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Research Article

Development of HPLC Method for Estimation of Furonocumarins in *Psoralea corylifolia* and *Ammi majus*

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ABSTRACT
Furonocumarins are widely used in the treatment of Vitiligo and Psoriasis. The objective of present study was to develop a reliable, accurate and reproducible HPLC method for the simultaneously estimation of four furanocaumarins (psoralen, isopsoralen, xanthotoxin and bergapten) in *Psoralea (P.) corylifoia* and *Ammi (A.) majus* plants. The furocoumarins were separated simultaneously on a reverse phase *Symmetry* C8 (150mm×4.6mm) column in isocratic method of methanol, acetonitrile and water solution as mobile phase having flow rate at 0.8 mL/min and detected with UV detector. Maximum psoralen and isopsoralen (Angelicin) were recorded in *P. corylifolia*, whereas maximum 8-methoxypsoralen (xanthotoxin) and 5-methoxypsoralen (bergapten) were found in *A. majus*. *P. corylifolia* is a good source of furanocoumarins psoralen and angelicin, whereas *A. majus* is the good source for 5-Methoxypsoralen and 8-Methoxypsoralen. Hence, both plants can be used in the treatment of Vitiligo and Psoriasis. The isocratic HPLC method was found more suitable, accurate, less time consuming and reproducible method for the estimation of above cited four furanocumarins simultaneously from the *P. corylifolia*, and *A. majus* plants.

Keywords: Furonocumarins, HPLC, *Psoralea corylifolia* and *Ammi majus*

INTRODUCTION

Furonocoumarins are a class of organic chemical compounds is present in various plants such as celery, parsley, figs, parsnips, and grapefruit[1-3]. The biochemically most important furanocaumarins are psoralen, 8-methoxypsoralen, 5-methoxypsoralen, and isopsoralen[4]. These compounds have been reported to possess diverse biological activities. The furocoumarins psoralen, isopsoralen, 8-methoxypsoralen and 5-methoxypsoralen are used in the treatment of skin disorders such as psoriasis and vitiligo[5-7]. These furanocaumarins are used in treatment of cutaneous T-cell lymphoma also. Psoralen, isopsoralen, 8-methoxypsoralen and 5-methoxypsoralen were isolated from the plant *Psoralea (P.) corylifolia* (8-9) and *Ammi (A.) majus*[10-12]. *Psoralea corylifolia* Linn. belongs to family Leguminoseae is a well known medicinal herb used in Indian Ayurveda medicine, Tamil Siddha medicine and Chinese medicine systems[13] to treat various diseases. *P. corylifolia* is traditional Indian and Chinese herbal medicine for the treatment of several skin disorders such as psoriasis, leukoderma, hair loss and leprosy[14]. It is also widely used as antitumor, antibacterial, cytotoxic and antihelmenthic properties[15-17]. *Ammi majus* Linn. belongs to family Apiaceae is widely used for the treatment of skin disorders such as psoriasis and vitiligo[18-19]. It is used as an emmenagogue to regulate menstruation, as a diuretic, and for treatment of leprosy, kidney stones, and urinary tract infections[20].

A number of HPLC procedures have been already developed for the estimation of individual furanocaumarin in seeds and herbal formulations by deferent workers such as HPLC method for Psoralen[16], Isopsoralen[21], 5-Methoxypsoralen and 8-Methoxypsoralen[4,8,22-24]. There is not a single HPLC procedure for the
estimation of four furanocaumarins simultaneously as per available literature is concerned. The objective of present study was to develop a accurate and reproducible HPLC method for the simultaneously estimation of above four furanocaumarins form *P. corylifoia* and *A. majus*.

MATERIALS AND METHODS

Chemicals: Standard psoralen, isopsoralen, xanthotoxin and bergapten were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). Other chemicals of analytical grade were purchased from Merk India Limited.

Biological materials: The *P. corylifolia* and *A. majus* plants were cultivated in Defence Institute of Bio-Energy Research, Defence R & D Organization, Field Station, Pithoragarh. Mature seeds of these plants were collected

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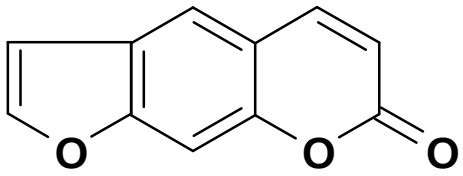
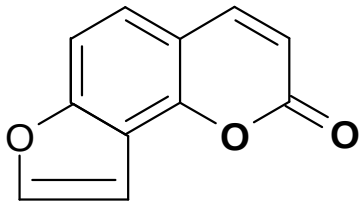


