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Research Article

RP-HPLC Analysis of Furanocoumarins-Psoralen, Bergapten and Xanthotoxin in *Ruta graveolens* L.

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ABSTRACT

Furanocoumarins namely psoralen, bergapten and xanthotoxin which are novel potent topoisomerase I inhibitors of *Ruta graveolens* is of interest because of its pharmacological function. Hence, Reversed – Phase HPLC analysis was done to analyse the content of psoralen, bergapten and xanthotoxin during different seasons of the year April 2014- January 2015, before and after flowering. The leaves harvested after flowering during the month end of September 2014 contained more of psoralen, bergapten and xanthotoxin. In general the stem and leaf of *Ruta graveolens* contained more of bergapten followed by psoralen whereas the concentration of xanthotoxin *was very less.*

Keywords- psoralen, bergapten, xanthotoxin, HPLC, *Ruta graveolens*

INTRODUCTION

Ruta graveolens L. , a member of Rutaceae is a native of the Mediterranean region but cultivated throughout Europe and many Asian countries including China, India and Japan. The components of *Ruta* species are of great interest in medicinal chemistry as these compounds show a broad range of biological activity and a number of them are used in medicine . Recently its extracts were shown to have potent anti-cancer activity [1, 2]. DNA topoisomerases are essential cellular enzymes required for cell proliferation and therefore it has recently emerged as important cellular targets for chemical intervention in the development of anti-cancer agents. There are very few topoisomerase – I inhibitors developed and applied clinically as anti-cancer agents. But their side effects remain a serious problem. Natural products from plant sources can be a valuable source of novel inhibitors. Psoralen, bergapten and xanthotoxin are identified as novel topoisomerase inhibitors[3]. No data has been reported for the separation of psoralen, bergapten and xanthotoxin from the methanolic extract of *Ruta graveolens* during different seasons of a year before and after flowering. This motivated the present investigation to analyze the content of furanocoumarins like psoralen, bergapten and xanthotoxin in stem and leaf of *Ruta graveolens* L. cultivated in Bengaluru, India, before and after flowering (April 2014 to January 2015) to select the best plant material for the extraction of more amount of psoralen, bergapten and xanthotoxin.

MATERIALS AND METHODS

Potted healthy plants were procured from University of Agriculture Sciences, which were maintained in the nursery, Nagarabhavi, Bengaluru. Required number of potted plants were maintained in the garden at home. Stem and fully mature leaves exposed to sunlight were collected during summer (April-June 2014), rainy (July-September 2014) and winter (December 2014-January 2015) seasons. They were shade dried and used for phytochemical analysis.

Chemicals

Reference standards like psoralen, bergapten, xanthotoxin and solvents like HPLC grade methanol and acetonitrile were purchased from Sigma company. The other analytical grade chemicals like methanol, distilled water were purchased from SD-Fine chemicals.

Preparation of crude plant extract

Crude plant extract was prepared by soxhlet extraction method. About 20 gms of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of methanol. The process of extraction was carried out for 2 ½ or 3 hours or till the solvent in the siphon tube of an extractor became colourless. After that the extract was taken in a round bottomed flask of rotovacuum evaporator and temperature was adjusted to 64 ^{0}c till all the solvent got evaporated**.** Methanolic extract was stored for further use.

Preparation of reference standard solution-psoralen, bergapten and xanthotoxin

4.8mg (4800 ppm) of psoralen was dissolved in filtered 1 ml methanol. 5 mg (5000 ppm) of bergapten was dissolved in filtered 1 ml methanol 4.7 mg (4700 ppm) of xanthotoxin was dissolved in filtered 1 ml methanol

Extraction of furanocoumarins- psoralen, bergapten and xanthotoxin

25 mg of methanolic crude extract of each sample was dissolved in 5 ml of methanol. Then the extracts were filtered through 2µ filter into a 1 ml vial and 10 µl sample was injected into HPLC.

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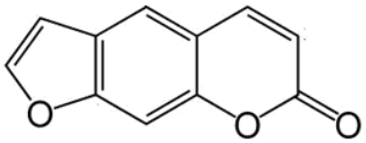
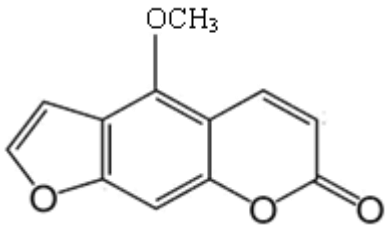
Table 1: The content of psoralen, bergapten and xanthotoxin in stem and leaf of *Ruta graveolens* L.

