepared separately by dissolving authentic compounds in methanol. Different concentrations ranging from 20μg to 160μg 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. Spectroscopy methanol grade (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 $AlCl_3$ 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 $AlCl_3$). 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 (ether and ethyl acetate sample) 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.

RESULT AND DISCUSSION:

Qualitative evaluation

All the experimental plant parts showed the presence of three flavonoids Viz. kaempferol, luteolin and quercetin. Three flavonoids spotted which were common in plant parts on thin layer chromatography. The Rf values of the spots matched with authentic standards and IR specta also matched with standard spectra so the isolated flavonoids identified as kaempferol,luteolin and quercetin.(**Table1 and Fig.1.2,3)**

Table 1: Chromatographic behavior and physicochemical characteristics of isolated flavonoids in *Corchorus olitorius*

Isolated Compounds	Rf Value			Physical appearance			Color after spray				Melting Point (°C)	IR Spectral Peaks (KBr) cm^{-1}
	S1	S2	S3	Daylight	UV ammonia	Iodine vapor	R1		R2			
							Visible	UV	Visible	UV		
Kaempferol	0.84	0.80	0.57	GN-YW	BT-YW	YW-BN	BN	BK	YW	YW-GN	276-278	(O-H) 3410 cm-1(270, 295, 344, 1690)
Quercetin	0.77	0.62	0.44	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	0.58	0.86	0.79	GN-YW	YW	YW-BN	TN	BK	DL-YW	YW-GN	326-329	3421, 2965, 1736 (Lactone), 1510 (Furan), 1461, 1388, 1360, 1274, 1242, 1187, 1136, 1028, 903, 850 cm-1

Abbreviations: S1- Benzene: acetic acid: water (125: 72: 3), S2- n-butanol: acetic acid: water (4: 1: 5), S3- Conc. Hydrochloric acid : acetic acid : water (3: 30: 10), R1- 5% FeCl3 solution, R2- %% alc. The AlCl3 solution, YW- Yellow, BK- Black, BN, Brown, BT-Bright, DL- Dull, GN- Green, GY- Gray

