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ARTICLE

A Comparative Bioefficacy of Aqueous and Methanolic Extract of *Trachyspermum ammi* Towards the Antioxidant Potentiality and Electrochemical Behaviour

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ABSTRACT

The quest for natural palatable cost-effective antioxidant, *Trachyspermum ammi,* a spice of Asian origin used by all kinds of race and ethnic groups of this continent and others, has became our area of interest. In this study, the antioxidant properties of the aqueous and methanolic extract of *Trachyspermum ammi* were thoroughly investigated by spectroscopic and electrochemical techniques. The priority of this study was to investigate and compare the changes in response to free radical and superoxide scavenging, iron chelation activities where all the observed chemical assay supported electrochemical behaviour in both extracts. The antioxidant potentiality in the traditional and standard spectroscopic processes showed the observations as, for the methanol extract 62.1 %, 51 %, 57 %, 36 % and for aqueous extract 55 %, 43 %, 46 %, 34.8 % iron chelating, hydrogen peroxide scavenging, DPPH free radical scavenging and superoxide scavenging activity respectively. The experimental results clearly inferred that the methanolic extract of *Trachyspermum ammi* has more antioxidant potentiality than the aqueous extract. This may be attributed due to the fact that antioxidant components in *Trachyspermum ammi* are more soluble in methanol than in water and therefore, methanolic extract exhibited more antioxidant potentiality.

KEYWORDS

Trachyspermum ammi, Antioxidants, Iron chelation, Free radical scavenging, Electrochemical assay.

INTRODUCTION

There is a growing interest in natural antioxidants found in herbs and spices because of the global trend toward the identification of antioxidant compounds that are pharmacologically effective and have low or no side effects for use in preventive medicine and the food industry [1,2]. These antioxidants act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species (ROS) [3,4] and therefore can boost the immune defence and lower the risk of cancer and degenerative diseases [5,6]. Natural source of antioxidants mainly come from medicinal plants [7] in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic

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acid and carotenoids [8]. Food industry also uses natural antioxidants as a substitute of synthetic antioxidants in food and considers natural products to be promising and a securer source [9].

Trachyspermum ammi belonging to family Apiaceae, is an inhabitant of Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan and India [10].

Based on above background study and current trends of alternative medicine and eco-friendly healthier approaches, this work have been initiated to study the antioxidant activity and iron chelation of aqueous and methanolic extract of *Trachyspermum ammi* in respect to the standard pure antioxidant compound *e.g.* ascorbic acid and ethylenediaminetetraacetic acid.

EXPERIMENTAL

Seeds of *Trachyspermum ammi* (local name: ajwain) were purchased from local market and ethylenediaminetetraacetic acid (EDTA), ferrozine, ferrous chloride ($FeCl_2$), hydrogen peroxide, 2,2-diphenylpicryl-1-picryl-hydrazyl (DPPH), nitrobluetetrazolium (NBT), NADH, phenazinemethosulphate (PMS), Folin-Ciocalteau reagent (Qualigens), gallic acid (Spectrochem) were purchased from Sigma-Aldrich (St. Louis, MO). All chemicals and reagents used are of analytical grade.

Sample collection and extraction: The seed of *Trachyspermum ammi* were collected, washed and dried. The samples were kept in both aqueous and methanol solution for 48 h at room temperature (25 °C) at stirring condition. The crude aqueous and methanolic extract was collected by centrifugation at 3000 rpm for 15 min.

Assay for iron chelating activity: The iron chelating activity of the extract on ferrous ion Fe^{2+} was determined following the process mentioned by Xiao and co-workers [11]. Various concentrations (20 µg, 40 µg, 60 µg, 80 µg, 100 µg) of both aqueous and methanol extract (0.5 mL) were separately mixed with 1.8 mL of ultrapure aqueous $FeCl_2$ (0.05 mL, 2 mM) and ferrozine (0.1 mL, 5 mM) solution. Ferrozine reacted with the divalent iron to form stable complex species (magenta coloured) which were soluble in water. After 20 min at room temperature, the absorbance of the ferrozine complex in the reaction mixture was measured at 562 nm. EDTA was used as a positive control and ultrapure aqueous was used in place of samples or EDTA as a blank.

