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ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OF *TRACHYSPERMUM AMMI* (L.) SPRAGUE AND *OCIMUM BASILICUM* L. AGAINST *ACIDOVORAX* SP.

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ABSTRACT

Antibacterial activity of essential oils of fruit of ajwain [*Trachyspermum ammi* (L.) Sprague] and leaves and inflorescence of basil (*Ocimum basilicum* L.) was studied against *Acidovorax* sp. using disk diffusion method. Two antibiotics namely streptomycin and kanamycin were used as reference compounds. Efficacy of the two antibiotics and the essential oils was assessed in terms of diameters of inhibition zones formed against the growth of the target bacterial species. Different concentrations of streptomycin (75, 150, 225, 300 µg 5µL^{-1}) and kanamycin (62.5, 125, 187.5, 250 µg 5µL^{-1}) formed inhibition zones of 12–22 mm and 16–17 mm, respectively. Efficacy of different doses (1.25, 2.5, 3.75, 5.0 µL) of essential oils of *T. ammi* fruit was better than either of the two antibiotics with inhibition zones of 21–29 mm. Likewise, inhibition zones formed by the same doses of essential oils of leaves and flowers of *O. basilicum* were 7.4–10 mm and 7–9.3 mm, respectively. The present study concludes that *T. ammi* and *O. basilicum* contain essential oils with potent antibacterial activity.

Keywords: *Acidovorax* sp., antibacterial activity, essential oils, *Ocimum basilicum, Trachyspermum ammi.*

INTRODUCTION

Essential oils are hydrophobic liquids containing volatile aroma compounds extracted from plants generally by distillation, mostly by using steam. It has been reported that many essential antimicrobial compounds like thymol, carvacrol, linalool, and eugenol are the integeral constituents of some plants' essential oils (Ouibrahim *et al*., 2013), that impart focal access in pharmaceutical, food and cosmetics industries. Besides, with increasing legislation on utlization of chemical preservatives and methods to combat phytopathogen, there has been increasing interest amongst the scientists to explore the antimicrobial potential of plant essential oils against the destructive plant pathogens (Uniyal *et al*., 2012). Whereas, it has been recommended to explore antimicrobial acivity of medicinally important plants owing to enrichment of novel antimicrobial compounds (Mitscher *et al*., 1987).

Trachyspermum ammi (L.) Sprague belongs to family Apiaceae is a traditional potential medicial herb, native to Egypt, while widely cultivated in India, Pakistan, Iraq and Afghnistan. Locally, it is famous as ajwain and its seed are extensively utilized in food as valued aromatic spice (Bairwa *et al*., 2012). The seeds and fruits of ajwain yield 2-4% brown colored essential oil with thymol (40-60%) as pricipal phenolic component (Ishikawah *et al*., 2001). The oil has been utilized for treatment of gastro-intestinal ailments, atonic dyspepsia, diarrhea (Bentely and Trimen, 1999) and bronchial problems due to anti-aggregatory (Srivastava, 1988), antimicrobial (Sivropoulo *et al*., 1996) and fungicidal (Singh and Singh, 2000) effects. The essential oil of different part of plant also showed significant activity against plant parasitic nematodes (Park *et al*., 2007), insects (Seo *et al*., 2009); bacteria (Paul *et al*., 2011) and fungi (Siripornvisal *et al*., 2010).

Another popular culinary herb and important medicinal plant is *Ocimum basilicum* L. (Lamiaceae) commonly named as basil. It is native to India and cultivated in many part of the world due to several antioxidants compounds (Grayer *et al*., 1996). The essential oil extracted from different aerial plant parts contained linalool, geraniol, cineole, allylanisole and limonene (Al-Maskri *et al*., 2011), with potent antioxidant, antiviral, and antimicrobial compound and great potential in treating cancer (De Almeida *et al*., 2007). In addition, antimicrobial activity of essential oil is well-recognized against many phytopathogenic fungi (Al-Maskri *et al*., 2011) and bacteria (Moghaddam *et al*., 2011).