Psoralen



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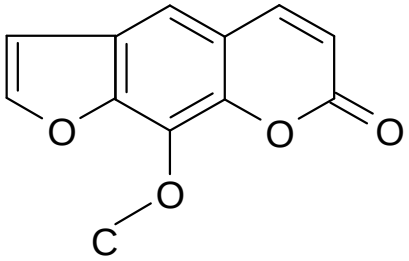
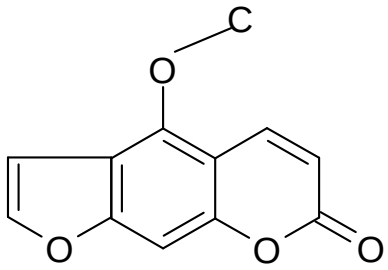


8-Methoxypsoralen (Xanthotoxin)



5-Methoxypsoralen (Bergapten)

and dried below 40 ^{o}C in oven then finely powdered with the help of mechanical grinder and stored in an air-tight container at - 4 ^{o}C.

Extraction of furanocaumarins: Approximate 1.0 g dried powder of seeds of each plants extracted with 10 ml solution of methanol and chloroform (50:50 V/V) in a 25 ml centrifuge tube it was then placed in an ultrasonicator for 30 minutes and then centrifuged. Supernatant was collected and residue obtained after centrifugation was re-extracted twice and exact 25 ml volume was made. Above extracts were filtrated through a 0.45 µm filter in to a 1 ml vial and 10 µl sample was injected in HPLC.

Determination of furonocumarins: Analysis of all samples were carried out by Water's HPLC (Quaternary gradient), which consists of a Photo-diode Array Detector (Waters 996), an Auto sampler (Waters 717 plus), and a C-18 Reverse phase symmetry column (Waters C-18 RP, 4.6 mm×150 mm, 5µm). Standard calibration curve was drawn by consecutively injecting different concentration of standard drugs (25, 50, 75, 100 and 125 PPM). Standard solution was prepared by using filtered methanol and chloroform. Injection volume was taken 10 µl. Water, methanol and acetonitrile (55:35:10,V/V) were used as mobile phase for separation of psoralen, Isopsoralen, xanthotoxin and bergapten from the extract of *P. corylifolia* and *A. majus* plants. Isocratic elution method was adopted with a flow rate of 0.8 ml/min for separation. The detection wavelength of photo-diode array was 254 nm and the column temperature was kept 30°C.

Data analysis: Data were analyzed using Waters Empower- 2 software. The results were shown as the means of three replicates.

RESULTS AND DISCUSSION

Based upon the finding, presently study concludes that the isocratic HPLC method was found reliable, accurate, less time consuming and reproducible method for the estimation of four furanocumarins (psoralen, 8-methoxypsoralen, angelicin and 5-methoxypsoralen) simultaneously from the medicinal plants like *P. corylifolia* and *A. majus*. The highest contents of psoralen and angelicin were recorded in the seed of *p. corylifolia*. While, maximum concentrations of 5-methoxypsoralen and 8-methoxypsoralen were recorded in the seeds of *A. majus*.

Figure 1 showed HPLC chromatograms of standard four furonocumarins (psoralen, 8-methoxypsoralen, angelicin and 5-methoxypsoralen). The HPLC are most important reliable, accurate and reproducible method for estimation of active ingredients from crude plant materials. Methods for the determination of coumarin and 7-hydroxycoumarin (7-HC) in biological fluids have been published extensively (25-26), but few have dealt with furocoumarin determination in crude materials. In the present study reliable, accurate and reproducible HPLC method was developed for determination of furanocumarins from extract of methanolic and choloroform (1:1) of the seeds of *p. corylifolia* and *A. majus.* The retention times of furanocaumarins psoralen, 8-methoxypsoralen, angelicin and 5-methoxypsoralen were found 11.9, 12.6, 13.6 and 19.8 minutes respectively. Hence, range of retention times of furanocumarins was recorded from 11 to 22 minutes. Similarly, earlier worker have also been estimated furonocaumarins in human urine during and after continuous oral administration of a Umbelliferae Chinese Medicine and retention times were found around 4 to 22 minutes of furanocaumarins (8).

