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Chemical Analysis and Antidiabetic Activity of Ethanolic Extract of *Bunium Persicum* Seeds

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KEYWORDS

Chemical Analysis, Antidiabetic Activity

ABSTRACT:

Introduction: Traditional use of Bunium persicum seeds are well known in urinary and digestive disorders. Limited studies in Iran and Pakistan revealed that supplementation of B .persicum seeds (1000 mg in capsules) to over-weight and obese patients with type 2 diabetic patients declined their fasting blood glucose, HOMA-insulin resistance and body mass index (1), needs further exploration.

Methodology: The seeds of B. persicum were procured, authenticated and washed several times with tap water and dried under Sun-light. The crude powder of dried seeds were extracted with 95 % ethanol. The ethanolic extract of the dried seeds was analyzed by GC-MS. Streptozotocin induced diabetic rats were used as a model for determining blood glucose lowering activity in crude ethanolic extract of B. persicum. Skeletal muscle cell lines (L6 myc) were used for determining cytotoxicity of crude ethanolic extract of B.persicum seeds.

Result: The major five compounds identified in ethanolic extract of B .persicum seeds were Cuminaldehyde, α -pinene beta myrcen, 3-carene, benzenemethaneamine, dl-limonene,1,8-cineole, gamma–terpinene , alpha-thujone, camphene, alpha terpinene, and 1,3,3-trimethylbicyclo(2.2.1) –heptan-2-ol,p-menth-2-en-1-ol. The ethanolic extract when given to Streptozotocin-induced diabetic rats at 250 mg/kg showed significant lowering in fasting blood glucose profile.

Conclusion: Crude powder of B.persicum seeds revealed 17 compounds whereas the major ones are five i.e. Cuminaldehyde, α -pinene beta myrcen, 3-carene, benzenemethaneamine, and demonstrated significant blood glucose lowering activity diabetic rats induced by streptozotocin.

Introduction

Bunium persicum is a branched and perennial herb that belongs to family Apiaceae at its seeds are widely used not only as as a spice but also for the treatment various disorders. Such purposes are well described in traditional and folklore medicine of different countries There are plenty of ethno-medical uses and pharmacological activities indications in the seeds of *B.persicum seeds* but are limited to in vitro studies **(2).** Moreover various bioactive compounds have been isolated and identified in *B. persicum*. However, their proved pharmacological activities are still lacking.. Compounds such as caffeic acid and p-coumaric acid present in *B.persicum* seeds have been reported for antioxidant activities (3**)**. Flavonoids present in the seeds of ***B. persicum*** have been reported for antioxidant, antidiabetic, (4)analgesic, anti-inflammatory **(5)** Terpenoids such as p-cymene and gamma-terpenes present in ***B. persicum*** seeds have been reported for analgesic, anti-inflammatory, and antimicrobial activities. **(6).** There is single report where supplementation of *B .persicum* seeds (1000 mg in capsules) to obese patients with type 2 diabetes mellitus has been shown to decline their blood glucose levels, HOMA-insulin resistance and body mass index **(7),**

Type 2 diabetes mellitus poses medical, physical, and psychological problems of obesity, a chronic and frequently progressive illness.Strong and consistent data suggests that managing obesity can stall the onset of type 2 diabetes mellitus from pre-diabetes mellitus. . it is very helpful in the treatment of type 2 diabetes. In people with type 2 diabetes and overweight or obesity, modest weight loss improves glycemia and reduces the need for glucose-lowering medications. ***Bunium persicum*** is one of the major constituent in the Unani preparation Arq-e-zeera which is being used in controlling obesity. It will of interest now to identify the compounds in ***B. persicum*** seeds responsible for antidiabetic activity. In an order to identify the bioactive components present in ***B. persicum*** seeds responsible for anti-diabetic activity, the ethanolic

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extract of seeds of ***B. persicum*** was prepared, analyzed for the identification and quantitation of compounds within and evaluated for antidiabetic activity on streptozotocin-induced diabetic rat model. (8)

Materials and Methods –

The seeds of ***Bunium persicum*** were procured from local markets in Srinagar, Kashmir. Male albino rats of Sprague Dawley strain were procured from CSIR-Central Drug Research Institute, Lucknow (U.P.). The source for getting Streptozotoxin was Sisco Research Laboratories, Mumbai. Glucometer and Glucostrips were purchased from Optium (Freestyle), India. Rest of chemicals and solvents used were of analytical grade.

