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Evaluation of Antimicrobial Activity of Trachyspermum ammi (Ajwain) through Disc Diffusion Method

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ABSTRACT

The study was on antimicrobial activity of Trachyspermum ammi through disc diffusion method. The disc diffusion method is a commonly used technique to assess the antimicrobial activity of plant extracts by observing the zone of inhibition around a paper disc impregnated with the extract. This method relies on the diffusion of the extract's antimicrobial compounds from the disk into an agar medium seeded with the test microorganism, leading to a clear zone where bacterial or fungal growth is inhibited if the extract is effective. All extract of Trachyspermum ammi and control solution as DMSO inhibited the production of OH-radicals. The % of free radical scavenging activity of hydro-methanolic extract of Trachyspermum ammi presented to Acetobacter, Citrobacter, E. coli, B. subtilis, S. aureus, Enterococcus has reduced power, the free radical OH- scavenging activity of the extract increases with increasing the concentration.

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Parekh and Chanda, 2007). Thousands of plant secondary metabolites have been identified and it is estimated that other thousands are yet to be discovered. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more "drug – likeness and biological friendliness than totally synthetic molecules" (Koehn and Carter, 2005), making them good candidates for further drug development.

Trachyspermum ammi (L.) also known as ajwain (Bairwa et al., 2012), is a highly reputable plant as a source of constituents with promising bioactivity to be exploited at pharmaceutical level. *T. ammi* belongs to the Apiaceae family and is an annual herb up to 90 cm tall, native to arid and semi-arid regions of Egypt (Ashraf, 2002); it is also widely distributed and cultivated in India, Iraq, Iran, Afghanistan, and Pakistan. The plant is a highly valued medicinally seed spice. The fruits, having a bitter and pungent taste, are used to flavour and preserve foods, in perfumery for the manufacture of essential oil and in medicine (Pruthi, 1992). In the area of origin of the plant, the fruits are believed to exert aphrodisiac effects. They possess stimulant, antispasmodic and carminative properties, and are used traditionally in the treatment of flatulence, atonic dyspepsia, diarrhoea, abdominal tumours, abdominal pains, piles, bronchial problems, lack of appetite, asthma and amenorrhoea (Bairwa et al., 2012). The fruits contain 2–5% of a brown coloured essential oil, responsible for plant odour and taste, known as 'ajwain oil'. It is used in the treatment of gastrointestinal ailments, lack of appetite, and bronchial problems (Bairwa et al., 2012). The main component of this oil is

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thymol (35–60%), which is a strong germicide, antispasmodic and fungicide agent. The non-thymol fraction contains p-cymene, γ-terpinene, α-pinene, β-pinene, and other minor components (Zarshenas et al., 2014). However, sometimes γ-terpinene and p-cymene exceed the thymol content (Omer et al., 2014, Moein et al., 2015), and in other cases thymol and p-cymene are not among the predominant components (Singh et al., 2008).The ajwain essential oil exhibited nematocidal (Park et al., 2007), sporicidal (Moazeni et al., 2012), antitermitic (Seo et al., 2009), antibacterial (Kumar et al., 2011, Paul et al., 2011, Moein et al., 2015), antifungal (Ashrafi Tamai et al., 2013, Moein et al., 2015, Kedia et al., 2015), and antioxidant (Chatterjee et al., 2013, Gandomi et al., 2014) effects. Interestingly, the oil showed appreciable spermicidal potential, which may be explored as an effective ingredient of male contraceptives (Paul and Kang, 2011, Paul and Kang, 2012). Ajwain oil also showed vapour toxicant and repellent effects against adults of *Anopheles stephensi* (Pandey et al., 2009), as well as larvicidal activity against *Aedes aegypti* (Seo et al., 2012), thus having promising applications in the management of malaria and yellow fever.

In the present work, we have evaluated the in vitro biological effects of ajwain oil, namely the antimicrobial and antioxidant activities, cytotoxicity on human tumour cells, and the induction of lymphocyte proliferation. These activities were evaluated by agar disc-diffusion, microdilution, DPPH, ABTS, FRAP, and MTT methods, and in vitro peripheral blood mononuclear cells (PBMC) proliferation assay, respectively. To complete the work, we have evaluated the inhibitory effects of ajwain oil on nicotinate mononucleotide adenylyl transferase (NadD), which is a promising new target for developing novel antibiotics. Therefore, we have plants to carry out evaluation and antimicrobial effect.

MATERIALS AND METHODS

Collection of *Trachyspermum ammi*

The experimental plant species *Trachyspermum ammi* was purchased from the local herbal market. The plant was authenticated and the voucher specimen was deposited in the herbarium of the central laboratory facility, Chhattisgarh council of Science and Technology, Raipur.