Separation of furanocoumarins from *P. corylifolia*: Figure 2 showed HPLC chromatogram of furanocumarins separated from the seeds of *P. corylifolia*. In the study, the furanocumarins psoralen, angelicin (isopsoralen) and 5-methoxypsoralen were recorded 2741.676mg, 2431.815mg and 16.141mg per 100g dried seeds powder of *P. corylifolia* respectively. Whereas, furanocumarin like 8- methoxypsoralen was not detected in seed of *P. corylifolia.* The psoralen furanocumarin (2741.676mg/100g) was found in highest concentration followed by angelicin (2431.815mg) in seed powder by

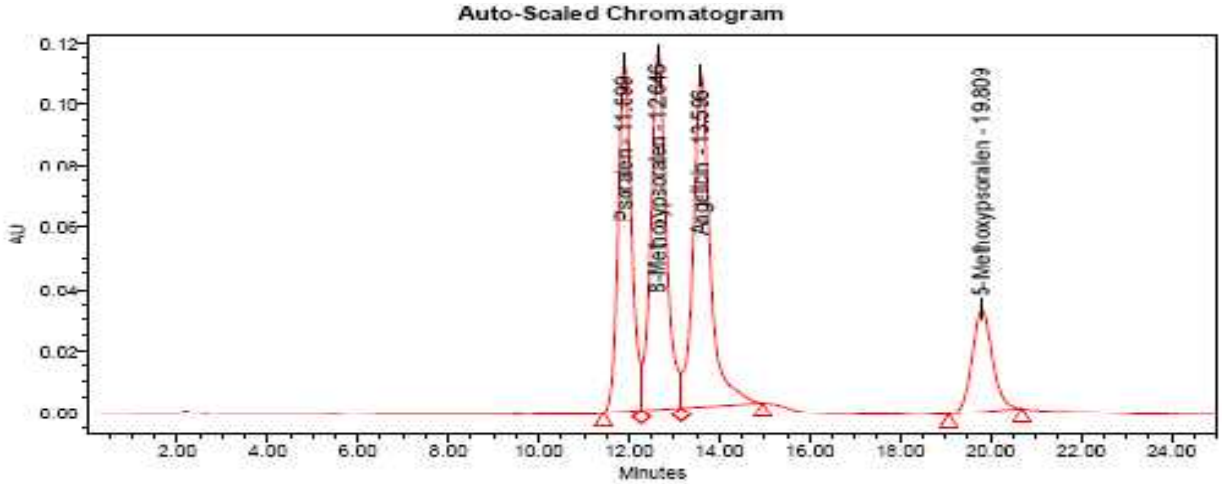


Fig. 1: Separation of furanocoumarins standard by isocratic method (methanol: acetonitrile: water; 35:55:10) of HPLC. Peak identified: Psoralen (11.9 minute), 8-Methoxypsoralen (12.6 minute), Angelicin (13.6 minute) and 5-Methoxypsoralen (19.8 minute).

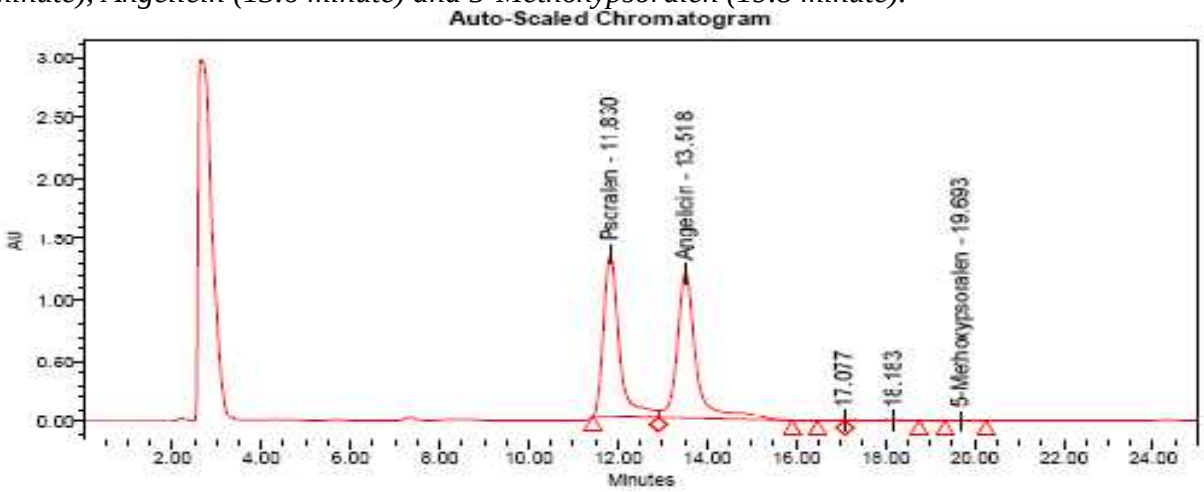


Fig. 2: Separation of furanocoumarins by isocratic method (methanol: acetonitrile: water; 35:5510) of HPLC in P. corylifoila. Peak identified: Psoralen (11.8 minute), Angelicin (13.5 minute) and 5-Methoxypsoralen (19.69 minute).