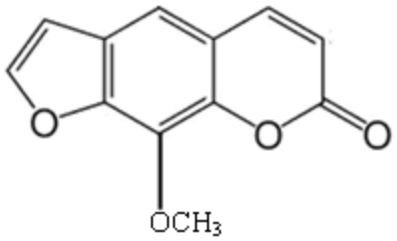
S. No.	Samples	Time of harvesting	Furanocoumarins Psoralen		Bergapten		Xanthotoxin	
			% Recovery	mg/ 100 g dry weight of the sample	% Recovery	mg/ 100 g dry weight of the sample	% Recovery	mg/ 100 g dry weight of the sample
1	STEM (B.F.)	April-June (summer season)	0.63	60	2.2	240	0.002	0.7
2	LEAF (B.F.)		0.39	70	2.5	518	0.01	1
3	STEM (A.F.)		0.5	15	2.1	63	0.02	0.8
4	LEAF (A.F.)		0.27	120	1.8	810	0.003	1
5	STEM (B.F.)	July – Sep (rainy season)	0.4	65	1.8	240	0.02	0.7
6	LEAF (B.F.)		0.6	150	2.2	750	0.01	0.9
7	STEM (A.F.)		0.91	78	1.9	170	0.06	5.1
8	LEAF (A.F.)		0.92	280	2.9	900	0.05	10
9	STEM (B.F.)	Dec –Jan (winter season)	0.2	20	0.5	50	0.01	1
10	LEAF (B.F.)		0.9	200	3.03	720	0.01	2
11	STEM (A.F.)		0.3	70	1.6	200	0.01	1.3
12	LEAF (A.F.)		0.7	250	2.0	800	0.02	2



Psoralen



Bergapten (5- Methoxypsoralen)



Xanthotoxin (8- Methoxypsoralen)

Determination of furanocoumarins- psoralen, bergapten and xanthotoxin

Analysis of all samples were carried out by Agilent 1200 series HPLC which consisted of a Photodiode array detector with Reversed phase column (C-18RP, 150x4.6x5 µm).

Isocratic elution method was adopted with a flow rate of 0.8m/min for separation. The detection wavelength of photodiode array was 254 nm and the column temperature was kept 28 ^{0}c**.**

Injection volume was taken 10 µl. Water, methanol and acetonitrile (55:35:10 v/v) were used as a mobile phase for separation of psoralen, bergapten and xanthotoxin from the stem and leaf extract of *Ruta graveolens*.

Data analysis

Data analysis were analysed using open lab chemstation software. The results were shown as the means of 3 replicates.

RESULT AND DISCUSSION

HPLC is the most important, reliable, accurate and reproducible method for estimation of active ingredients from crude plant materials. Isocratic HPLC method is reliable, accurate, less time consuming and reproducible for the estimation of four furanocoumarins namely psoralen, bergapten and xanthotoxin, angelicin [4]. In the present study Reversed-phase HPLC is used wherein psoralen, bergapten and xanthotoxin were quantified at 254nm using peak area by comparing the calibration curve of respective reference standards. Figure 1 shows

Auto-scaled Chromatogram

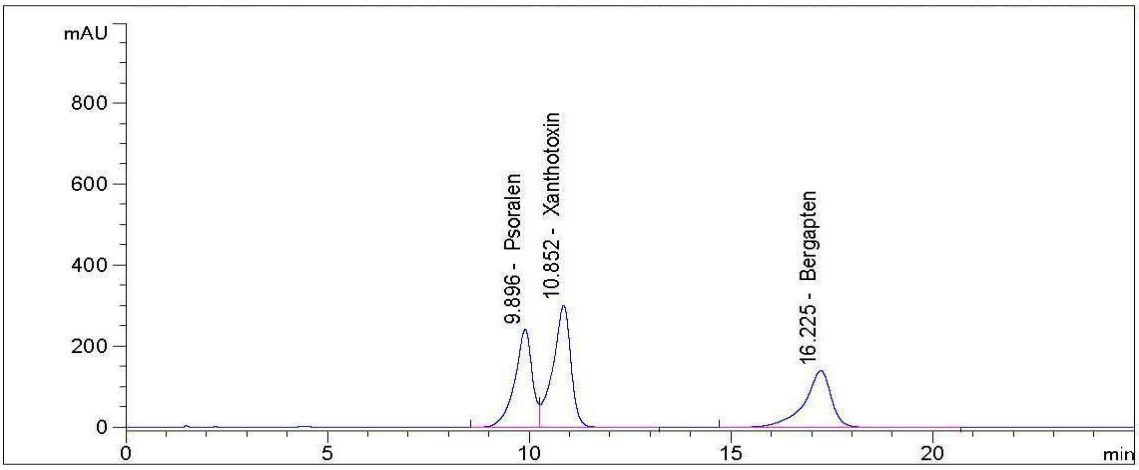


Figure 1: Separation of psoralen, bergapten and xanthotoxin standards by isocratic method (water : methanol : acetonitrile-55:35:10) of HPLC. Peak identified: Psoralen (9.8 minute), Bergapten (16.2 minute) and Xanthotoxin (10.8 minute)