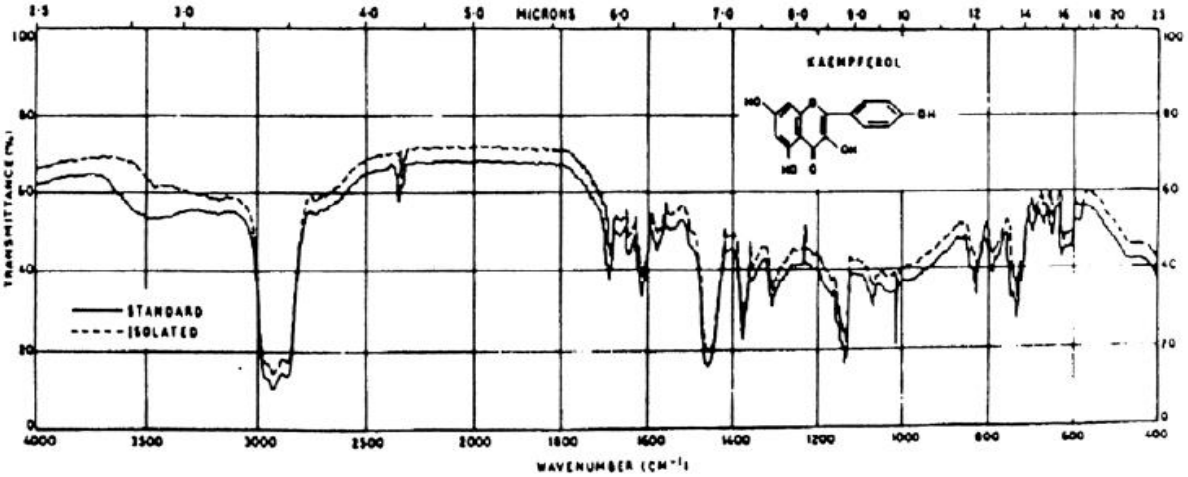

Fig.1: IR Spectra of Kaempferol

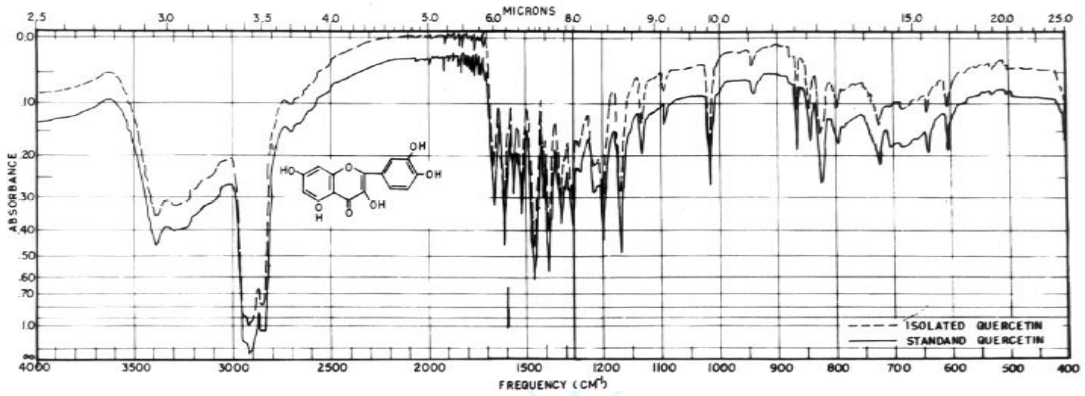

Fig.2: IR Spectra of Quercetin

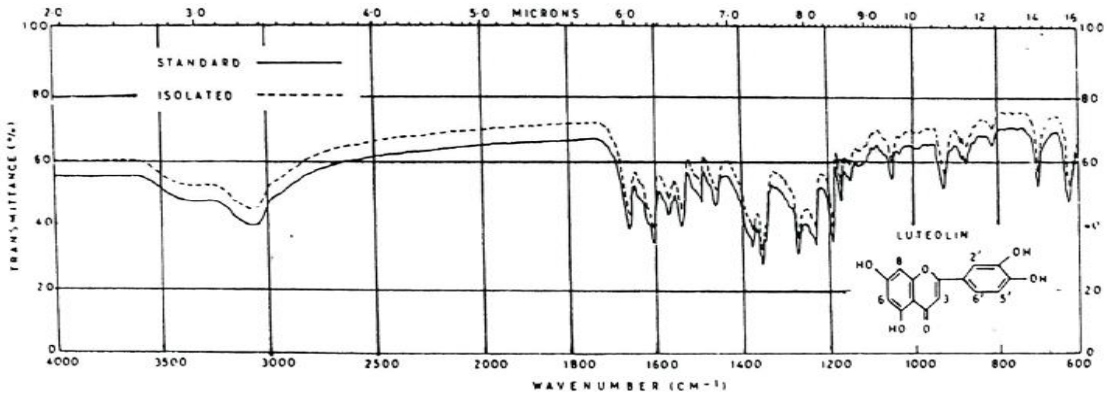

Fig.3: IR spectra of Luteolin

GC-MS analysis:

Thirty three compounds were found in GC-MS analysis of flavonoids from fruits of *Corchorus olitorius*. Compound that was found to be maximum was Ethyl Oleate with % area of 14.65 %, at the retention time of 20.68 and the compound which was minimum in amount is Squalene of 0.53% area, at the retention time of 30.50. **(Table 2and Fig 4)**.

Table 2: GCMS analysis of flavonoids from fruits of *Corchorus olitorius*

S. No.	RT	Compound Name	Area	Area %
1	6.89	Hydroquinone	404119	1.32
2	7.74	2-Methoxy-4-vinylphenol	356794	1.16
3	10.01	Benzene, 1-nitro-4-(phenylmethoxy	286532	0.93
4	15.35	2(4H)-benzofuranone, 5,6,7,7A-tetrahydro	775441	2.53
5	16.17	2,6,10-Trimethyl,14-ethylene-14-pentadecne	792247	2.59
6	16.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	461471	1.51
7	16.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	777965	2.54
8	17.75	Name Unknown	385394	1.26
9	17.99	n-Hexadecanoic acid	385394	1.26
10	18.09	Ethyl 9-hexadecanoate	601449	1.96
11	18.37	Hexadecanoic acid, ethyl ester	2794855	9.12
12	19.08	Hexadecanoic acid, trimethylsilyl ester	1014069	3.31
13	19.75	9,12-Octadecadienoic acid (Z,Z)-	233545	0.76
14	19.84	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	512175	1.67
15	20.01	Phytol	3373535	11.01
16	20.62	Linoleic acid ethyl ester	1747256	5.70
17	20.68	Ethyl Oleate	4490548	14.65
18	21.00	Octadecanoic acid, ethyl ester	1274433	4.16
19	21.30	Oleic acid, trimethylsilyl ester	560374	1.83
20	23.12	9-Octadecenamide	142116	0.46
21	23.49	Hexanedioic acid, bis(2-ethylhexyl) ester	327082	1.07
22	25.56	Bis(2-ethylhexyl) phthalate	1444260	4.71
23	30.50	Squalene	161392	0.53
24	31.36	Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacosa-1(25),3,5	663257	2.16
25	31.57	Cholesta-3,5-diene	355210	1.16
26	32.46	Cholest-5-ene, 3-methoxy-, (3.beta.)-	310708	1.01
27	33.11	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	1021401	3.33
28	33.61	Stigmasta-5,22-dien-3-ol, acetat, (3-beta,22-z)	529836	1.73
29	33.99	Stigmast-5-en-3-ol, (3.beta.)-	305185	1.00
30	34.57	Stigmast-5-en-3-ol, oleat	2102968	6.86
31	35.18	Vitamin E	960706	3.13
32	41.81	Betulin	574814	1.88
33	42.03	4-(1,3,3-Trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one	486335	1.59