Preparation of Plant Powder

Fresh *Trachyspermum* plants were washed thoroughly in tap water followed by distilled water and were then shade dried until all the water content was lost completely. Dried plants were crushed and powdered using a blender. Fine powder was obtained after sieving and stored in an airtight container until further use.

Preparation of Experimental Plant Extracts

The plant powder was extracted with methanol solvent with an increasing polarity. The successive extraction was done by cold maceration process for seven days with regular agitation. After a seven-day old cold maceration process it was filtered through sterile muslin cloth and the solvent was evaporated using a Soxhlet apparatus. The residues obtained after evaporation were stored at -20°C until used for experimentation.

Test Microorganisms

To evaluate the antimicrobial activity of Trachyspermum ammi extracts, six species/strains of microorganisms were selected, namely *Acetobacter, Citrobacter, E. coli, Bacillus subtilis, S. aureus, Enterococcus.* All these bacterial strains were collected from the clinical lab and subculture in nutrient agar medium and used for antimicrobial susceptibility tests.

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Antimicrobial Assay

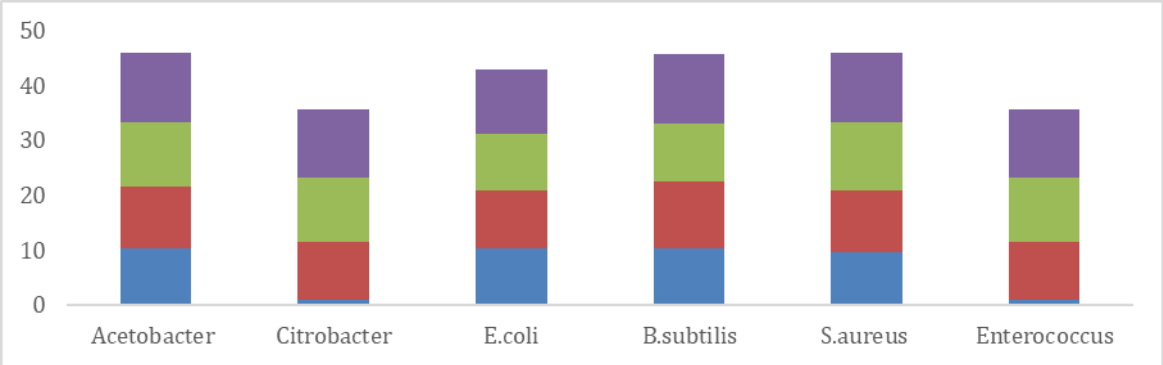
The potential antimicrobial activity of Trachyspermum ammi extract was studied through well diffusion method (Murray, et. al.,1995). The sterile petri dishes water filled with 25 ml of agar and allowed the agar to get solidified. Prior to streaking the plates with bacterial culture, 5mm diameter wells were punched in the medium using a sterile borer. After the agar gets solidified the bacterial culture were inoculated by spreading in the petri plates using sterile cotton swabs. Then 0.1ml of plant extract in peptone water was directly applied to the well made on the surface of agar containing bacterial lawn. Positive control was maintained with different antibiotics and Wells containing solvent alone was maintained as negative control. The inoculated plates were incubated overnight at 37° C for allowing the bacterial growth and the diameter of zone of inhibition was measured in mm.

RESULTS

The result of the effect of the examined Trachyspermum ammi extract as well as control solution on OH- radical production. They show that all extract of Trachyspermum ammi and control solution as DMSO inhibited the production of OH-radicals. The % of free radical scavenging activity of hydro-methanolic extract of Trachyspermum ammi presented in Table 1 have reduced power, the free radical OH- scavenging activity of the extract increases with increasing the concentration.

Table 1: The study of antibacterial activities of Trachyspermum ammi extracts using disc diffusion method (Mean + SE)

S1	Bacterial strain	Bacterial use	Zone of inhibition(In MM)			
			25%	50%	70%	100%
1	Gram negative (-)	*Acetobacter*	10.33±0.56	11.33±0.56	11.66±1.15	12.66±0.56
		Citrobacter	0.966±0.58	10.66±0.56	11.66±0.57	12.33±0.56
		E.coli	10.33±0.56	10.66±0.56	10.33±0.56	11.66±0.57
2	Gram positive (+)	*B.subtilis*	10.33±0.56	12.33±0.56	10.33±0.56	12.66±0.56
		S.aureus	09.66±0.58	11.33±0.56	12.33±0.56	12.66±0.56
		Enterococcus	0.966±0.58	10.66±0.56	11.66±0.57	12.33±0.56



Graph shows the study of antibacterial activities of Trachyspermum ammi extracts

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DISCUSSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners from one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc. It has been known that superoxide dismutase SOD is a Metallo protein that is involved in the antioxidant defines mechanism, which plays an important role in the protection of cells against reactive oxygen system (ROS) by lowering the steady state of superoxide anions. Also, SOD converted superoxide radical to hydrogen peroxide and molecular oxygen which in turn can be counteracted by catalase or glutathione peroxidase reaction thereby reducing the level of cellular damage (Loliger, 1991).