Assay for hydrogen peroxide radical scavenging activity: Scavenging activity of peroxide radical using the plant extract was determined. Samples (1 mL) of both aqueous and methanolic extract prepared in distilled aqueous at various concentration (20 µg, 40 µg, 60 µg, 80 µg, 100 µg) was mixed with 2 mL of 20 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) [12] and incubated for 10 min. The absorbance of the solution was taken at 230 nm.

Assay for DPPH free radical scavenging: The DPPH radical scavenging activity of seed extracts were measured according to the previous method [13]. Phosphate buffer (2 mL, pH 6.86), freshly prepared DPPH in ethanol solution (2 mL, 0.5 mM) and samples (0.5 mL) with different concentrations were mixed in a glass tube. The mixture was vigorously shaken and left to stand at room temperature for 0.5 h. The reduction of the DPPH radical was measured by monitoring the absorbance at 517 nm [14].

Assay for superoxide anion scavenging activity: About 1 mL of nitrobluetetrazolium (NBT) solution (156 mM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL NADH solution (468 mM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of sample solution were mixed initially. The reaction was started by adding 100 µL of phenazinemethosulphate (PMS) solution (60 µL PMS in 100 mM phosphate buffer, pH 7.4) to the mixture [15]. The reaction mixture was incubated at 25 °C for 5 min and the absorbance at 560 nm was measured against blank.

Total phenol estimation: Total phenol values are expressed in terms of gallic acid equivalent (in mM range of concentrations), which is a common reference compound. The exact detection range for standardization is predicted to be between 0.2 to 2.0 mM concentration, as the standard curve was prepared by 0, 200, 400, 800, 1000 µM solutions of gallic acid in 95 % methanol (v/v).

The both extract (0.15 mL of different dilutions) were mixed with Folin-Ciocalteu reagent (300 µL, diluted to 10 %, with distilled aqueous) for 5 min and aqueous Na_2CO_3 (0.7 M, 1.2 mL per reaction mixture) were then added. Ratio for each reaction mixture should be 1:2:8 and each component was added in a proper sequence [16]. The mixture was allowed to stand for 1 h of incubation and the phenols were determined by UV-visible spectrophotometry (JASCO R-630) at 765 nm.

Cyclic voltammetry: Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed on an Autolab type III FRA2 potentiostat/galvanostat using a closed standard three electrodes cell. An indium doped tin-oxide (ITO) (4 cm × 1 cm), bearing resistance 8-12 Ω/sq was used as the working electrode and platinum as the counter electrode. All potentials are referred to an Ag/AgCl 3 M KCl as reference electrode (Methrom). Prior to use, the working electrode was washed in milli-Q aqueous sonicated then in methanol. This cleaning procedure was always applied before any electrochemical measurements. The scan rate was 100 mV S^{-1} and the initial scan was taken from -100 mV to 100 mV past the first anodic peak and was repeated 3-5 times in sequence. Further scans were taken to 1200 mV to look for the presence of subsequent redox processes at more positive potentials. Background cyclic voltamograms were taken in the phosphate buffer solution and were recorded on the same day as the standards or the wine in question. The voltamograms were analyzed for peak potentials (all reported *vs.* Ag/AgCl) and currents as described below.

Procedure: Both the extracts and standard compounds were studied in phosphate buffer 0.1 M (pH 7.0) solution. For the calibration, standard solutions were prepared (ascorbic acid and gallic acid, concentration range, 0.1 to 1 mM in buffer). For the working extracts, the concentration was maintained between 10 to 70 mg/mL in buffer. All solutions were analyzed immediately after preparation and the electrochemical responses were recorded after the ITO electrode immersion, to minimize absorption of species onto the electrode surface prior to the run. Cyclic voltammetry was used for characterizing the electrochemical responses between 0 and 1.2 V, at

0.1 V s^{-1}, whereas the antioxidant potentiality was evaluated by differential pulse voltammetry, using the following operating conditions for DPV were set at 60 mV pulse amplitude and 0.030 V s^{-1} as scan rate.

