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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 730-733

www.thepharmajournal.com
Received: 01-09-2017
Accepted: 02-10-2017

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Extraction, isolation and spectral analysis of the psoralen compound from *Ficus carica* Linn. Leaves

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Abstract
Fig leaves (*Ficus carica* Linn.) belong to the family Moraceae which constituted one of the largest genera of the medicinal plants. *Ficus carica* is an important member of the genus Ficus. The common fig is one of the first plants that were cultivated by humans. Preliminary phytochemical screening of ethanolic extract shows the presence of carbohydrates, alkaloids, flavonoids, glycosides, proteins, saponins and terpenoids. Different biologically active compounds are also present in fig. The leaf of *Ficus carica* consists of various volatile compounds which are identified and distributed by distinct chemical classes. Different spectral analysis like HPLC, UV, IR, HNMR, C^{13} and Mass Spectrometry were done. From the results, it was confirmed that the isolated compound is Psoralen.

Keywords: *Ficus carica* Linn, Moraceae, Volatile compound, Spectral analysis, Psoralen

Introduction
Ficus constituted one of the largest genera of angiosperms, with almost 800 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurring in the tropics and subtropics worldwide [1]. It is a small or moderate sized deciduous tree, 3-10 m high with broad ovate or nearly orbicular leaves, more or less deeply 3-5 lobed, rough above and pubescent below; fruits axillary, usually pear shaped, variable in size and color [2]. The fruit of *Ficus carica*, like those of other species of *Ficus*, is a syconium a fleshy hollow receptacle with a narrow aperture at the tip. The bark is a cylindrical and pale grey coloured [3].
Ficus carica has been cultivated for a long time in various places worldwide for its edible fruit. It is supposed to originate from Western Asia and spread to the Mediterranean by humans [4]. *Ficus carica* possibly originated from the Middle East, which is one of the early cultivated fruit species [5]. *Ficus carica* commonly known as Anjir (Hindi) is cultivated in many parts of North-Western and south India [2]. *Ficus carica* pollinates by the pollinator wasp *Blastophaga psenes* (L.) [6]. *Ficus carica* (fig tree) has been extensively investigated for its proteolytic enzymes [7], amino acids, minerals and sugars [8], triterpenes [9], and organic acids [10]. The present investigation was undertaken to isolate the bioactive compound Psoralen from the leaves of *Ficus carica* leaves.

Materials and methods
Plant material
The leaves of the plant of *Ficus carica* were collected from the local surroundings at Kunzer area of Baramulla District of Jammu and Kashmir, during the month of August-September 2014. The plant was identified by Dr. Bikrama Singh, Scientist at Department of Botany, Indian Institute of Integrative Medicine (IIIM–CSIR) Jammu. The voucher specimen no. RRLH-22990 is kept in the herbarium of Indian Institute of Integrative Medicine (IIIM–CSIR) Jammu for futurereference.

Preparation of plant material
The live plants collected were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then the plant was shade dried without any contamination for about 3-4 weeks.
The dried plant sample was powdered (coarse) and subjected to maceration using ethanol and water. Preliminary phytochemical screening of ethanolic extract shows the presence of carbohydrates, alkaloids, flavonoids, glycosides, proteins, saponins and terpenoids.

The extracts obtained were then evaporated in rotary evaporator to get a powdery mass. The powder extract obtained was then mixed with water and fractionized with ethyle acetate. After this process the extracts was evaporated in rotary evaporator to get dried extracts.

Each extract was examined through the TLC using solvent system EtOH: Hexane (30:70) ratio to confirm the presence of different compounds present in them. After the TLC, column chromatography was done by the solvent system ethyl acetate: Hexane mixtures 05:95, 10:90, 20:80, 30:70 and 40:60 respectively.

