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Short Title: Determination of antioxidant activity of some commercial plant essential oils in Saudi Arabia markets

RESEARCH ARTICLE



## Determination of antioxidant activity of some commercial plant essential oils in Saudi Arabia markets

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### Abstract

Commercial plant essential oils have been widely studied for antioxidant activities as substitutes for artificial preservatives in food, pharmaceutical, and cosmetic industries. The antioxidant properties of the commercial essential oils sourced in Saudi Arabia (specifically Cinnamon (Cin) (*Cinnamomum verum*), Fennel (Fen) (*Foeniculum vulgare* L.), Thymus (Thy) (*Thymus camphoratus*), and Pomegranate (Pom) (*Punica granatum*)) were examined in the present study. These oils were extracted in vitro utilizing ethanol, hexane, and water in spectrophotometrically analysed methods to evaluate TLAC and TWAC. Out of all solvents, ethanol-extracted Cinnamon oil has the highest antioxidant activity while the Thy, Fen, and Pom oils have lower levels across all solvents. These findings reinforce the ability to determine the effective doses of natural preservatives and functional ingredients that exhibit high antioxidant activity and demonstrate that efficacy is a function of the compound concentration and specificity to each oil. The findings underscore the significance for future research to clarify the role of essential oils beyond their food components in various health-related applications due to their antioxidant activity.

**Keywords:** Antioxidant, Essential oils, Cinnamon, Fennel, Pomegranate, Thymus, Phenolic compounds, Free radical scavenging, Natural antioxidants, Plant extracts

### Introduction

Essential or volatile oils (e.g. aromatic liquids) are oils which are concentrated and aromatic liquids containing a variety of volatile compounds. These fragrant oils come from plants based on leaves, flowers, buds, seeds, roots and pericarps. They are rich sources of a plethora of bioactive agents present that exhibit antioxidative and antimicrobial properties (Cassel and Vargas 2006, Di Leo Lira, et al. 2009). The composition varies from plant to plant, especially aromatic, leading to different colours and smells depending on their constituents. The yield of these oils is also significantly variable with various plant species, thus affecting the market rates based on the supply of these oils (Bousbia, et al. 2009). Some bioactive compounds such as cinnamaldehyde in cinnamon and carvacrol in

thyme oil have been reported to possess potential antimicrobial activity against foodborne pathogens based on their natural activities (Llana-Ruiz-Cabello, et al. 2015). Well characterized ingredients in these oils may potentially be used as natural replacements for food safety and shelf life in preserving processes (Mith, et al. 2014). In these essentials are cinnamon, thyme, clove, sage, rosemary, and vanillin with established efficacy against bacterial strains.

Because of their strong antioxidant capacities that minimize the risks of diseases, play the part in reducing CVD, cancer development, inflammation, and other adverse conditions from harmful free radicals generated by cellular reactions (Kumar, et al. 2019). A wide range of health benefits from natural plants are available. This work aims to study how these compounds can act upon the antioxidant properties of these oils as chemical neutralizing factors to counteract the formation of cellular forms of harmful free radicals and lead to protective effect against CVD risk to mitigate these harmful free radical formation in humans (Kumar, et al. 2019). According to study by Smail, et al. 2011, Imane, et al. 2020 identified phenolic compounds present in certain extracts of plants as powerful antioxidant compounds.

The study of Khalil and Ahmed, 2024 on the physical and chemical properties of clove thyme marjoram antioxidants through gas chromatography/mass spectrometry proved advantageous. Free radical scavenging capabilities and reduction capacities were identified in three major components under study. The extracts of citrus cultivars produced from another study by Yang and Park, 2004 showed that they had better antioxidant activity than the other varieties. Chronic diseases can largely blame oxidative damages in lipids, proteins, nucleic acids caused by free radicals' actions, as many findings have shown (Langseth, 2000). Among the many studies, a common goal have been to analyze the beneficial effects of dietary antioxidants. Spices herbs are identified as important natural sources of antibacterial and antioxidant compounds; their efficacy is largely ascribed to their polar phenolic compounds and essential oils (Santoyo, et al. 2006, Elmastas, et al. 2006, Pillai and Ramaswamy 2011). In this study, it was aimed to evaluate potential antibacterial antioxidative effects of selected commercial plant essential oils.

## Materials and Methods

### Sample preparation

**Commercial plants:** The essential oils of four medicinal plants were Cinnamon (Cin) (*Cinnamomum verum*) from the Lauraceae family, Fennel (*Foeniculum vulgare* L.) from the Apiaceae family, Thymus (*Thymus camphoratus*) from the Lamiaceae family, and Pomegranate (*Punica granatum*) from the Lythraceae family sourced from herbal product retailers in Jeddah, Saudi Arabia.

### Determination of antioxidant activity

Preparation of ethanol, hexane and water utilized stock solutions consisting of  $\alpha$ -tocopherol and L-ascorbic acid and evaluated spectrophotometrically at 294 nm, in accordance with the method described by Windholz, 1976. Essential oil extracts were stored at temperatures around 4°C prior to adding either hexane, ethanol, or water at ratios of approximately two milliliters per gram homogenizing suspensions, then transferred into glass tubes where they were shaken for 1 hour under dark conditions at low temperatures, and centrifuged at high speed, allowing for supernatants to be evaluated following Alshehri, et al. 2019 research methodology first developed.

