



Identify the role of *Trachyspermum ammi* L. and *Helianthus annuus* phytoconstituent on microbial cultures

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Abstract

The extant study pacts with the role of *Trachyspermum ammi* L. and *Helianthus annuus* buffer extracts on microbial cultures. *Trachyspermum ammi* L. Buffer Extract (TABE) and *Helianthus annuus* Buffer Extract (HABE) found to be presence of terpenoids, Glycosides, flavonoids, carbohydrates and steroids. But only TABE withholds polyphenol compounds and HABE contains only saponins respectively in the extracts when it subjected to preliminary phytoconstituent characterization. Surprisingly both TABE and HABE confirm more than 10 peaks in analytical assay performed with GC-MS and HPLC instrumentation technique. Which intern states that more phytochemical compounds are extracted in the buffer extraction of TABE and HABE. Importantly, TABE and HABE contain several minerals like aluminium, cadmium, zinc, barium and etc.... Moreover, TABE and HABE exhibit anti-microbial property by inhibiting the microbial culture growth such as *E. coil*, *S. aureus*, *pseudomonas*, *salmonella* and *shigella* species. Furthermore, TABE and HABE found to be non-toxic in nature as it unable to break RBC cells when we analyse *in-vitro* study by using packed RBC.

Keywords: *Trachyspermum ammi* L. Buffer Extract (TABE), *Helianthus annuus* Buffer Extract (HABE), GC-MS, RP-HPLC, Antimicrobial property and Non-toxic property

Introduction

Trachyspermum ammi L. commonly called as Ajwain belongs to Apiaceae family and order Apiales Genus *Trachyspermum* and species *ammi* ^[1]. It is an annual herbaceous plant; hence it belongs to the highly valued and utmost medicinally important herbal ^[2]. It is widely distributed and cultivated in various regions of worldwide importantly India, Iran, Pakistan and Afghanistan as well as in the European countries ^[3]. Ajwain is generally grown in the month of October-November and it also can be harvested in the month of May-June ^[4]. Ajwain is the most commonly used herbal as traditional medicine for its variety of medicinal and pharmacological properties ^[5]. In Traditional Persian medicine (TPM), it is well known from thousands of years. Persian prationers usually used seeds of Ajwain as the most useful part of the herb ^[6]. According to its temperament, Ajwain is hot and dry in the third degree and also possesses some bitterness and acidity; it is also

widely used in the treatment of paralysis ^[7].

Helianthus annuus generally termed as sun flower seeds belongs to Asteraceae family and Order Asterales Genus *Helianthus* and species *annus* ^[8]. Out of 1900 genera, it bears of over 32,000 known species, thus it belongs to large family of flowering plants. It is a leading oil seed crop which is cultivated primarily for the rich content of oil in seeds ^[9]. It possesses several medical importance as it is used to treat diuretic, expectorant, cough, throat and lung infections. The healing effect of sunflower seeds makes wide broad spectra in the usage of therapy for bronchitis, thrombophlebitis, carbuncles, catarrh, blindness, sinusitis diarrhea, dysentery, dysuria, scorpion stings, snakebite, inflammation, urogenital ailments, leg ulcer, whitlow and wounds ^[10].

Materials and Methods

All the chemicals used were of analytical grade.

Preparation of TABE and HABE: *Trachyspermum ammi* L. and *Helianthus annuus* seeds were purchased from local market and it was powdered. The obtained powders were extracted by using phosphate buffer using Soxhlet extraction method. The finally obtained *Trachyspermum ammi* L. and *Helianthus annuus* buffer extracts were termed as TABE and HABE respectively and it utilized for further assays.

Preliminary phytochemical screening of TABE and HABE: TABE and HABE were screened for terpenoids, phytosterol, tannin, phenolic, glycoside, Saponin, flavonoid, carbohydrates, proteins, steroids and lipids ^[11-15].

Reverse phase high performance liquid chromatography analysis of TABE and HABE: TABE and HABE were subjected to RP-HPLC using C₁₈ column (150mm×3mm, particle size 2.7µm) with VWD detector in Agilent 1260-infinity II. The column was pre-equilibrated with HPLC water and Acetonitrile and sample was eluted at the flow rate of 1ml/min in linear gradient mode ^[16].

