INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

GC-MS ANALYSIS, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF *BUNIUM PERSICUM* FROM KASHMIR, INDIA

ZA. Bhat, Riehana Gani* and M. Iqbal Zargar

Department of Pharmaceutical Sciences, Pharmacognosy Division, University of Kashmir, Hazratbal, Srinagar-190 006, Jammu and Kashmir, Srinagar, India.

ABSTRACT

This work was carried out to evaluate chemical composition, antibacterial and antifungal activity of *Bunium persicum* essential oil. The oil was extracted by hydrodistillation which was analysed through GC-MS. The antibacterial and antifungal activity was evaluated by Agar well diffusion method and Minimum inhibitory concentration (MIC) was evaluated by Agar dilution method. P cymene, Gamma terpinene and cumic aldehyde were the major constituents present in the oil. The oil showed significant antibacterial and antifungal activity.

Keywords: *Bunium persicum*, GC-MS, Antibacterial and Antifungal Activity.

INTRODUCTION

Bunium is a genus of flowering plants in the family Apiaceae, with 45 to 50 species. *Bunium persicum* is a perennial herb with white or pink flowers and small brownish fruits which grows in warm climate zones of Iran. The plant is common in southeastern Europe, Siberia and Western Asia. It is native of native Baluchistan, Afghanistan, Kashmir and Himachal-Pradesh.The ripe Kala zeera fruits contain an essential oil (5-14%) rich in monoterpene aldehyde. The main components are cumin aldehyde, p- mentha-1, 3-dien-7-al and p-mentha-1,4-dien-7-al. Comparative analysis of major volatiles from wild and cultivated sources of zeera fruits revealed that the flavour of Kala zeera fruits is mainly due to γ- terpinene and p-cymene. The oil from a cultivated source was more superior to that from wild sources. The fruits of cultivated origin contained cuminaldehyde (27-34%) followed by p-mentha-1,3- dien-7-al and p-mentha-1,4-dien-7-al (29-36%), whereas the fruits from the wild mainly contained γ- terpene (25-42%) and p-cymene (24-27%) and less aldehydes. They also reported that the straw of Kala zeera also contains 1-20 per cent oil that resembled the oil from fruits (Jassbi *et al*., 2001) analyzed essential oils from *B. elegans* and *B. caroides* and found that their essential oils mainly contained sesquiterpene hydrocarbons such as germacrene-d and E, caryophyllene amounting to 24.1%, 38% for B. elegans, 22.1% and 26.6% for *B. caroides* respectively. *B. elegans* oil also contained traces of monoterpenes, while *B. caroides* oil contained various phenylpropenoids such as asaricin (7.5%) and dillapiolle (10.2%) as important constituents. Similarly, it has been reported that the essential oil derived from *B. cylendricum* mainly consists of Myristin (43.1%), β-phellandrene (20%), β-pinene (15.6%) and α-pinene (10.7%). The supercritical fluid extraction method for volatile components from *Bunium persicum* was used and found that a total of 16 compounds accounting for about 99.36%. Main components were 4-terpine (37.98%) followed by cuminaldehyde (11.48%) and α-methyle-benzenemethanol (25.55%), whereas, the rest was accounted for by components such as α-pinene, β-pinene, myrcene, α-terpinene, α-cymene, limonene, α-terpinolene, β-sinensal, β-selinene, Germacrene-B, and Dillapiole. The components, namely γ-terpinene, p-cymene and β- pinene, are thought to reduce the quality of the spice.

TRADITIONAL USES OF BUNIUM PERSICUM

Kala Zera (*Bunium Persicum*) is a high value herbaceous spice widely used for culinary, flowering, perfumery and Carminative purposes.Several therapeutic effects including those on digestive disorders, urinary tract disorders, diuretic, gynaecologic, anti-convulsion, anti-helmentic and also anti-asthma and dyspnea have been described for the fruits of *Bunium persicum.*

1. MATERIALS AND METHODS

1.1 PLANT MATERIAL

The dried fruits of *Bunium persicum* were collected from Tral region of Kashmir valley in juiy-2013. The plant sample was identified and authenticated by Dr. A.R. Naqshi, taxonomist under specimen vocher number-KUIK01. The specimen has been deposited in the herbarium of pharmaconosy and phytochemistry laboratory, Department of Pharmaceutical Sciences, University of Kashmir.