Preparation of Ethanolic Extract of *B.persicum* seeds

The procured seeds were authenticated by the Scientists at CSIR-CIMAP, India. A voucher specimen is kept in the laboratory. Seeds were washed several times with tap water and finally with distilled water. Seeds were dried under sun light and the dried seeds were powdered into a mechanical grinder. The crude powder was filtered through muslin cloth. 1.0 kg of fine powder was extracted with five times of 95 % ethanol (w/V) for 24 h and this process was repeated five times. The filtrate from each extraction was pooled. The pooled filtrate was concentrated to dry mass by evaporating the alcohol using a rotary evaporator in a water bath at 40°C. The dried mass is stored in screw capped glass tubes and termed ethanolic extract.

Cytotoxicity determination of ethanolic extract *Bunium persicum* seeds

The ethanolic extract of B. persicum seeds was dissolved in 10 % DMSO. L6 myc cell line was considered for cytotoxicity evaluation.. L6 myc cells were seeded at 1×10^4 cells/well in a 96-well culture plate and allowed to get confluence. Cells were treated with different concentrations of ethanolic extract i.e. 10 µg to 50µg/ml and incubated at 37°C for 24 h and. after incubation, 10 µl of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide) solution (5 mg/ml in PBS) was added into each well, covered with aluminum foil, and incubated at 37°C for a further period of 4 h. After aspiration of culture medium from each well 100µl of DMSO was added to each well and incubated tc dissolve the formazan crystals. The absorbance was compared at 570 nm in an ELISA plate reader.

Antidiabetic efficacy determination of ethanolic extract of *B.persicum* seeds on STZ-induced diabetic albino rats

Healthy male and female albino rats of Sprague-Dawley were acclimatized for 10 days in the animal colony under standard conditions (12 hour dark/12 hour night cycle, temperature 25+2º C, humidity 60 %, air changes 10 to 12 per hour) before these were considered in the study. Animals were starved overnight (16 hours) and their fasting blood glucose levels determined. Streptozotocin was dissolved in citrate buffer pH 4.5 and injected intraperitoneally into overnight starved albino rats at 50 mg/kg dose. Two days later the animals were again fasted overnight and next morning their blood glucose levels determined by glucostrips using glucometer. The animals showing over 250 mg/dl blood glucose profile were grouped into two. The animals of Group II was orally treated with 100 mg /kg ethanolic extract of *B.persicum* seeds suspended in 1.0 % gum acacia whereas the animals of group I was orally treated with an equivalent amount of gum acacia. Blood glucose level of each followed at hourly intervals till 06 hours and thereafter food was given into cages. The next morning the animals were again determined for their blood glucose levels. The blood glucose profiles of each animal was plotted versus time. The average % lowering in oral glucose tolerance in B.persicum treated group compared to sham treated group determined blood glucose lowering activity of ***B.persicum*** seeds.

Qualitative and quantitative analysis of Ethanolic Extract of *B. persicum* seeds

The qualitative analysis of ethanolic extract of ***Bunium persicum*** seeds was performed by using gas chromatography-mass spectrometry (GC-MS). A thermo trace ultra gas chromatograph coupled with TSQ quantum mass spectrometer (triple quadrupole . the mass was detector was operated at 70Ev ionization energy ,0.132s/scan in full scan mode.over the mass range 40-500Da, the chromatograph equipped with a thermo TR-5 MS fused silica column (length 3.0m.i,d., 0.25 mm; and film thickness 0.25µm). the stationary phase was 5% phenyl polyphenylene–siloxane. The temperature of oven increased from 40˚ C/min, the temperature injector 250˚C.Helium was used as carried gas ,with the flow rate of 1.mL/min , and split flow of 25 mL/min which corresponded to a split ratio of 25.the

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transfer line temperature was set at 250˚C.The compound were identified through the comparison of their mass spectra with the reference mass spectra of several libraries.(Rawan Al Nemari et al., 2020)

The qualitative analysis of the essential of ethanolic extract od ***B. persicum*** was performed using the Agilent 8890 GC system and the column HP-88 diameter 0.250 mm, 100 mm length, and 0.20 µm film. The carrier gas flow was 1.0 ml/min. The temperature of the injector was operated at 240°C and set 60°C for the oven. Then, the temperature was increased gradually for 40 min. For analysis, 1.0 mg/ml w/v solution of ethanolic extract and 100 ul was injected for identification of compounds. Peaks were marked and their retention time was compared to attached NIST library with GC-MS instruments.