GC-MS analysis of TABE and HABE: TABE and HABE analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionisation Total Ion Chromatography (EITIC) mode with capillary column (30m length×0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.999%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector temperature 350 °C; Auxiliary temperature 250 °C, oven temperature initially 50 °C (4min hold) with an increase in temperature of 10 °C/min to 150 °C (4min hold), thereafter 20 °C/min to 200 °C (4min hold), 25 °C/min ramp to 250 °C (4 min hold), 30 °C/min ramp to 280 °C (4 min hold). Total run time 35.5 min. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 70ev; a scan interval of 2.92s ^[17].

Direct hemolytic activity of TABE and HABE

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of TABE and HABE (0-100µL) for 1hr at 37 °C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37 °C ^[18]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

Antimicrobial assay of TABE and HABE

The bacterial cultures (*E. coli*, *Salmonella*, *Pseudomonas*, *Shigella* and *S. aureus*) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made on the solidified agar using a cork borer. The test

solution was made by dissolving 37mg and 44mg of TABE and HABE respectively in 1.0mL of methanol to get 37mg/mL and 44mg/mL concentration respectively followed by sonication for 2min. The 100µL of this test solution containing 3700µg and 4400µg of TABE and HABE respectively were added into the respective wells (3-10mg). The standard antibiotic drug Amoxycillin was kept as positive control and tested against all the pathogens. These plates were incubated at 37 °C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded ^[19]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

ICP-OES analysis of TABE and HABE

TABE and HABE were analyzed in Agilent Make ICP-OES instrument, model number 5110. To evaluate the content of minerals in the extract, the samples were aspirated at 12 RPM pump speed, 25 seconds sample uptake time, 30 seconds of rinse time, 5 seconds, read time, 1.2 KW RF power, 15 seconds stabilization time, Axial viewing mode, 8mm viewing height, 0.7 L/Min nebulizer flow, 12 L/Min plasma flow, 0.75 L/Min Aux flow.

Results and Discussion

Phytochemical and mineral analysis of TABE and HABE: TABE and HABE found to be presence of terpenoids, Glycosides, flavonoids, carbohydrates and steroids. But only TABE withholds polyphenol compounds and HABE contains only saponins respectively in the extracts when it subjected to preliminary phytoconstituent characterization (Table 01). TABE and HABE show the presence of several minerals like aluminium, cadmium, zinc, barium, boron and etc., (Table 02).

Table 1: The extracts when it subjected to preliminary phytoconstituent characterization

SL. No	Phytochemical analysis	TABE	HABE
1.	Terpenoid	Present	Present
2.	Phytosterol	Absent	Absent
3.	Tannin	Absent	Absent
4.	Phenolic	Present	Absent
5.	Glycoside	Present	Present
6.	Saponin	Absent	Present
7.	Flavonoid	Present	Present
8.	Carbohydrates	Present	Present
9.	Proteins	Absent	Absent
10.	Steroids	Present	Present
11.	Lipids	Absent	Absent

Table 2: TABE and HABE show the presence of several minerals like aluminium, cadmium, zinc, barium, boron and etc.

SL. No	Name of the Metal	TABE in ppm	HABE in ppm
1.	Aluminum	0.55	0.51
2.	Boron	0.24	0.12
3.	Barium	0.21	0.10
4.	Cadmium	0.00	0.00
5.	Copper	0.10	0.17
6.	Iron	0.74	1.13
7.	Manganese	0.62	0.18
8.	Molybdenum	0.01	0.01
9.	Nickel	0.02	0.03
10.	Lead	0.01	0.01
11.	Zinc	0.61	0.65

RP-HPLC analysis of TABE and HABE

TABE and HABE HPLC chromatograms conforms the presence of 10 and 11 different types of compounds by eluting 10 and 11 peaks at different retention time

respectively in reverse phase HPLC attached to Variable Wavelength Detector. Sample was eluted at 216nm at room temperature (Fig.01 and Fig.02).