1.2 Essential oil isolation

The dried fruits of *Bunium persicum* were used and the essential oil was obtained by hydrodistillation in a clavenger type apparatus as recommended by European Pharmacopeae. The yield of oil, as calculated on fresh weight basis (v/w), was 1.22%. The oil sample was dried over anhydrous sodium sulphate and stored in a sealed glass vial in a refrigerator at 4°C prior to analysis.

1.3 Chemical Composition

1.3.1 GC-MS Analysis

GC-MS analysis was carried on a Varian Gas Chromatograph series 3800 fitted with a VF-5ms fused silica capillary column (60mx0.26mm, film thickness 0.25µm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.20µl with split ratio 1:60, helium as carrier gas at 1.0ml/min constant flow mode, injector temperature 230°C, oven temperature 60°C to 280°C at 3°C/min.

1.4 Antimicrobial assay

1.4.1 Microbial strains and culture media

Gram positive and Gram negative bacterial strains and the fungal strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH) Chandigrah, India. The bacterial strains used were *Pseudomonas aeruginosa* MTCC 1688, *E. coli* MTCC 407, *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Proteus vulgaris* MTCC 426, *Klebsiella pneumonia* MTCC 19. The fungal strains used were *Saccharomyces cerevisiae* MTCC 1023, *Candida albicans* MTCC 6258, *Pencillium crysogenum* MTCC 1380, *Aspergillus fumigates* MTCC 9001. The Muller Hinton Agar and Sabouraud Dextrose Agar media were used for the determination of antibacterial and antifungal activity respectively. The bacterial strains were grown on MHA plates and MHA slants at 37°C and later on refrigerated until further use. The fungal strains were grown on SDA plates and SDA slants at 28°C and maintained under refrigeration.

1.4.2 Antimicrobial activity and determination of Minimum inhibitory concentration (MIC)

AGAR WELL DIFFUSION ASSAY

The antimicrobial susceptibility tests were carried out using the Agar well diffusion assay (Irith Wiegand *et al.,* 2008). The bacterial cultures were developed for 24 hours and fungal cultures were developed for 48 hours and later transferred into boiling tubes containing 20 ml of liquid MHA and 20 ml of SDA respectively. The contents of the tubes were transferred to petriplates. After 5 minutes of solidification of the agar, petriplates were punched in the form of wells. Later these wells were filled with different concentration of oils (10µl, 20µl, 30µl) for bacterial assay and (20µl, 30µl) for fungal assay. The incubation was carried out for 24hours at 37°C for bacteria and for fungi incubation period was 48 hours at 28°C. After the incubation period, the antimicrobial activity was evaluated by measuring the width of zone of inhibition. The aqueous solution of streptomycin sulphate (6µl) was used as positive control in case of antibacterial activity while as Nystatin (50µg /disc) was used as positive control for antifungal activity. However DMSO was used as negative control.

MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAY

The essential oil of *Bunium persicum* displayed significant and broad spectrum antibacterial and antifungal activity against different bacteria and different fungi used. Minimum Inhibitory Concentration of oils was determined by Agar Dilution Method, recommended by Clinical Laboratory Standards Institute (CLSI) (Irith Weigand *et al.,* 2008). A series of two fold dilutions of the oils ranging from 0.2-25.6 mg/ml was prepared in MHA at 48°C and in SDA at 40°C for antibacterial and antifungal activity

respectively. Plates were dried at room temperature for 30 minutes prior to spot inoculation with 3µl and 2µl aliquots of culture containing approximately 10^5cfu/ml and 10^3cfu/ml of each organism for antibacterial and antifungal activity respectively.The bacterial plates were incubated at 37°C for 18 hours while the fungal plates were incubated at 28°C for 48 hours and were read visually and MIC was determined. Experiments were performed in triplicate. Inhibition of bacterial growth and fungal growth in the plates containing test oil was judged by comparison with growth in blank control plate. The MICs were determined as the lowest concentrations of oil inhibiting visible growth of each organism on the agar plate. The MIC for different strains of bacteria and fungi are presented in tables 3 and 5.