Amongst the phytopathgens, bacteria are well-known for spreading destructive diseases in economically important plant. *Acidovorax* is a genus of proteobacteria consisting of four species and many subspesies, that are aerobic and common inhabitant of soil, water, activated slduge and clinical environment (Fegan, 2006). Most of species are responsible for causing leaf spot and streak, seedling blight and bacterial fruit blotch in economically important plant families including Poaceae (Song *et al*., 2004) and Cucurbitaceae (Burdman and Walcott, 2012). Number of cultural and chemical options and some bio stimulus are in practice for the management of *Acidovorax* without satisfactory control (Hopkins *et al*., 1996, 2003; Fessehaie and Walcott, 2005). Few reports are available on antimicrobial action of plant essential oil against *Acidovorax* (Dehghanzadeh *et al*., 2012), while literature is scars regarding the antibacterial activity of *T. ammi* and *O. basilicum* against *Acidovorax.* The present study was, therefore, carried out to check *in vitro* antibacterial activity of essential oils of fruit of *T. ammi*, and leaves and inflorescence of *O. basilicum* against *Acidovorax* sp.

MATERIALS AND METHODS

Extraction of essential oils

The essential oils were extracted by hydro-distillation method (de Miranda *et al.*, 2014) with slight modifications. Hundred grams of each of fruit of ajwain, and leaves and inflorescence of basil were crushed in pestle and mortar. Materials were separately transferred to round bottom flask containing distilled water. Essential oils were extracted using Dean-Stark type hydro-distillation assembly. Oil layer from distilled extract was taken into a microfuge tube and centrifuged at 14000 rpm for 15 min. After centrifugation, upper essential oil layer was separated from lower aqueous layer and stored at 4 °C till further use.

Antibacterial bioassays of essential oils

Culture of a Gram negative bacterium *Acidovorax* sp. was procured from Fungal Culture Bank, University of the Punjab Lahore, Pakistan. Subculturing of the bacterium was done on Luria Bertani Agar (LBA) medium (yeast extract 2.5 g, trypton 5 g, agar 7 g, sodium chloride 2.5 g, distilled water 500 mL, pH 6.7). Disc diffusion method was used to assess the antibacterial activity of the essential oils. For comparison two antibiotics namely streptomycin (50 mg mL^{-1} stock solution) and kanamycin (60 mg mL^{-1} stock solution) were used. Different dilutions of the essential oils and the antibiotics were prepared.

Dilutions of the essential oils were:
0% = 50 µL methanol only
25% = 37.5 µL methanol + 12.5 µL oil
50% = 25 µL methanol + 25 µL oil
75% = 12.5 µL methanol + 37.5 µL oil
100% = 50 µL oil only

Dilutions of the antibiotics were:
0% = 50 µL distilled H_2O
25% = 37.5 µL H_2O + 12.5 µL antibiotics solution (60 mg mL^{-1} kanamycine or 50 mg mL^{-1} streptomycine)
50% = 25 µL H_2O + 25 µL antibiotic solution
75% = 12.5µL H_2O + 37.5 µL antibiotic solution
100% = 50 µL antibiotic solution

Filter paper discs of 6 mm were soaked in antibiotics and essential oils solutions (5 µL each, applying 2.5 µL at a time). Overnight grown culture of *Acidovorax* sp. in LB broth was inoculated (100 µL) on LBA Petri plates and spread evenly using sterile spreader. After drying the plates, dried filter paper discs (pre-soaked with essential oils or antibiotics) were placed in the center of the plates. Inoculated plates were then incubated at 37 °C for 24 h. Next day, clear zone around the discs were recorded.

Statistical analysis

All the data were analyzed by one way of analysis of variance. Treatment means were separated by Tukey's HSD Test at P0.05 using computer software Statistix 8.1.