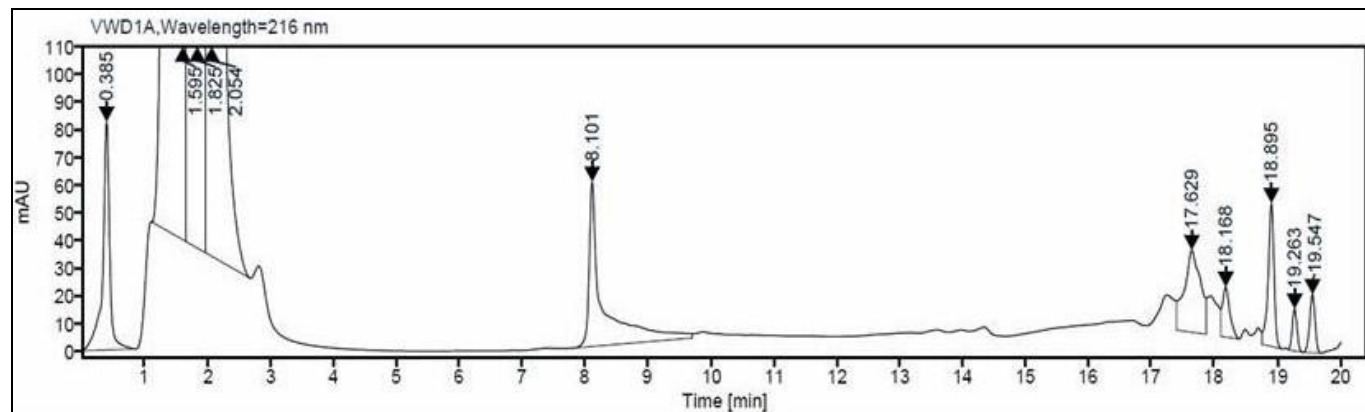


Fig 1: RP-HPLC chromatogram of TABE

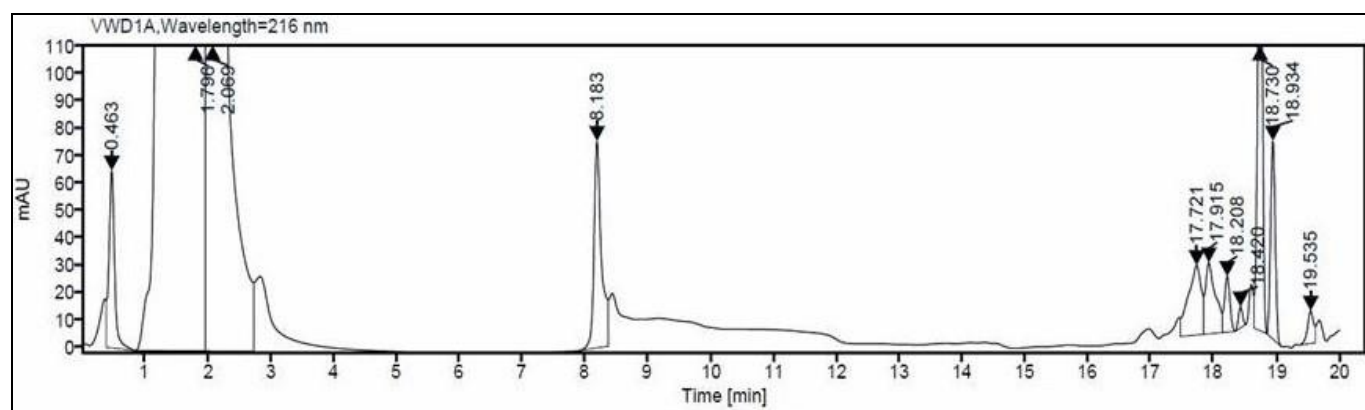


Fig 2: RP-HPLC chromatogram of HABE

GC-MS analysis of TABE and HABE

TABE and HABE found to exhibit 15 and 16 different set of compounds respectively as per GC-MS analysis. Samples were eluted at the retention time of 1.94, 2.33, 2.79, 7.86,

9.37, 10.06, 13.12, 13.57, 14.84, 20.29, 23.51, 26.61, 28.25, 28.62, 28.74 and 2.01, 2.36, 2.77, 3.42, 5.63, 7.87, 9.05, 9.48, 10.83, 13.15, 13.75, 14.50, 15.29, 18.04, 26.61, 28.65 respectively (Fig.03 and Fig.04).