Results and Discussion

Fig 1 presents the effect of ethanolic extract of ***B. persicum*** seeds on L6 myc cell lines. The cytotoxic effect was monitored by uptake of MTT by L6 myc cells. . The cells were incubated for 24 hours with various concentrations of ethanolic extract i.e. 10 to 50 µg/ml, and 50 µg/ml in the wells . As evident from the figure 1 that treatment of the ethanolic extract of ***B.persicum*** seeds at the concentrations ranging from 10 µg to 50 µg/ml had no adverse effect on the viability of L6 myc cell and considered to be safe 50 ug concentrations.

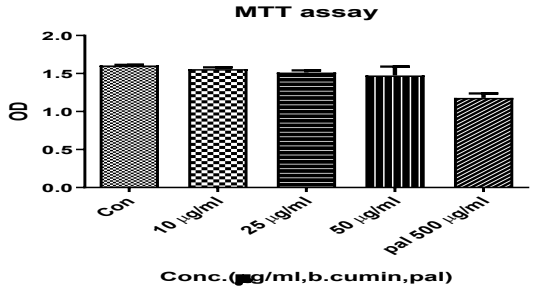
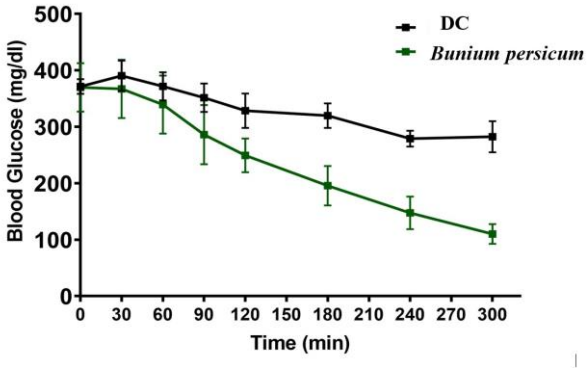
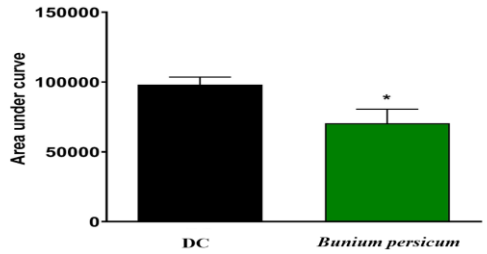


Fig 2 presents the blood glucose profile at hourly intervals of B. persicum treated and sham treated control. It is evident from the blood glucose profile that declined post treatment of ethanolic extract of ***B.persicum*** seeds and significant lowering can be observed from 120 min till the termination of experiment i.e. 300 min. Comparing the area under curve between treated and control group, it revealed around lowering in blood glucose profile post treatment of ethanolic extract of ***B. persicum*** (Fig