RESULTS AND DISCUSSION

In the present study, two antibiotics viz. streptomycin and kanamycin were used as reference compounds to compare their efficacy against *Acidovorax* sp. with those of essential oils of *T. ammi* and *O. basilicum*. Both the antibiotics significantly suppressed the bacterial growth as indicated by their inhibition zones. Streptomycin was found comparatively more effective against the test bacterial species than kanamycin. Different concentrations of streptomycin formed inhibition zones of 12–22 mm. On the other hand different concentrations of kanamycin formed inhibition zones of 16–17 mm (Fig. 1A & B). The antimicrobial activity of both antibiotics could be due to impairment of the bacteria membrane integrity with the increase in osmotic stress and inhibition of protein synthesis by aminoglycoside (Whitfield and Roberts, 1999). The less antibacterial activity of kanamycin than the streptomycin has also been reported previously (Ahmed *et al*., 2002; Rehman *et al*., 2008; Lu *et al*., 2008).

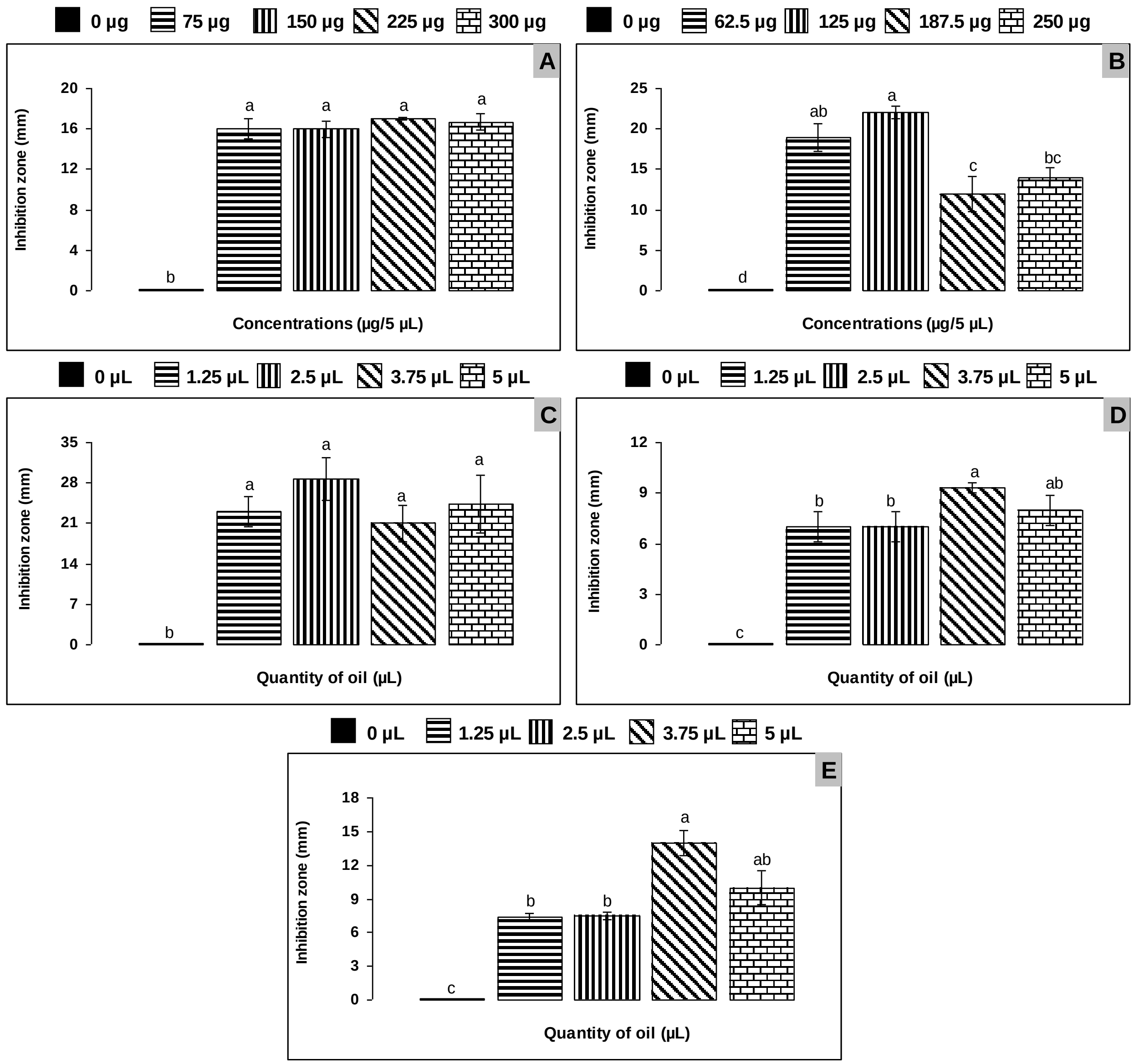

Fig. 1 (A-E). Effect of different concentrations of antibiotic Kanamycin (A), antibiotic streptomycin (B), essential oil of *Trachyspermum ammi* (C), essential oil of inflorescence of *Ocimum bacilicum* (D), and essential oil of leaves of *Ocimum bacilicum* (E) on inhibition zone against growth of *Acidovorax* sp. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's HSD Test.

Mechanisms by which specific aminoglycoside-modifying enzymes in kanamycin and streptomycin impose physiological stress and inhibit protein synthesis are slightly different (Lu *et al*., 2008), that probably consequences in different antimicrobial effect on the cells. Moreover, increasing dose of antibiotics was found to be less effective against bacteria. Induction of resistance to reduce antibiotic uptake through membrane impermeabilization and the subsequent accumulation inside the cell has been suggested with increasing dose of antibiotic (Lu *et al*., 2008).

Essential oil of *T. ammi* fruit exhibited pronounced antibacterial activity. Diameter of inhibition zone formed by different oil concentrations was 21–29 mm that was markedly higher than the diameter of inhibition zones formed by either of the two reference antibiotics (Fig. 1C). Essential oils of both leaves and flowers of *O. basilicum* showed significant antibacterial