At uniform interval, the eluents (each of fifty ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC) using solvent system ethyl acetate: hexane (30:70) and iodine vapour as detecting agent. Different fractions like Fr-I Fr-II, Fr-III, Fr-IV and Fr-V were eluted. Fraction Fr-IV eluted with methanol: chloroform 20:80 showed single spot on TLC and afforded a fraction 52.1 mg.

Results and discussion

Spectral analysis and structural elucidation of isolated compounds

Identification of the compounds usually involves a combination of different techniques including UV, IR, HNMR, C^{13} and mass spectrometry. These techniques were done with the assistance of instrumentation department at Indian Institute of Integrative Medicine (IIIM-CSIR) Jammu, India.

Table 1: Weight of plant material after drying and percentage loss of *Ficus carica* Linn

S. No.	Description	Weight (g)	% loss
1	Weight of plant material in wet, fresh condition	5600 g	
2	Weight of plant material after drying at room temperature	1500 g	73.21
3	Loss in weight on drying	5600-1500=4100 g	

Table 2: Showing ash content of *Ficus carica* Linn

Name of plant	Wt. of powered material	After burning in the crucible (ash)	Percentage of ash content
Ficus carica Linn.	10 g	1.248 g	12.48

Table 3: Phytochemical screening of crude extract of ethanol from *Ficus carica* Linn

S. No.	Tests	Observation for extract
		Ethanol
1	Test for carbohydrates	
	Fehling's test	+
2	Test for alkaloid	
	Wagner's test	+
	Mayer's test	+
3	Test for flavonoids	
	Shinoda test	+
	Alkaline reagent test	+
4	Test for terpenoids	
	Salkowski test	–
5	Test for saponins	
	Foam test	+
6	Test for proteins	
	Biuret's test	+
7	Test for C-glycosides	
	Modified Borntrager's test	+

Table 4: Separation of constituents from ethyl acetate crude fractions of *Ficus carica* Linn. fraction from column chromatography

S. No.	Solvent System	Ratio	Fraction	Yield
1	Ethyl acetate: Hexane	0.274306	Fr-I	No residue after evaporation
2	Ethyl acetate: Hexane	0.479167	Fr-II	22.3 mg
3	Ethyl acetate: Hexane	0.888889	Fr-III	52.1 mg
4	Ethyl acetate: Hexane	30:70	Fr-IV	32.7 mg
5	Ethyl acetate: Hexane	40:60	Fr-V	197.4 mg

UV spectra of compound

The UV visible spectra of the compound were performed over a wavelength range of 200-500 nm. The typical UV spectra of the compound are shown in Fig 1-3. The various bands observed in the spectra showed both linear and angular nature. The peak 266.20 indicates the presence of lactonic functional group. The peaks at 289.40 and 296.00 indicate the presence of furan ring system in the compound. Thus the first peak is attributed by lactonic functional group. While peaks at 296.00 and 289.40 are due to furan ring system. Hence UV spectra indicates the presence of furan ring system with lactonic functional group in the compound and thus confirms its structure and provides additional support for F-2 compound besides other spectral analysis (Tables1-4).

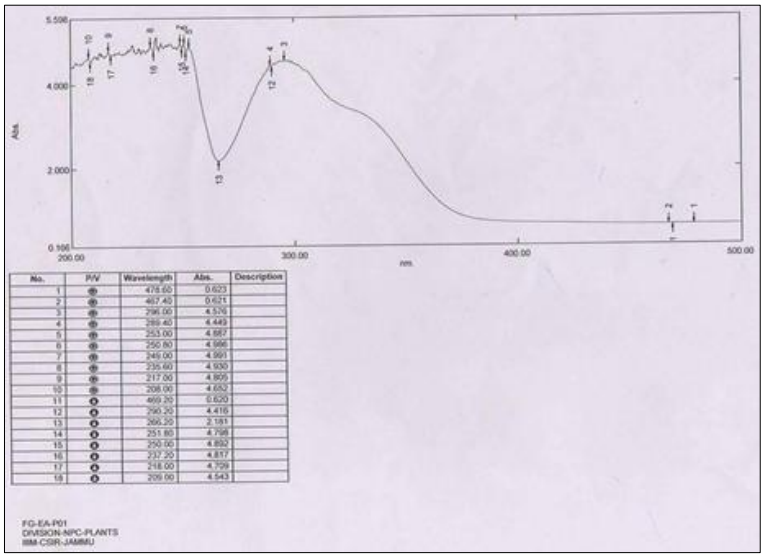


Fig 1: Showing UV spectroscopy of isolated compound from *Ficus carica* Linn.