2. RESULTS AND DISCUSSION

The different essential oil constituents of the fruits of *Bunium persicum* are shown in table-1, in order of their elution from RTX-5 column. GC-MS analysis led to the identification of 5 chemical constituents accounting for 100% of the total oil composition.

Table 1: Chemical composition of essential oil of *Bunium persicum* by GC/MS

S. No.	Peak Name	RI (min)	Area	%age	Method of identification
1	Sabinene	16.177	1187	1.978	MS,RI
2.	p-cymene	18.999	28263	47.087	MS,RI
3.	Gamma terpinene	20.790	9656	16.087	MS,RI
4.	Cumic aldehyde	30.305	16913	28.178	MS,RI
5.	Phenyl glycol	32.586	4004	6.670	MS,RI
	Total identified percentage			**100%**	
	Class composition				
	Monoterpene hydrocarbons			65.152%	
	Sesquiterpene hydrocarbons			34.848%	

RI- Retention indicies in elution order from RTX-5 columns,
COI-Coinjection, Retetion time identical to Authentic compounds

Results presented are the means of three replicative isolations, %, relative percentage obtained from peak obtained from peak area.

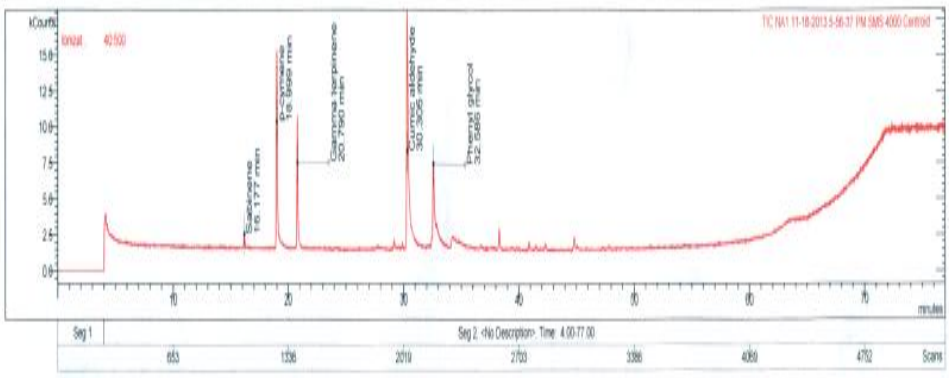

Fig. 1: The GC/MS Chromatogram of the *Bunium persicum* essential oil

Table 2: In vitro antibacterial activity of *Bunium persicum* essential oil and reference antibiotic determined with agar well diffusion method

S. No.	Test Organisms	Concentration of essential oil used in µl to determine zone of inhibition (diameter in mm)			Standard used (streptomycin in µl)
		10 µl	20µl	30µl	6µl
01.	*Staphylococcus aureus* MTCC 96	18±0.57	20±0.33	21±0.88	27±0.57
02.	*Bacillus Subtilis* MTCC 441	15±0.57	16.6±0.3	17±0.57	22±0.57
03.	*Pseudomonas aeruginosa* MTCC 1688	17±0.57	19±0.57	23±0.66	28±0.57
04.	*Klebsiella pneumoniae* MTCC 19	18±0.57	19±0.57	22±0.33	25±0.57
05.	*Proteus vulgaris* MTCC 426	19±0.57	20±0.57	22.6±0.33	29±0.57
06.	*Escherchia coli* MTCC 443	16±0.33	18.6±0.3	19±0.57	29±0.57