RESULTS AND DISCUSSION

Antioxidant potentiality of the aqueous and the methanolic extract of seeds of *Trachyspermum ammi* was caused for the presence of polyphenols. Both extract were assessed by radical scavenging and cyclic voltammetry methods. Electrochemical results are in accord with the data obtained with the assay using spectrophotometric analysis. Both clearly reveal that the extract of seeds of *Trachyspermum ammi* collecting from our country exhibited prominent antioxidant potential and their usage as potent natural antioxidant agents.

Ferrous ion chelating activity: The antioxidant activities of the aqueous and methanolic extract of *T. ammi*, were evaluated based on chelating of ferrous ion. Fig. 1 shows the antioxidant abilities at different doses. The aqueous and methanolic extract showed 62.1 % and 55 % effects on ferrous ion chelating (Fig. 1), which showed the highest scavenging activity between all.

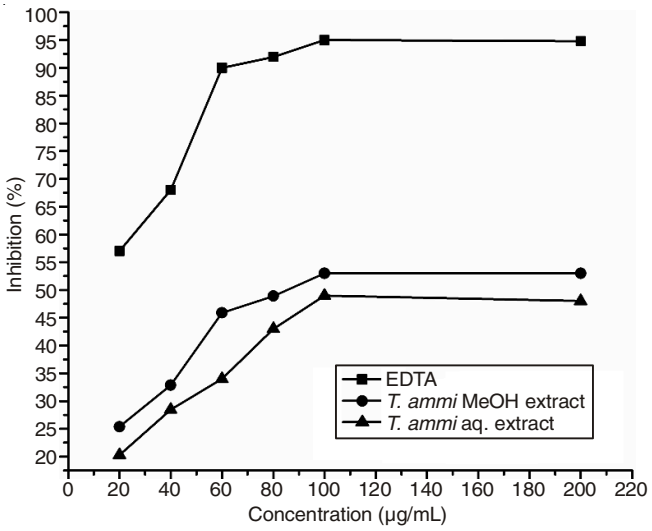

Fig. 1. Ferrous ion chelating activity of aqueous and methanolic extract of *Trachyspermum ammi*

Hydrogen peroxide scavenging: Scavenging of H_2O_2 by extracts may possibly endorsed to their phenolic part, which can contribute electrons to H_2O_2, thus neutralizing it. Extracts showed 51.1 and 43.2 % scavenging effects on H_2O_2 (Fig. 2). Although hydrogen peroxide itself is not very reactive, it can sometimes motive cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very significant all through food systems [17].

DPPH free redical scavenging: DPPH is a stable nitrogen-centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [1,18]. It was found that the radical- scavenging activities of extracts increased with increasing concentration. Extracts showed 57 and 46 % (Fig. 3) on DPPH respectively.

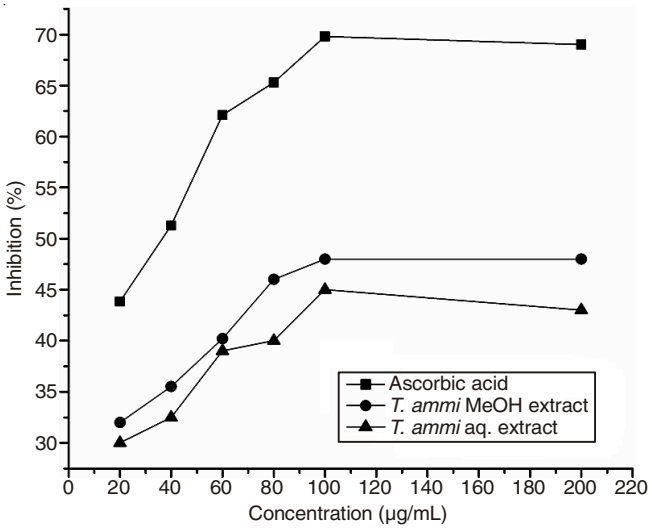

Fig. 2. Peroxide scavenging activity of aqueous and methanolic extract of *Trachyspermum ammi*