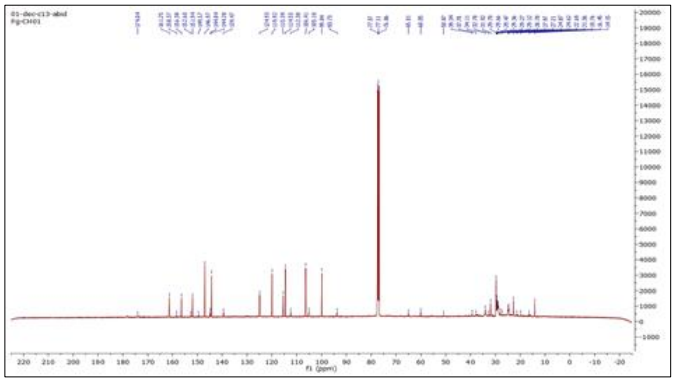


Fig 2: Showing ^{13}C of isolated compound from *Ficus carica* Linn.

Sample ID: Psoroline Injection Vol.: 10 Method;
Time 0 40 50
C% 0 100 0
Flow rate: 1 ml/min,
Coloumn: Eclipse XDB-C-18, 5 UM, 9.4 × 250 mm.

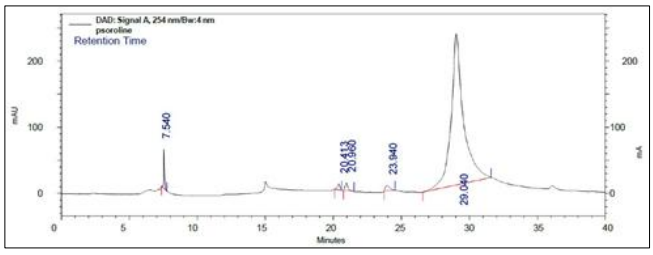


Fig 3: Showing HPLC of the isolated compound from *Ficus carica* Linn. leaves

IR spectra of isolated compound

The IR spectrum of isolated compound (Fig 4 and Table 5) reveals the following information. The weak sharp bands observed at 3326.57
cm^{-1}, 3155.50 cm^{-1}, 3121.42 cm^{-1}, 2919.70 cm^{-1}, 2850.73 cm^{-1} are assigned to C-H stretching. A sharp band at 3554.87 cm^{-1} may be due to OH stretching of moisture content (water). In the region below 1700 cm^{-1}-1800 cm^{-1}, several stretching bands were observed which may be due to carbonyl group stretching. The medium band intensities between 1600 cm^{-1}-1650 cm^{-1} have been found due to C=C stretching's as has been reported earlier [11]. The low intensity band at 1576 cm^{-1} is due to aromatic ring stretching. The characteristics changes observed in the spectral range for C-O-C stretching mode have been found between 1400 cm^{-1}-1200 cm^{-1}. The bands found mainly in the lower region of spectrum are due to out of plane vibrations of C-H and C-O stretchings.

The above data clearly indicates that the isolated compound contains carbonyl groups and lactone bonding arrangements in a fused aromatic ring system. This indicates that the compound may be most probably Psoralen.