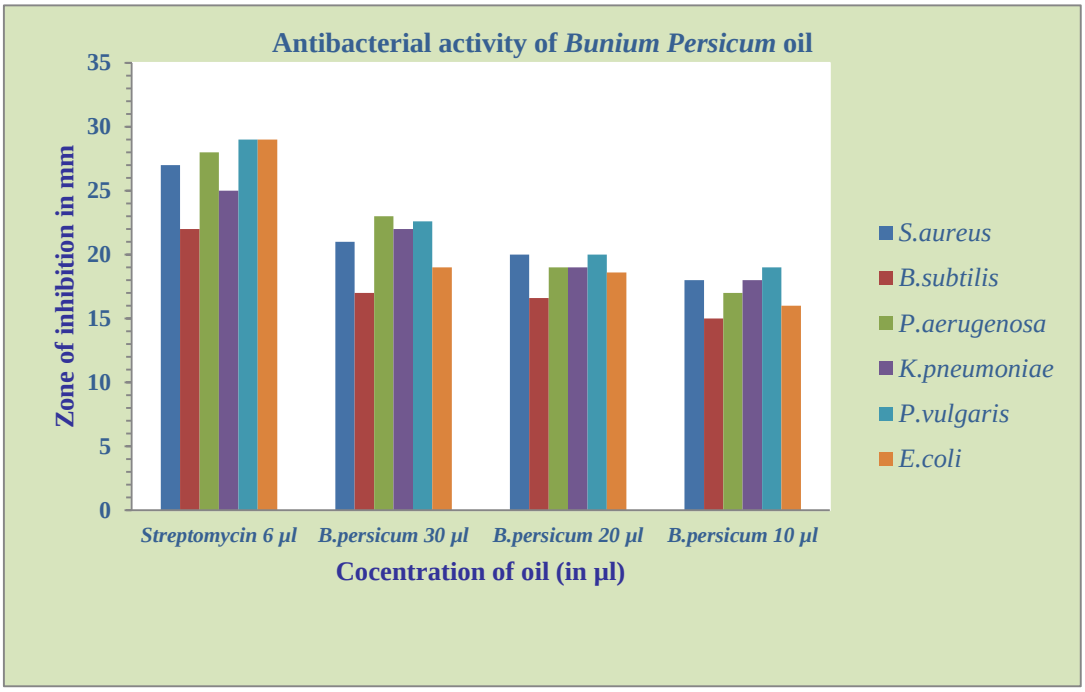

Fig. 2: Graphical representation of zone of inhibition of the *Bunium persicum* oil against different bacterial strains

Table 3: Minimum inhibitory Concentration (MIC) of *Bunium persicum* oil (mg/ml) against different bacterial strains

Oil name	Micro oraganisms					
	S. aureus MTCC 96	*A. Subtilis* MTCC 441	*K. pneumoniae* MTCC 19	*P. vulgaris* MTCC 426	*P. aeruginosa* MTCC 1688	*E. coli* MTCC 443
Bunium persicum	3.2mg/ml	1.6mg/ml	3.2mg/ml	3.2mg/ml	3.2mg/ml	1.6 mg/ml

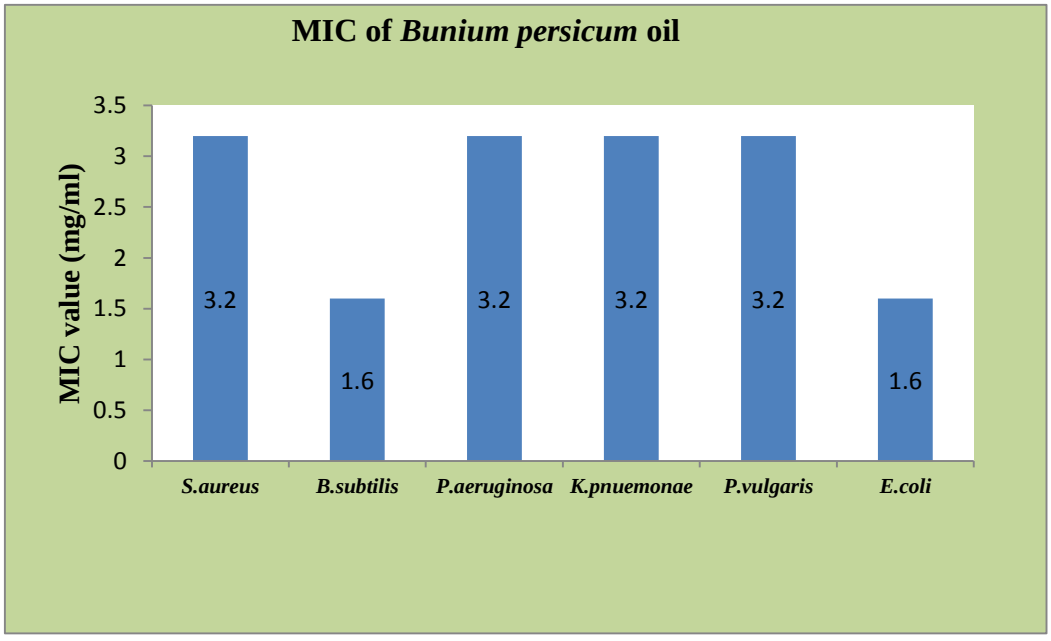

Fig. 3: Graphical representation of Minimum inhibitory concentration (MIC) value of *Bunium persicum* oil against various bacterial strains