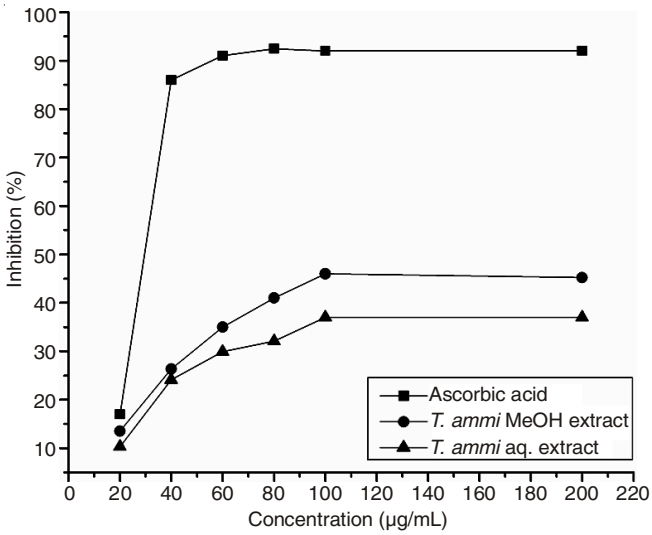

Fig. 3. DPPH free redical scavenging activity of aqueous and methanolic extract of *Trachyspermum ammi*

Superoxide free radical scavenging: The aqueous and methanolic extracts showed 36 and 34.8 % superoxide free radical scavenging (Fig. 4) respectively, which is lower than DPPH, iron chelating and H_2O_2 scavenging activity. These values were less than the same dose of ascorbic acid and EDTA. Both the extract showed the maximum antioxidant activity at 100 µg/mL. For these free radicals, aqueous extract has a close value of scavenging activity with methanolic extract.

Total phenolic compound: The spectrophometric quantification was revealed with significant amount of phenolic compound as 2.5 and 1.4 mg gallic acid equivalent for methanolic extract and aqueous extract respectively.

Electrochemical behaviours of *T. ammi* extract: Cyclic voltammetry and differential pulse voltammetry showed that there was relative generation of current in these extracts which leads to support the biochemical antioxidant assays, with an effective presence of electroactive organic compounds like polyphenols (Figs. 5 and 6). And the current generation was relatively lower than standard ascorbic acid, whereas the presence of antioxidant compound generated the reducing

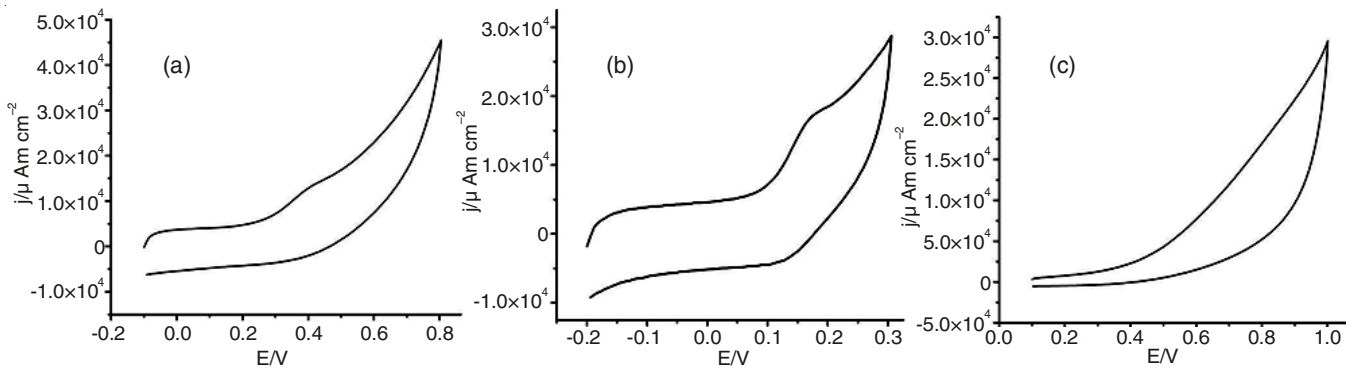

Fig. 5. Cyclic voltamogram at 0.3 V s^{-1} of *Trachyspermum ammi* (a) gallic acid (b) and ascorbic acid (c) in 0.1 M phosphate buffer (pH 7.0)