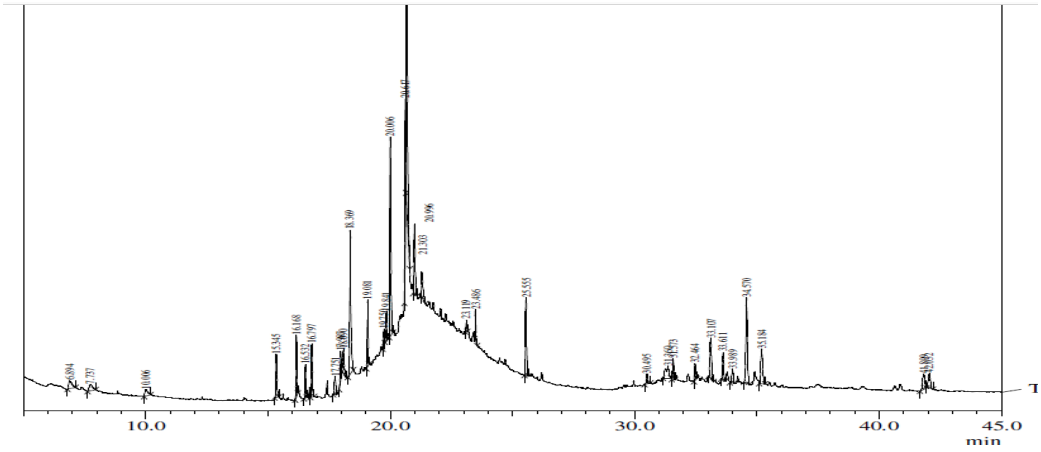

Fig.4:GCMS analysis of flavonoids from fruits of *Corchorus olitorius*

Quantitative evaluation:

Total flavonoid content (free & bound) was found to be slightly more in stem (0.55 mg/gdw) than in fruit (0.30 mg/gdw). Flavonoid content in its bound form was more as compared to the free form in plant parts. Individually, all the isolated flavonoids were more in stem with the highest level of quercetin (0.30 mg/gdw) followed by kaempferol (0.12 mg/gdw) **(Table-3 and Fig.5).**

Table 3: Flavonoids content (mg/gdw) in different plant parts of *Corchorus olitorius*

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.	**Fruit**	0.02	0.06	0.02	**0.10**	0.05	0.12	0.03	**0.20**	**0.30**
2.	**Stem**	0.02	0.02	0.01	**0.05**	0.12	0.30	0.08	**0.50**	**0.55**

Abbreviations: K-Kaemferol, Q-Quercetin, L-Luteolin

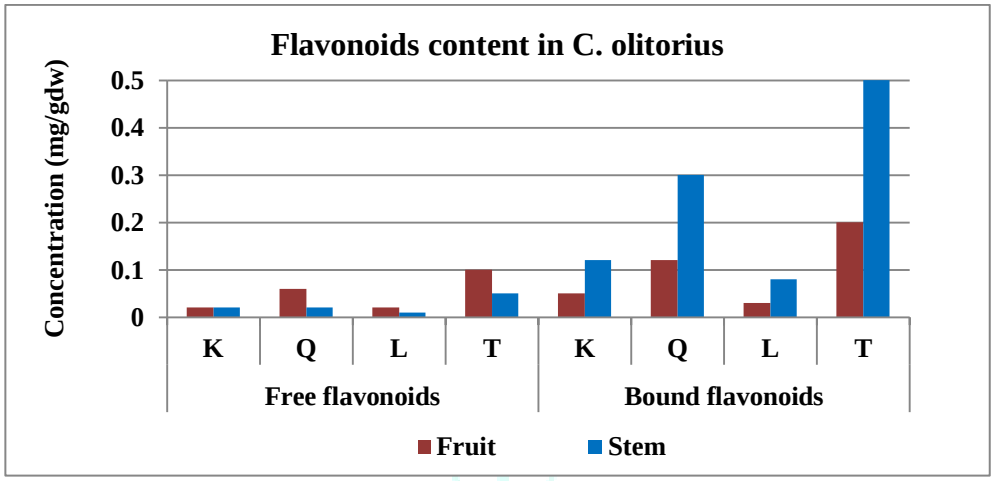

Fig. 5: Flavonoids content (mg/gdw) in different plant parts of *Corchorus olitorius*

CONCLUSION

Flavonoids are play remarkable role in biological activities .They are abundantly present in the human diet. Nutrient group of flavonoids is most famous for its health benefits, as well as its contribution of vibrant color to the foods we eat. Quercetin, kaempferol, catechins, and anthocyanidins are some of the best-known flavonoids. They know for their antioxidant and anti-inflammatory health benefits as well as the support of the cardiovascular and nervous systems. Flavonoids also help in detoxification of potentially tissue-damaging molecules, their intake has often, although not always, been associated with decreased risk of certain types of cancers, including lung and breast cancer.In present study *Corchorus olitorius* found good source of flavonoids quantitatively and qualitatively.

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