Our results of the effects of the examined Trachyspermum ammi extract as well as control solutions on OH- radical production. They show that all extract of Trachyspermum ammi and control solutions as a DMSO inhibited the production of OH- radicals. The % of free racial scavenging activity of hydromethanolic extract of Trachyspermum ammi presented in Table 1 have reducing power, the free radical OH- scavenging activity of the extract increases with increasing the concentration. The discovery, development and clinical use of antibacterial during the 20th century have substantially reduced mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitro-glycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered and developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. Parallel to this there has been an alarming increase in resistance of bacteria, fungi, viruses and parasites to multiple existing agents (Tanwar et. al., 2014). Another set of experiment antimicrobial growth was considered as zero hour and further accordingly reading was taken.

Our present study shows that antibacterial activity of 50% methanolic extract of *Trachyspermum ammi* against *Citrobacter* is best in 100% concentration after 12 hrs. (12.33 mm zone of inhibition), Although 75% concentration is having mild effect as 11.66 mm zone of inhibition. In *S. aureus* 100% concentration of extract is having good antibacterial activity at maximum zone of inhibition 12.66 mm. On the other hand, 75% is showing static activity from, with a zone of inhibition of 12.33 mm. For E. coli 100% concentration of extract shows a maximum zone of inhibition 11.66 mm. Although the same effect of 75% concentration of extract is also revealing as showing a zone of inhibition 10.33 mm. In the case of *Acetobacter* 75% and 100% concentration of extract show good activity with zones of inhibition of 12.66 mm and 11.66 mm respectively.

The above observations suggest that different concentrations (50%, 75% & 100%) were having good antibacterial activity against *Acetobacter, Citrobacter, E. coli, B. subtilis, S. aureus* and *Enterococcus*. Thus, the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. But extract is not as potent as erythromycin and amoxicillin in these conditions. This work provides an insight to understanding some molecular basis of therapeutic properties of *Trachyspermum ammi* in traditional medicine. Furthermore, detailed studies on the isolation and characterization of the

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plant extract as well as in vivo assays will be necessary in discovering new biological antioxidants and low cast antibacterial drugs.

CONCLUSION

According to the teachings of Ayurveda, every human being has four biological and spiritual instincts: religious, financial, pro-creative and the instinct toward freedom. Balanced good health is the foundation for the fulfilment of these instincts. Ayurveda helps the healthy person to maintain health, and the diseased person to regain health. It is a medical-metaphysical healing life-science, the mother of all healing arts.

The practice of Ayurveda is designed to promote human happiness, health and creative growth. Through studying the teachings of Ayurveda, the practical knowledge of self-healing may be acquired by anyone. By the proper balance of all energies in the body, the processes of physical deterioration and disease can be impressively reduced. This concept is basic to Ayurvedic science: the capability of the individual for self-healing. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause.

Plant produces various anti-oxidative compounds to counteract reactive oxygen species (ROS) in order to survive. ROS, which include free radicals such as superoxide anion radicals (02-), hydroxyl radicals (OH.) and non-free-radical species such as H2O2 and singled oxygen, are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and the aging process. In foods, ROS can cause lipid per-oxidation, which leads to the deterioration of the food. Our results of the effects of the examined *Trachyspermum ammi* extract as well as control solutions on OH- radical production. They show that all extract of *Trachyspermum ammi* and control solutions as a DMSO inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Trachyspermum ammi* presented in Table have reducing power, the free radical OH- scavenging activity of the extract increases with increasing the concentration. Our observations for antimicrobial suggest that different concentration (50%, 75% & 100%) were having good antibacterial activity against *Acetobacter, Citrobacter, E. coli, B. subtilis, S. aureus* and *Enterococcus*. Thus, the extract is showing varying activity against all microorganisms.

On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. But extract is not potent as erythromycin and amoxicillin in these conditions. Experimental data revealed that there might be correlation between total phenolic and antioxidant capacity of different extracts of lemon grass. However, some literature demonstrated that antioxidants were not solely dependent on phenolic content but it may be due to other phytoconstituents such as tannins, triterpenoid or combine effect of them. Another set of experiments, the antibacterial study was also done which shows a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and shown antibacterial susceptibility to lemongrass plant extracts with clear zone of inhibition.

So, in future it can be used as an alternative to synthetic antioxidants and antibiotics. Much effort has needed to increase lemongrass plant as a dietary supplement in food so as to

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acquire antioxidant potential in our body naturally to fight against oxidative stress and other harm generated by free radicals and to resist human pathogenic bacterial disease.

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