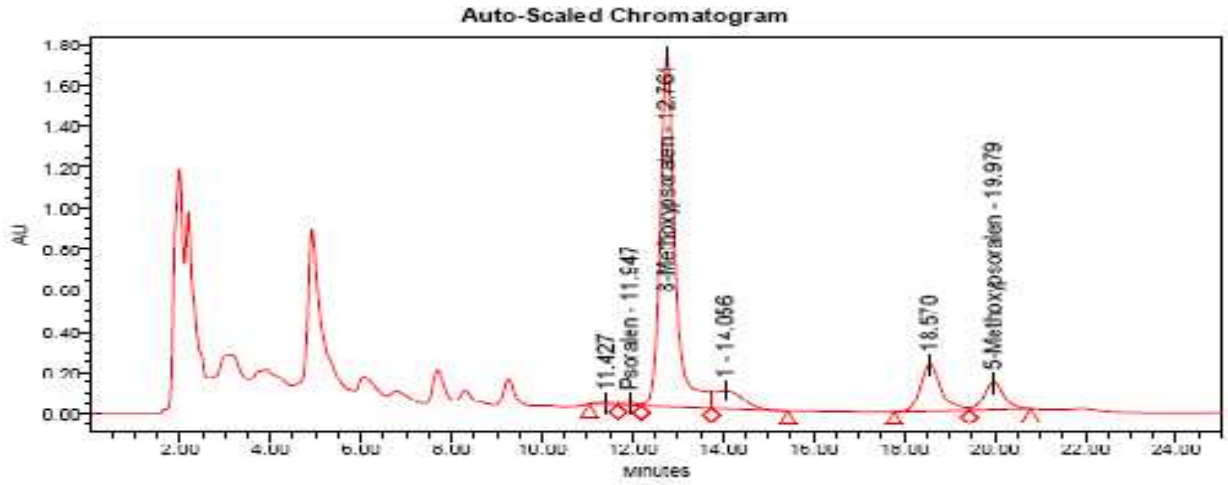


Fig. 3: Separation of furanocoumarins by isocratic method (methanol: acetonitrile: water; 35:55:10) of HPLC in A. majus. Peak identified: Psoralen (11.9 minute), 8-Methoxypsoralen (12.7 minute) and 5-Methoxypsoralen (19.9 minute).

HPLC method. Whereas, 5-methoxypsoralen was recorded in lowest concentration (16.141mg) in *P. corylifolia* seeds powder as compared to other furanocumarins. Similarly, Renmin Liu *et al.,* (2000) (27) has been reported that 39.6 mg psoralen and 50.8 mg isopsoralen were recorded in 100 mg crude extract of *P. corylifolia*, at over 99% purity by HPLC.

Separation of furanocoumarins from *A. majus*: Figure 3 showed HPLC chromatogram of furanocumarins separated from the seeds of *A. majus* cultivated at DIBER, Field Station Pithoragarh. In the study, furanocumarins psoralen, 8-methosxypsoralen and 5-methoxypsoralen were recorded 139.7695mg, 4124.50755mg and 739.374mg per 100g in dried powder of seeds respectively. Whereas, furanocumarin like angelicin was not found in seed of *A. majus.* Our study is well supported by Królicka *et al.,* (2001) (28), who had reported that furanocoumarins (psoralen, xanthotoxin (8-methoxypsoralen), bergapten (5-methoxypsoralen) and imperatorin) were found in the seeds of *A. majus.* In the present study, 8-methoxypsoralen (4124.50755mg/100g) was found in highest concentration followed by 5-methoxypsoralen (739.374mg/100g) in seed powder of *A. majus* by HPLC method. Whereas, psoralen was recorded in lowest concentration (139.7695mg/100g) in *A. majus* seeds powder as compared to other furanocumarins. Similarly, Ekiert & Gomółka (2000) (12) has been reported that maximum concentration of xanthotoxin was found 3010.41 mg/100 g in the fruit of *A. majus* by HPLC.

CONCLUSION

The results of the present study, we concluded that the isocratic HPLC method was developed for the estimation of four furanocumarins simultaneously from the medicinal plants is found reliable, accurate, less time consuming and reproducible method. In addition, the higher concentrations of psoralen (2741.676mg/100g seed) and angelicin (2431.815mg/100g seed) were recorded in *p. corylifolia* as compared to *A. majus*. Whereas, concentrations of 5-methoxypsoralen (739.374mg/100g) and 8-methoxypsoralen (4124.50755mg/100g) were recorded higher in the seeds powder of *A. majus* as compared to *P. corylifolia*. Hence, it can be concluded from this study that *P. corylifolia* is a good source of furanocoumarins psoralen and angelicin, whereas *A. majus* is the good source for 5-methoxypsoralen and 8-methoxypsoralen. Due to ample concentration of furanocoumarins in these plants, both plants can be used in the treatment of skin diseases Vitiligo and Psoriasis.

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