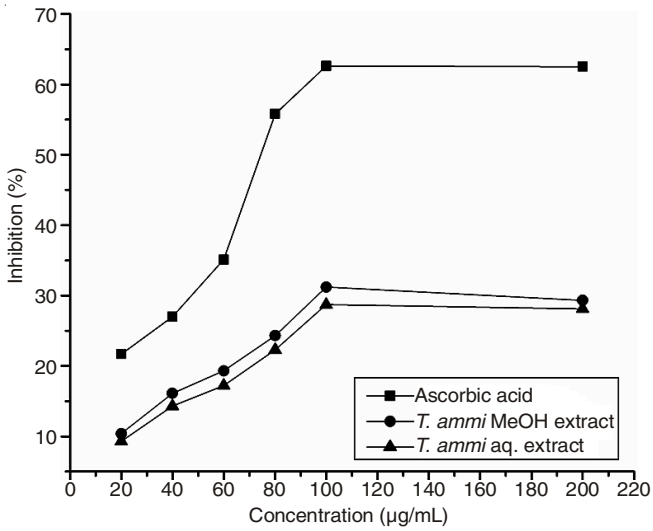

Fig. 4. Superoxide free radical scavenging of aqueous and methanolic extract of *Trachyspermum ammi*

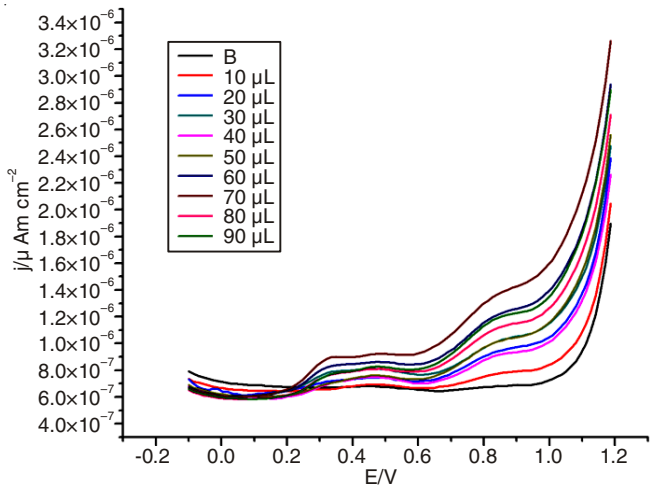

Fig. 6. DPV at 0.3 V s^{-1} of *Trachyspermum ammi* in 0.1 M phosphate buffer (pH 7.0) with different concentration

power of the compound which relatively reflected through the differential pulse voltammogram of the aqueous extract which showed the two different oxidative phase. Moreover, at high scan rates the voltammograms presented distorted shapes. This could be related with the dependence of the oxidation peak current with the logarithm of the scan rate, which must be close to 0.5 for a diffusion-controlled process. This is accomplished approximately only for few but in most cases the experimental value is greater than 0.5. These facts can be explained if adsorption processes are involved in the oxidations, causing the distortion at high scan rates and the dependences of the peak potentials towards positive values. This must also be reflected in the dependence of the voltammograms with the reactant concentration.

Conclusion

This study clearly indicates that both extracts of *T. ammi*, are good source of natural phytochemicals, having a considerable antioxidant potentiality. In particular, spectrophotometrical analysis identified these extracts have potent antioxidant activity, achieved by scavenging abilities observed against DPPH, Superoxide and iron chelating with a dose-dependent manner for *in vitro* system. Further the electrochemical behaviour supports the antioxidant activities of these extracts. The methanolic extract is more potential than the aqueous extract in term of the percentage of inhibition in antioxidant assay and iron chelation assay. Overall both extracts of *Trachyspermum ammi* have antioxidant potentiality which one can be gained by the regular use of this spice.

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