activity against the test bacterium species as compared to control. Essential oil of leaves showed comparatively better antibacterial activity than essential oil of inflorescence. Inhibition zones formed by different doses of essential oils of leaves and inflorescence were 7.4–10 mm and 7–9.3 mm, respectively (Fig. 1D & E). Greater antibacterial activity of plants essential oil could be due to hydrophobicity nature of oil components, which enable them to partition lipids of the bacterial cell membrane and mitochondria. While partitioning increases permeability from bacterial cell that could result in leakage of ions and other cell contents (Lambert *et al*., 2001) with or without loss of cell viability, depending on exit ratio of critical molecules and ions (Denyer and Hugo, 1991). The greater antibacterial activity of essential oils of *T. ammi* fruit than both leaves and inflorescence of *O. basilicum* could be due to difference in growth stages of plants that could affect composition and extraction contents of the final essential oil. It has been documented that in family Lamiaceae, the day length increases the content of their extractions, and the duration of day light augments the quality of their active materials (Moghaddam *et al.*, 2011). Such physiological difference with different chemical structure of the individual essential oil components affects their precise mode of action and antibacterial activity (Dorman and Deans, 2000). Generally, the strongest antibacterial activity of essential oil is correlated with a high percentage of phenolic compounds such as carvacrol, eugenol and thymol (Lambert *et al*., 2001). It seems that potential phenolic components in essential oil of *T. ammi* fruit would be more than in both leaves and flowers of *O. basilicum*, therefore, former showed more inhibitory action against the tested bacterial species. Antibacterial action of phenolic compounds is reported to act by disturbing cytoplasmic membrane, electron flow, active transport, proton motive force and coagulation of cell contents (Skkema *et al*., 1995). Different components of essential oil also act differently on cell proteins embedded in the cytoplasmic membrane (Bajpai *et al*., 2011). This study concludes that essential oils of *T. ammi* fruit are highly effective in controlling growth of *Acidovorax* sp.

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