Table 4: In vitro antifungal activity of *Bunium persicum* essential oil and reference antibiotic determined with agar well diffusion method

S No.	Test Organisms	Concentration of essential oil used in µl to determine zone of inhibition (diameter in mm)		Negative Control used (DMSO)	Standard Used
		20µl	30µl	40 µl	Nystanin 50µg/ Disc
01.	*Saccharomyces cerevisiae* MTCC 1023	17.6±0.3	18.6±0.33	0mm	14±0.57
02.	*Candida albicans* MTCC 6258	15.6±0.3	17.6±0.33	0mm	18±0.57
03.	*Pencillium Crysogenum* MTCC 1380	28.6±0.3	32±0.57	0mm	22±0.57
04.	*Aspergillus fumigatus* MTCC 9001	12.6±0.3	15.6±0.33	0mm	18±0.57

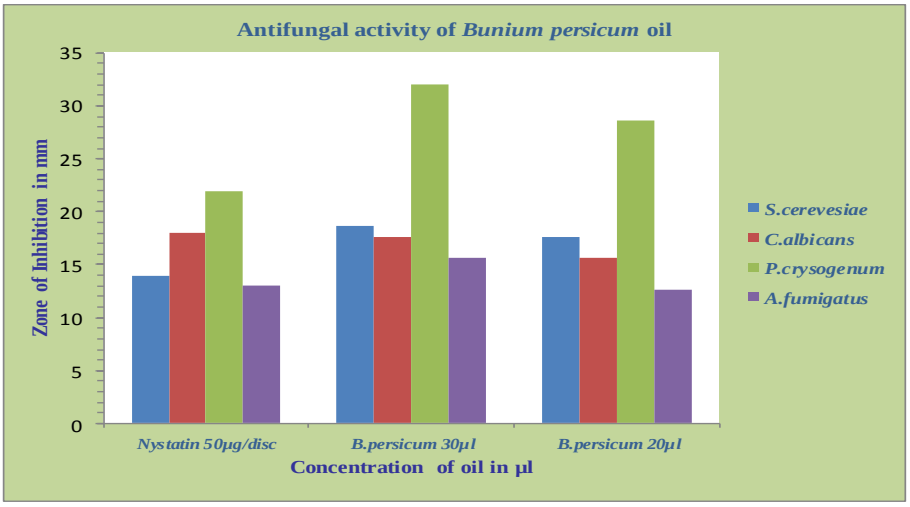

Fig. 4: Graphical representation of zone of inhibition of the *Bunium persicum* oil against different fungal strains

Table 5: Minimum Inhibitory Concentration (MIC) value of *Bunium persicum* oil (mg/ml) against different fungal strains

Oil name	Fungal organisms			
	S.cerevisiae MTCC 1023	*C.albicans* MTCC 6258	*P.crysogenum* MTCC 1380	*A.fumigatus* MTCC 9001
Bunium persicum	3.2mg/ml	3.2mg/ml	3.2mg/ml	1.6mg/ml