Auto-scaled Chromatogram

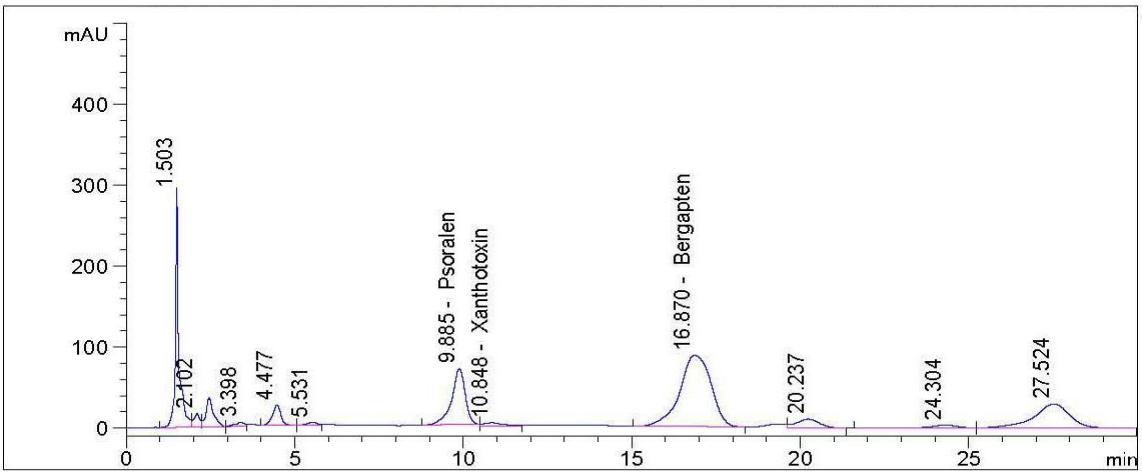


Figure 2: Separation of psoralen, bergapten and xanthotoxin by isocratic method (water : methanol : acetonitrile-55:35:10) of HPLC. Peak identified: Psoralen (9.8 minute), Bergapten (16.8 minute) and Xanthotoxin(10.8 minute)

HPLC chromatogram of standard 3 furanocoumarins-psoralen, bergapten and xanthotoxin. The retention times of psoralen, bergapten and xanthotoxin are 9.8, 16.2 and 10.8 minutes respectively. Figure 2 shows HPLC chromatogram of psoralen, bergapten and xanthotoxin separated from the leaf of *Ruta graveolens*, which were harvested after flowering during the month end of September. The retention times of psoralen, bergapten and xanthotoxin are 9.8, 16.8 and 10.8 minutes respectively. Data on the content of psoralen, bergapten and xanthotoxin in *Ruta graveolens* during different seasons from the stem and leaf before flowering and after flowering are represented in Table 1. The leaves harvested after flowering during the month end of September 2014 contained more content of psoralen, bergapten and xanthotoxin.(Fig.2) The highest content of bergapten was recorded 900 mg/100 g dried leaves powder followed by psoralen recorded 280 mg/ 100 g dried leaves powder and xanthotoxin of very low concentration recorded 10 mg/ 100g dried leaves powder of *Ruta graveolens*. From the above result it is observed that production of psoralen, bergapten and xanthotoxin in *Ruta graveolens* vary before and after flowering in stem and leaf and also during different seasons. Environmental conditions affect the plant growth as well as the formation of secondary metabolites as they are mostly formed in young and actively growing tissues [5], the age and the stage of the plant generally have an impact on phenolic contents[6]. Factors such as water level in the soil, evapotranspiration rate, light intensity, photosynthetic efficiency, plant water potential and plant stage directly respond to these variations. According to Gobbo- Neto and Lopes (2007)[7] and Neube et al., (2010)[8] the physiological characters associated with the genetic conditions of plants are responsible for the variations in the production of secondary metabolites which arise due to different climatic conditions. Neube et al,. (2010)[8] have found variation in the production of polyphenols in *Tulbaghia violacea*, *Hypoxis hemerocallidea*, *Merwilla plumbea* and *Drimia robusta* in different seasons.

Other authors Ma et al,. (2003) [9], Brooks and Feeny (2004)[10], Ercsli et al,. (2008)[11], Ruiz-Terah et al,.(2008) [12] , Santos and Kaye (2009) [13] , Sialka and Kasparova (2010)[14], Chavarria et al., (2011)[15], Thiago et al,.(2013)[16] have also attributed to the variation in the production of secondary metabolites to environmental factors.

B.F. (Before flowering), A.F. (After flowering). The explanation precisely lies in the climate differences, biotic and environmental conditions, in addition to the genetic aspect. The present work is in conformity with the above view.

CONCLUSION

There is a variation in the production of psoralen, bergapten and xanthotoxin in stem and leaves of *Ruta graveolens* during different seasons of the year and also before and after flowering. The present work showed more production of psoralen, bergapten and xanthotoxin in leaves which were harvested after flowering during rainy season, September 2014. In conclusion the stem and leaves of *Ruta graveolens* contain more content of bergapten followed by psoralen. But xanthotoxin concentration is very less.

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