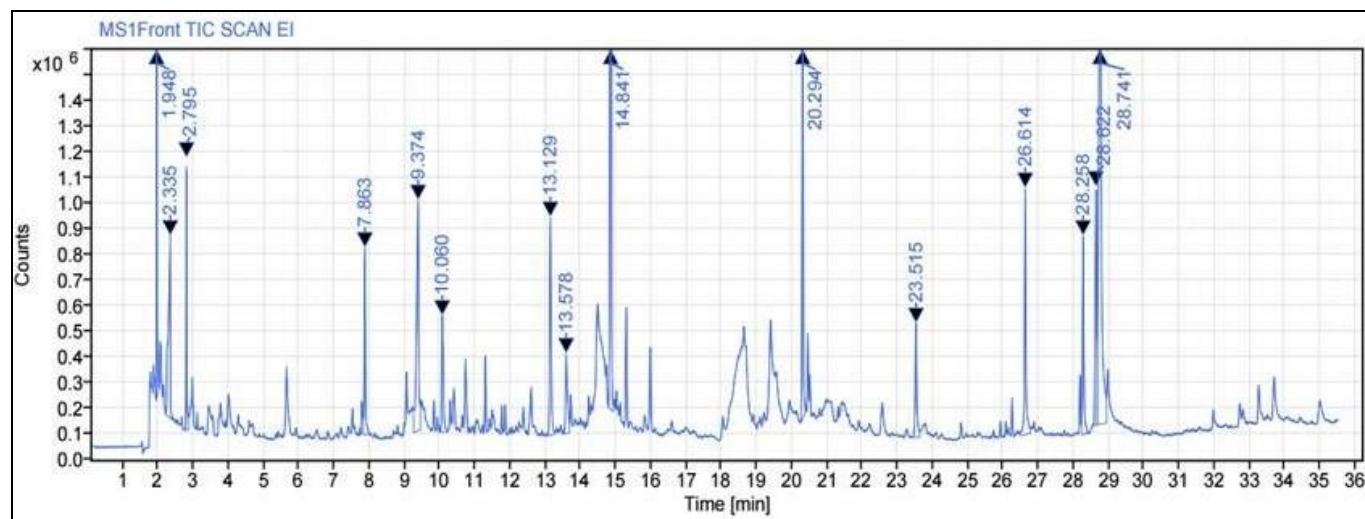


Fig 3: GC-MS chromatogram of TABE

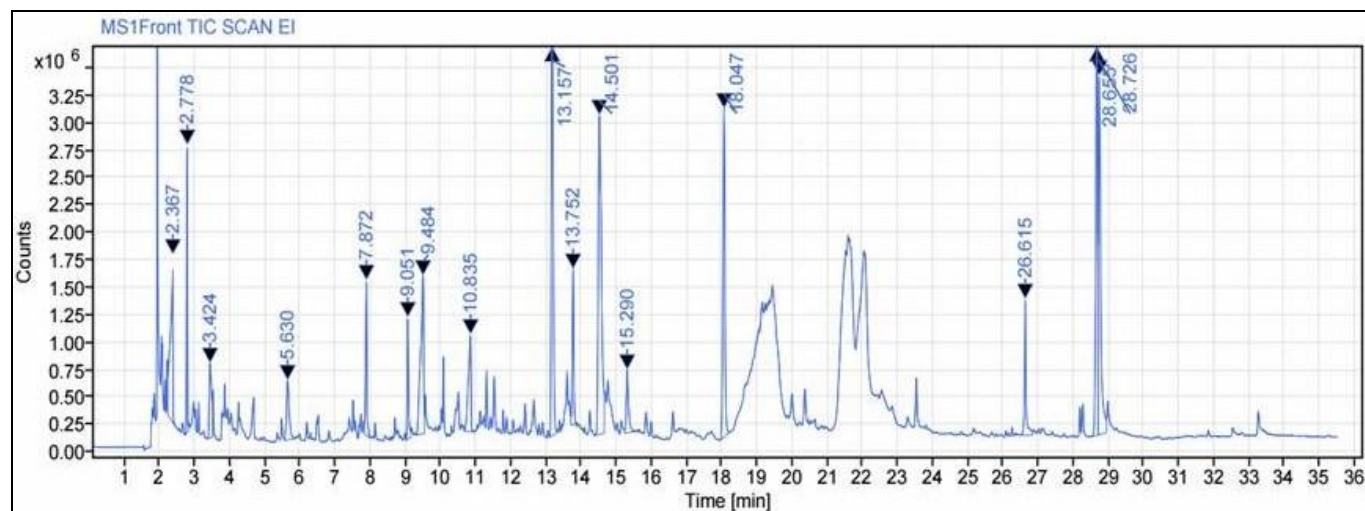


Fig 4: GC-MS chromatogram of HABE

Non-toxic property of TABE and HABE

Moreover, TABE and HABE did not hydrolyze RBC suggested its nontoxic property (Fig.05 and Fig.06).