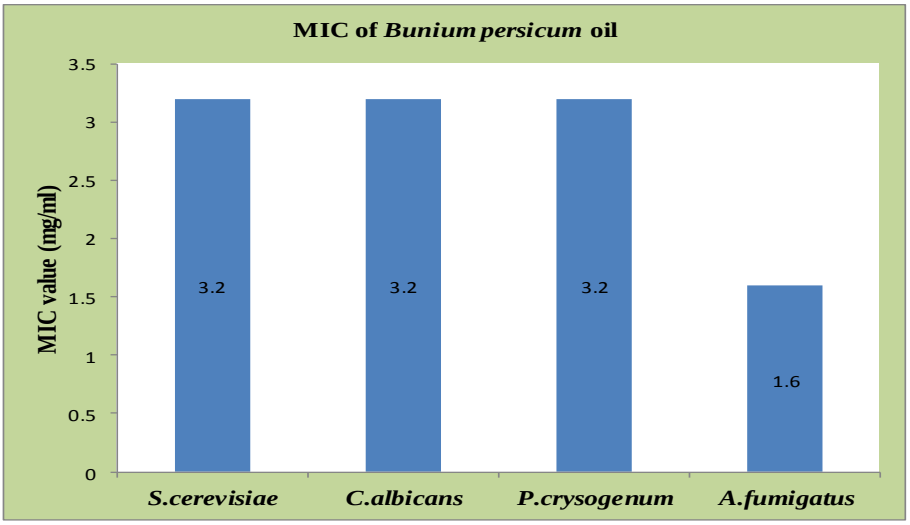

Fig. 5: Graphical representation of Minimum inhibitory concentration (MIC) value of *Bunium persicum* oil against various fungal strains

REFERENCES

1. Zargari, Medicinal Plants, Tehran, Tehran University Press, Vol. 3, pp. 106-108 (1990).
2. Adams, R. P. (2001). Identification of essential oil components by gas chromatography and mass spectrometry. Carol Stream, IL: Allured.
3. Azizi, M., Davareenejad, Gh., Bos, R., Woerdenbag, H. J., & Kayser, O. (2009). Essential oil content and constituents of Black zira (*Bunium persicum* [Boiss.] B. Fedtsch.) from Iran during field cultivation (domestication). Journal of Essential Oil Research, 21, 78–82.
4. B.E. Abduganiew, U.A. Abdullaev, K.N. Aripov, K.H.C. Baser and T. Oezek, J. Essen. Oil Res.,9, 597 (1997).
5. Baser, K. H. C., Özek, T., Abduganiev, B. E., Abdullaev,U.A., & Aripov,K.N. (1997). Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. From Tajikistan. Journal of Essential Oil Research, 9, 597–598.
6. Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. International Journal of Food Microbiology, 94,223–253.
7. Bicchi and P. Sandra, Capillary Gas Chromatography in Essential Oil Analysis. Heidelberg, Basel, New York, Alfred Huethig Verlag (1987).
8. Hajhashemi, V.; Sajjadi, S.E.; Zomorodkia, M. Antinociceptive and anti-inflammatory activities of Bunium persicum essential oil, hydroalcoholic and polyphenolic extracts in animal models. Pharm. Biol. 2011, 2, 146–151. [CrossRef] [PubMed]
9. Irith Weigand; Kai Hilpert & Robert E W Hancock. “Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances”. 3 (2008): 163-75.
10. Moghtader M, Mansori A, Salari H, Farahmand A. Chemical composition and antimicrobial activity of the essential oil of *Bunium persicum* Bioss seed. Iran J Med Arom PI 2009; 25 (25): 20-8.
11. R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, USA, Allured Publ. Corp., Carol Stream. (1995).
12. Santos FA, Jeferson FA, Santos CC, Silveira ER, Rao VSN (2005): Antinociceptive effect of leaf essential oil from Croton sonderianus in mice. Life Sciences 77, 2953–2963.
13. Seenivasan Prabuseenivasan, Manickkam Jayakumar and Savarimuthu Ignacimuthu. “In vitro antibacterial activity of some plant essential oils”. 3 (2007): 143-47.
14. Shahsavari, N.; Barzegar, M.; Sahari, M.A.; Naghdibadi, H. Antioxidant activity and chemical characterization of essential oil of *Bunium persicum.* Plant Foods Hum. Nutr. 2008, 63, 183–188. [CrossRef] [PubMed]
15. Sharififar F, Yassa N, Mozaffarian V. Bioactivity of major components from the seeds of *Bunium persicum* (Bioss) Fedtch Pak J Pharm Sci.2010; 23 (23): 300-4.
16. Thappa, R. K., Ghosh, S., Agarwal, S. G., Raina, A. K., & Jamwal, P. S. (1991).Comparative Studies on the major volatiles of Kalazira (*Bunium persicum*) seed of wild and cultivated sources. Food Chemistry, 41, 129–134.
17. V. Mozaffarian, A Dictionary of Iranian Plants Names, Tehran, Farhang Moaser Publishers (1998)
18. Wang R, Wang R, Yang B (2009): Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. Innovative Food Science and Emerging Technologies 10, 289–292.