### TLAC determination

The total antioxidant capacity of plant extracts of the essential oils was measured spectrophotometrically as reported by Prieto, et al. 1999 and is based on the formation of blue-green phosphomolybdenum complexes. Total lipid-soluble antioxidants of the hexanic extract were tested by mixing 5–200  $\mu$ L of hexanic extract with 1 mL of phosphomolybdenum reagent (32 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid). They stirred the mixture and incubated it at 95°C (> 90 minutes). First, researchers used absolute ethanol to perform control reactions, followed by absorbance measurements at 695 nm. They expressed TLAC as a function of  $\alpha$ -tocopherol concentration and created standard curves (A<sub>695</sub> vs.  $\mu$ M  $\alpha$ -tocopherol) from various concentrations in ethanol. They quantified average extinction coefficient ( $\epsilon$ ) of 137  $\mu$ M<sup>-1</sup> ( $r^2=0.9998$ ). Lastly, TLAC was determined per gram of plant material in terms of the following equation:

$$\text{TLAC } (\mu\text{mol } \alpha\text{-tocopherol/g}) = A_{695} \times \epsilon^{-1} \times RV \times SV^{-1} \times EV \times m^{-1}.$$

A<sub>695</sub>=Absorbance at 695 nm;  $\epsilon^{-1}$ =Absolute inverse extinction coefficient (137  $\mu$ M<sup>-1</sup>); RV=Overall reaction volume; SV=Sample volume used in the reaction; EV=Solvent volume; and m=Amount (grams) of fresh plant material extracted.

Using TLAC<sub>37</sub> (37°C) rather than 95°C yielded a greater overall antioxidant capacity resulting from potent lipid-soluble antioxidants (Prieto et al., 1999). All measurements were taken in triplicate.

### TWAC determination

Water extracts (5–200  $\mu$ L) were added to 1 mL of phosphomolybdenum reagent, the mixture was agitated and incubated at 95°C for 90 minutes. As a control for the experiments, the researchers utilized pure water and measured the absorbance at 695 nm. TWAC was expressed as the equivalent of L-ascorbic acid. Standard curves (A<sub>695</sub> vs.  $\mu$ M L-ascorbic acid) were created by dissolving L-ascorbic

acid at various concentrations in water, and the extinction coefficient was determined as  $213 \mu\text{M}^{-1}$  ( $r^2=0.9996$ ). The TWAC per gram of fresh plant material was calculated with the formula:

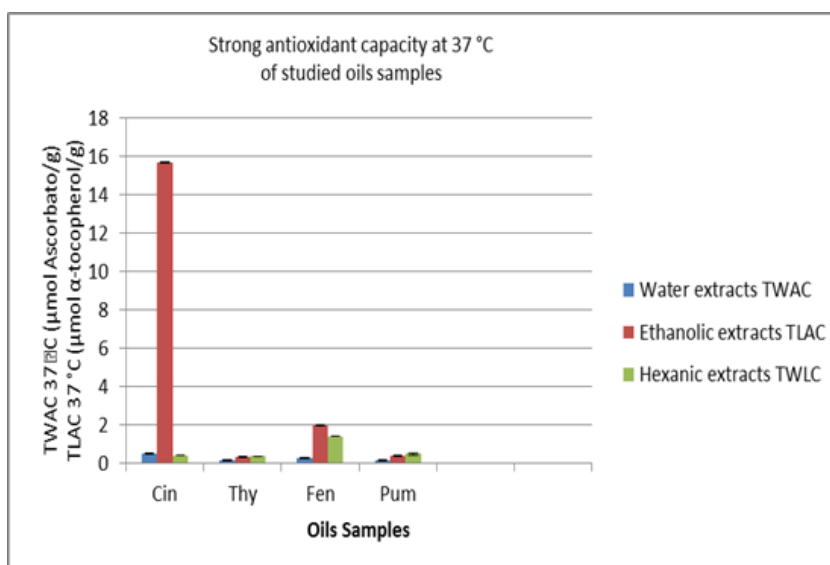
$$\text{TWAC } (\mu\text{mol L-ascorbic acid/g}) = A_{695} \times E^{-1} \times RV \times SV^{-1} \times EV \times m^{-1}.$$

For our tests at  $37^\circ\text{C}$  (TWAC 37) and not  $95^\circ\text{C}$ , we measured total antioxidant capacity, as these are high water-soluble antioxidants (Prieto, et al. 1999). All were made in triplicate.

## Results and Discussion

The antioxidant potential of extract of cinnamon (*Cinnamomum verum*), fennel (*Foeniculum vulgare* L.) and pomegranate (*Punica granatum*) depended heavily on the solvent used (ethanol, hexane, or water); polar solvents tended to be the more active solvent since phenolic compounds and flavonoids can be extracted more efficiently using solvent. Some extracts exhibited the strongest antioxidant activity by aqueous extracts while other extracts were less effective, it could be attributed to the low solubility of polar antioxidants. The conclusions highlight the significance of solvent selection in enhancing antioxidant extraction in functional foods, pharmaceuticals, and natural preservatives.