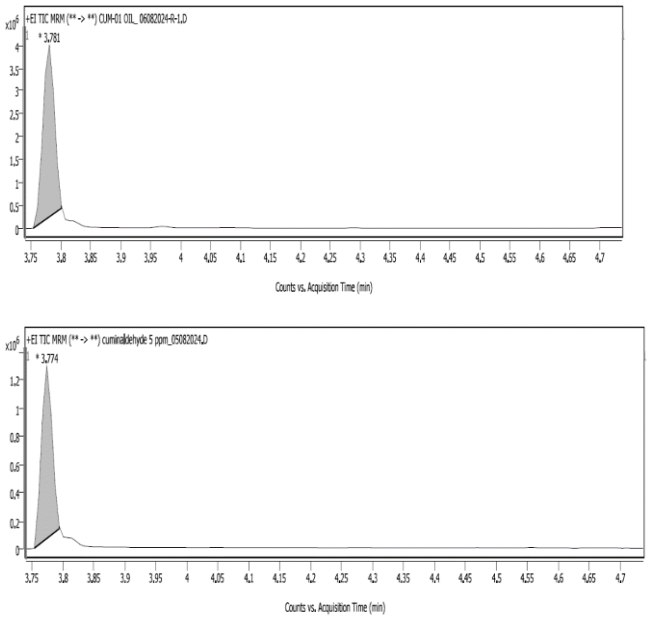


Fig 4. Chemical profiling of ethanolic extract of *Bunium persicum* by GC-MS

The ethanolic extract *of B.persicum* seeds showed 17 detectable peaks. Their chemical structure, mass, retention time (RT) and area of peaks are shown in Table 1. By comparing the RT, the 17 peaks were identified as Beta myrecene, dl –Limonene, Gamma terpinene, 1,3,3-

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trimethylbicyclo [2.2.1] -heptan-2ol, (1RS,4SR,6RS)-6-BROMO-1,3,3trimethyl-2-oxabicyclo[2.2.2.]octotane, 4 Terpineol, z beta-ocimenol , 1-dimethoxymethyl)-2 isopropylbenzene, Cumilaldehyde,2-isopropylbenzaldehyde, 5,5-dimethyl-3methylene-1cyclohexane -1 carbaldehyde, 2-7 dimethyl-1-cycloheptane-1carbaldehyde, 1R, 2S, 4R, 6S)-4-isopropenyl-1-methylbicyclo [4.1.0] heptane-2-ol, 4,8,dimethyl-4,7 nonadienal, 3,4 dimethylbenzenacetic acid, 4,5-epoxy -1isopropyl-4methyl-1 cyclohexane, 4-o (Methoxyphenyl) cyclopropane. Among these seventeen, five were present in major amounts i.e. dl –Limonene, 1-dimethoxymethyl)-2 isopropylbenzene, Cumilaldehyde,5,5-dimethyl-3methylene-1cyclohexane,2-isopropylbenzaldehyde.

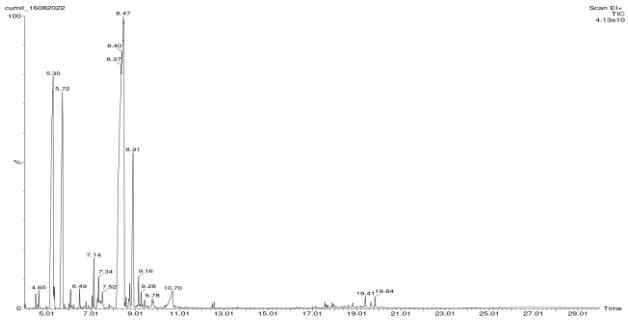
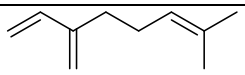
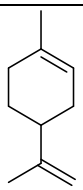
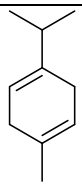
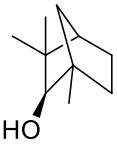


Fig 4. GC-MS chromatograph of ethanolic extract of *Bunium persicum* seeds

Identification and quantification of the compound *Bunium persicum*

Table 1: Chemicals present in ethanolic extract of *Bunium persicum* seeds as assessed by GC-MS.

S. no	Compounds	Mass	RT	% area
1.	 beta-Myrcene	136	4.65	7.32
2.	 dl-Limonene	136	5.30	**89.82**
3.	 gamma- terpinene	136	5.679	75.3
4.	 HO 1,3,3-trimethylbicyclo[2.2.1]-heptan-2-ol	154	6.491	8.75

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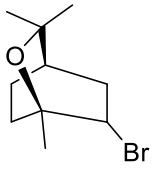
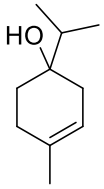
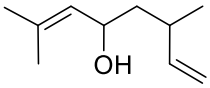
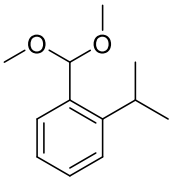
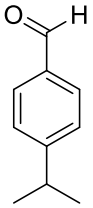
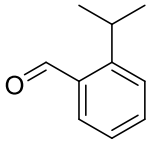
5.	 (1RS,4SR,6RS)-6-bromo-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane	232	7.141	18.5
6.	 4-Terpineol	154	7.344	11.6
7.	 Z-.beta.-ocimenol	154	7.520	5.68
8.	 1-(Dimethoxymethyl)-2-isopropylbenzene	148	8.373	**81.67**
9.	 Cuminaldehyde	148	8.400	**85.9**
10.	 2-Isopropylbenzaldehyde	148	8.470	**96.7**