Table 5: IR spectra of isolated compounds

1	3300-2800	Weak sharp bands	C-H stretching
2	1757-1723	Very weak sharp bands	C=O stretching
3	1680-1615	Weak bands	C=C stretching
4	1576.85-1541.78	Medium bands	Aromatic ring stretching
5	1464-1200	Weak medium bands	C-O-C stretching
6	1200-1100	Sharp bands	C-O stretching

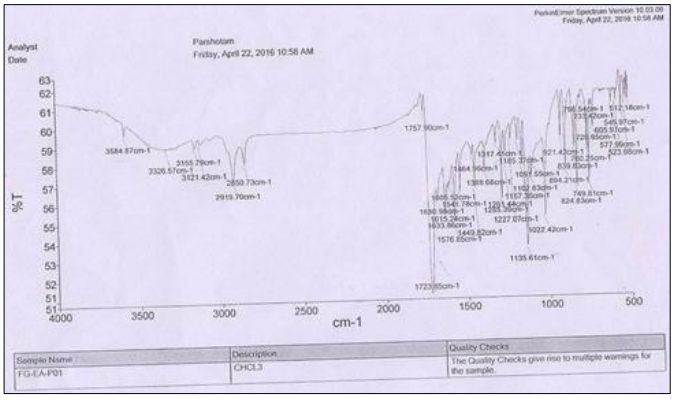


Fig 4: Showing IR spectroscopy of isolated compound from *Ficus carica* Linn.

Mass spectroscopy of isolated compound

The ESI mass of the compound was monitored and displayed in Fig 5. The molecular ion peak was visible in the spectra at m/z [m^+] 186.93. equalent to its molecular weight ($C_{11}H_6O_3$). The molecular ion peak underwent fragmentation and gives fragment ion peaks at 181.02, 163.15,
131.07 and 113.83. Thus mass spectral analysis provides additional information besides IR for the structure of compound.

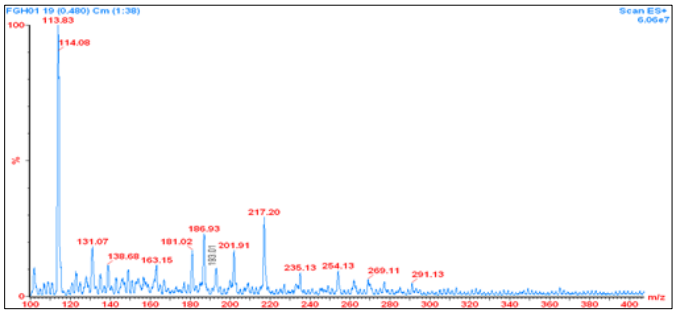
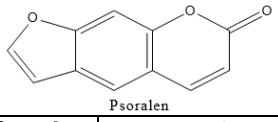


Fig 5: Showing mass spectroscopy of isolated compound from *Ficus carica* Linn

1HNMR interpretation of isolated compound

1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J*=9.5 Hz, 1H), 7.62 (s, 2H), 7.40 (s, 1H), 6.77 (s, 1H), 6.31 (d, *J* =9.5 Hz, 1H).

Elucidated structure of isolated compound



Psoralen

Chemical formula	$C_{11}H_6O_3$
Molecular weight	186.93
Melting point	158-161°C
Physical Description	White crystals
Solubility	Methanol, Chloroform, Water
Name of Compound	Psoralen

Conclusion

Ficus carica is considered one of the most important medicinal plants of the worldwide. Its usage not only fulfills the nutritive value of the human being but the presence of different types of bioactive compounds makes this plant medicinally very important for the human being. Different types of human diseases can be cured by the usage of this plant like heart, skin, kidney, memory, cancer, fungal, viral diseases etc.

As on literature various types of bioactive compounds are present in the leaves of *F. carica* Linn. and Psoralen is one of the compound which is present in this plant. By using different methods and investigated through different spectral analysis the compound which was isolated from the leaves of *F. carica* Linn. is Psoralen.

Acknowledgements

Authors are thankful to the Director General MPCST, Bhopal and Director General Indian Institute of Integrative Medicine (IIIM-CSIR) JAMMU for supported financially to carry this research work.

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