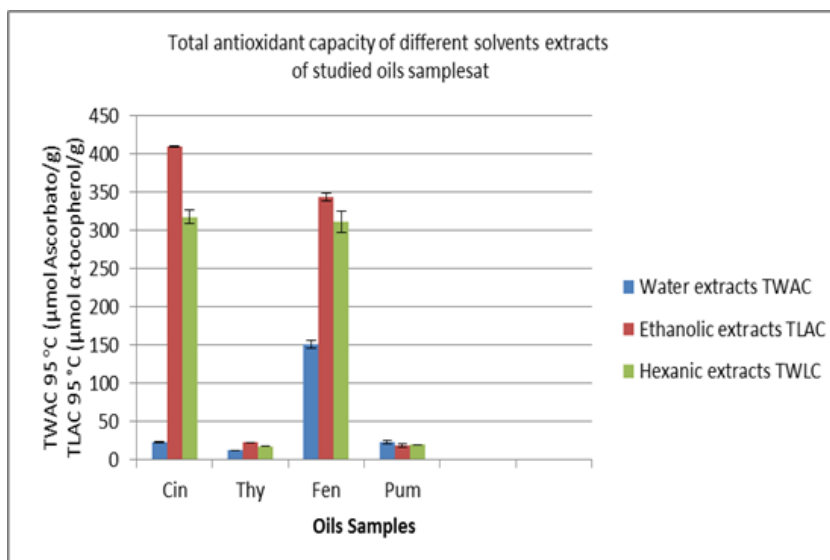
The analysis addressed both strong water-soluble and lipid-soluble antioxidants. Figures include the TWAC<sub>37</sub> and TLAC<sub>37</sub> values associated with strong water-soluble antioxidants and strong lipid-soluble antioxidants of type ascorbic acid and  $\alpha$ -tocopherol. As shown in Fig. 1, extracts from the essential oils of chosen herbs exhibited mixed potent antioxidant activity in TWAC<sub>37</sub> and TLAC<sub>37</sub>. The activity of cinnamon oil isolated from ethanol was the highest in terms of antioxidants. This aligns with some previous research indicating that cinnamon improves antioxidant action (Shan, et al. 2005, Rousse, et al. 2009, Noorolahi, et al. 2012). Thyme, fennel, and pumpkin oils showed significantly less antioxidant activities for the three solvents evaluated (water, ethanol, and hexane), contrasting with previous researches (Mohamed, et al. 2007). The discrepancy can be attributed to the decreased concentration of potent antioxidant chemicals in some products as a result of the extraction procedures of specific herbalists using essential oils. Consequently, aqueous extracts showed reduced antioxidant activity for all the commercial essential oils screened, indicating that water is a poor choice as solvent in extracting antioxidant compounds from it.



**Figure 1.** Analysis of the antioxidant capacity in oil extracts: Data represent means of three independent determinations  $\pm$  SD and concentrations are relative to their weights. Expressed TWAC<sub>37</sub> are equivalent to L-ascorbic acid ( $\mu\text{m/gm}$ ) and TLAC<sub>37</sub> to  $\alpha$ -tocopherol ( $\mu\text{m/gm}$ ).

### Total water-soluble and lipid soluble antioxidants capacity

Results of TWAC and TLAC of the four extracts using multiple solvents, including water, ethanol, and hexane, are presented in Fig. 2. The Cin and Fen commercial oils exhibited high total antioxidant activity, notably when first extracted with ethanol, then with hexane (Shan, et al. 2005, Rousse, et al. 2009, Noorolahi, et al. 2012). Results showed extracts with water remain inconsistent with previous data on the plant material (Sacchetti, et al. 2005, Singh, et al. 2007, Mohamed, et al. 2007). Ethanol would be the most suitable solvent for the extraction and Thy, Pum showed low antioxidant activity on comparison with Cin and Fen.



**Figure 2.** Comparative determination of antioxidant capacity of various extracts: Data represented as means  $\pm$  SD and concentrations are relative to their weights. Expressed TWAC are equivalent to L-ascorbic acid ( $\mu\text{m}$  per gram) and TLAC to  $\alpha$ -tocopherol ( $\mu$  per gram).

## Conclusions

The antioxidant potential of extract of cinnamon (*Cinnamomum verum*), fennel (*Foeniculum vulgare* L.) and pomegranate (*Punica granatum*) depended heavily on the solvent used (ethanol, hexane, or water); polar solvents tended to be the more active solvent since phenolic compounds and flavonoids can be extracted more efficiently using solvent. Some extracts exhibited the strongest antioxidant activity by aqueous extracts while other extracts were less effective, it could be attributed to the low solubility of polar antioxidants. The conclusions highlight the significance of solvent selection in enhancing antioxidant extraction in functional foods, pharmaceuticals, and natural preservatives.

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