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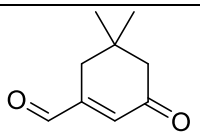
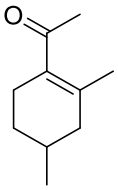
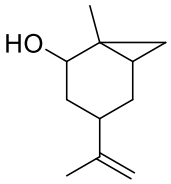
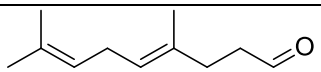
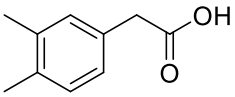
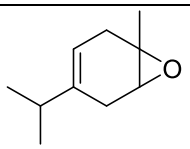
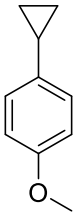
11.	 5,5-Dimethyl-3-methylene-1-cyclohexene-1-carbaldehyde	150	8.910	**51.6**
12.	 2,7-dimethyl-1-cycloheptene-1-carbaldehyde	152	9.160	12.6
13.	 (1R,2S,4R,6S)-4-Isopropenyl-1-methylbicyclo[4.1.0]heptan-2-ol	166	9.28	7.64
14.	 4,8-Dimethyl-4,7-nonadienal	166	9.78	4.75
15.	 3,4-Dimethylbenzenacetic acid	164	10.70	9.45
16.	 4,5--epoxy-1-isopropyl-4-methyl-1-cyclohexene	152	19.41	4.52
17.	 4-o-(Methoxyphenyl) cyclopropane	148	19.84	3.49

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Discussion -

The present work indicates that seeds of *B.persicum* has the property of blood glucose lowering and many of the compounds identified in the ethanolic extract may be advocated for the blood glucose lowering activity. Phytochemical investigation revealed the presence of certain compounds in the ethanolic extract. Notably, Beta-myrcene, a terpene present in a *Bunium persicum* extract, demonstrates a wide spectrum of pharmacological activities including analgesic, sedative, anti-inflammatory, and antioxidant properties. Gamma-terpinene (γ-terpinene) is a monoterpene found in various *Bunium persicum* essential oils, exhibiting wide spectrum of pharmacological activities includeing antimicrobial, antioxidant, anti-inflammatory, and antinociceptive effects. Gamma-terpinene (γ-terpinene) is a monoterpene alcohol present in numerous essential oils, demonstrating a variety of pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, and antinociceptive effects. It also has potential anti-platelet action. 4-Terpineol, commonly known as terpinen-4-ol, is a chemical substance found in a variety of plants.. It is a key component of tea tree oil and is valued for its antibacterial, antifungal, and anti-inflammatory effects. Cuminaldehyde generally found in cumin seeds acts as an antiseptic, analgesic, anti-inflammatory, and sedative and is used against stomach disorders, diarrhoea, diabetes and spasms 2-Isopropylbenzyl aldehyde is often referred to as Cuminaldehyde or Cuminic aldehyde .Compounds have a variety of pharmacological characteristics, including anti-inflammatory, anti-depressant, anti-arrhythmic, anticancer, antifungal, and antibacterial**.(9)** Some research indicates that 3,4-dimethylbenzaldehyde may have antibacterial, anti-inflammatory, and antioxidant activities.10.4-Terpineol, also known as Terpinen-4-ol, is a bioactive molecule having numerous pharmacological activities, including antibacterial, anti-inflammatory, antioxidant, and anti-cancer actions. It is a major component of tea tree oil and has shown promise in various therapeutic applications. **(10).** It also demonstrates antifungal activity against various fungi, such as Candida albicans and Aspergillus flavus. Some of the active ingredients in *Bunium persicum* is the ability to lower blood sugar levels, \which helps people with diabetes. The limited preclinical and clinical research shows that the seeds of ***Bunium persicum*** and its active compound help with metabolic syndrome. Though there was no report on harmful properties in *Buium persicum* seeds has no harmful properties except one report showing hazardous when given to to pregnant women **(11)**

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