HABE antimicrobial property were performed with *E. coli*, *Salmonella*, *Pseudomonas*, *Shigella* and *S. aureus*. TABE and HABE found to show zone of inhibition against all five microbial cultures (Fig.07 and Fig.08).

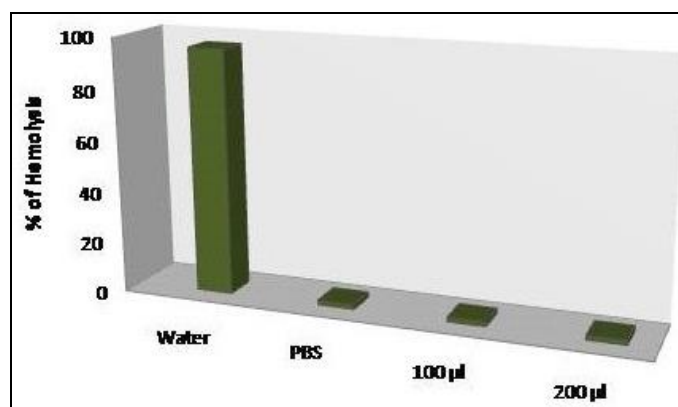
Antimicrobial activity of TABE and HABE: TABE and

Fig 5: Hemolytic activity of TABE

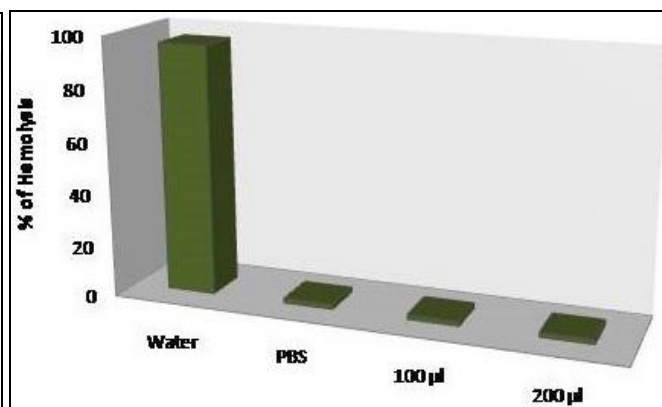


Fig 6: Hemolytic activity of HABE

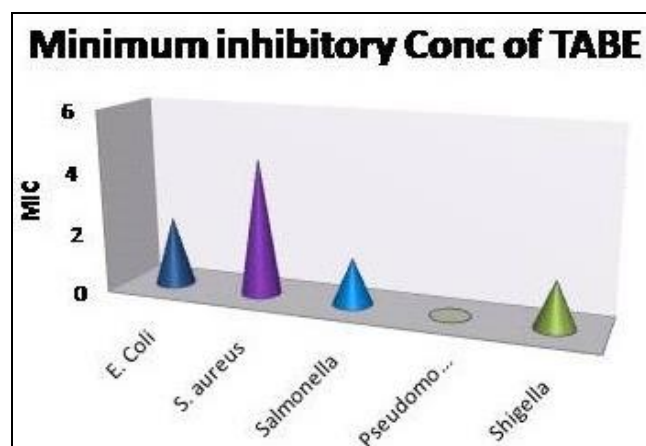


Fig 7: Anti-microbial property of TABE

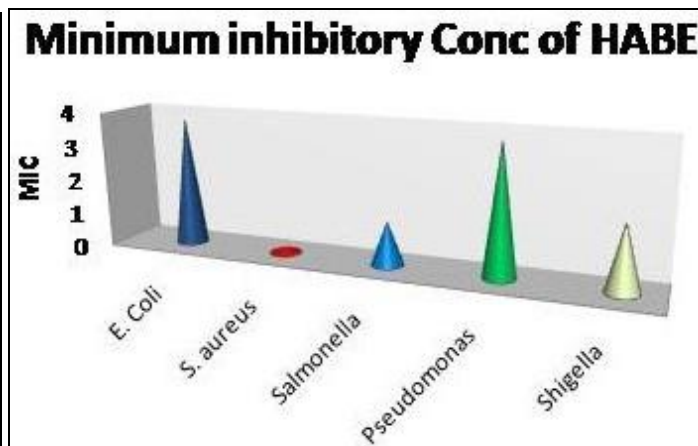


Fig 8: Anti-microbial property of HABE

Conclusion

In conclusion, this study demonstrates the preliminary characterization of TABE and HABE and its antimicrobial property with its non-toxic nature.

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Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

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