Journal of Medicinal Plants Research Vol. 6(5), pp. 768-775, 9 February, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1341
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Nutritional, phytochemical potential and pharmacological evaluation of *Nigella Sativa* (Kalonji) and *Trachyspermum Ammi* (Ajwain)

Shabnam Javed[1]*, Ahmad Ali Shahid[1], Muhammad Saleem Haider[1], Aysha Umeera[1], Rauf Ahmad[2] and Sobia Mushtaq[1]

[1]Institute of Agricultural Sciences, University of the Punjab, Quaid-e- Azam Campus, Lahore-54590 Lahore. Pakistan.
[2]Pakistan Council of Scientific and Industrial Research, Lahore, Pakistan.

Accepted 14 December, 2011

Nigella sativa **and** ***Trachyspermum ammi*** **were screened for phytochemical constituents and nutritional analysis. Tests for flavonoids, tannins, steroids were positive in both spices except saponins. The nutrition analysis indicated higher energy value (459.29 and 314.55%), carbohydrates (39.04 and 47.54%), protein content (24.05 and 20.23%), fat content (21.67 and 4.83%), moisture content (5.4 and 11.6%), fibre content (5.5 and 4.3%) and ash content (4.34 and 11.5%) in** ***N. sativa*** **and** ***T. ammi*** **seeds, respectively. Aqueous, methanolic, ethanolic, n-Hexane extracts of** ***N. sativa*** **and** ***T. ammi*** **and also essential oil of** ***T. ammi*** **were subjected to** ***in vitro*** **antifungal and antibacterial assay. Micro well dilution assay was adopted against three human pathogenic fungal strains** ***Aspergillus flavus, Aspergillus niger, Candida albicans*** **and four bacterial isolates** ***Escherichia coli, Staphylococcus sp., Pseudomonas syringae and Bacillus subtilis.*** **Among all the four extracts of** ***N. sativa*** **tested, methanolic extract showed maximum inhibitory potential against all the test fungi and bacteria. In case of** ***T. ammi*** **extracts and essential oil, all the aqueous, organic extracts especially ethanol, n-hexane and essential oil depicted marked antimicrobial potential against all pathogens. Anti bacterial activity of n-Hexane extract was maximum for all bacteria (4.76 cm)** ***P. syringica*****, (4.03 cm)** ***B. subtilis*****, (3.73 cm)** ***E. coli*** **and (4.76 cm)** ***S. sp.*** **Bactericidal action of different fractions of** ***T. ammi*** **decrease in the order n-Hexane extract>essential oil>ethanol extract>methanol extract>aqueous extract.**

Key words: *Nigella sativa*, *Trachyspermum ammi*, phytochemical analysis, nutrition value, antifungal/antibacterial.

INTRODUCTION

Spices have valued for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age suppressed their importance. In the present time herbal products are considered as safe alternatives of synthetic drugs that are regarded as unsafe to human and environment. However in the recent past increasing research evidence is getting accumulated, which clearly indicate the positive role of spices and plant extracts for health care. The use of plant drugs is increased in many of the developing countries because modern lives saving drugs are beyond the reach of three quarters of the third world's population. Many of such developing countries spend 40 to 50% of their total wealth on drugs and health care reference. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. *Nigella sativa* (Klonji) is a valuable spice, having distinctive aroma and taste; its seeds have been used in pickles, bread recepies and savoury dishes. *N. sativa* is regarded as a valuable remedy for various ailments, the seeds, oil and extracts have played an important role over the years in ancient Islamic system of herbal medicine. Bukhari reported that Holy Prophet Muhammad (peace be upon him) told" There exists, in the black grains, health care of all the diseases, except death (Gheznavi, 1988). The dried

*Corresponding author. E- mail: shabnamjaved@yahoo.com.

seeds are used as astringent, bitter, diuretic, emmemagogue; stimulant and anthelmintic. Its decoction is useful in paralysis, jaundice, intermittent fever, dyspepsia, piles and skin diseases (Paarakh, 2010; Ali et al., 2003; Gilani et al., 2001; Hosseinzadeh et al., 2007). *N. sativa* seeds are reported to contain numerous chemical compounds. Active ingredients are nigellone, beta-sisterol, and thymoquinone. Other compounds include 2-methyl-4-isopropyl-p-quinones, anthraquinones, saponins, glycosoides, melanthin, essential oil, fixed oil, tannins, protein, mucilage resins and glucose, etc (Saeed, 1969; Ikram and Hussain, 1978; Ghaznavi, 1988; Akhtar, 1988). Thymoquinone improves respiration, decrease serum levels of glucose, cholesterol and reduce blood pressure (ElTahir et al., 1993; Al-Shabanah et al., 1998; ElTahir et al., 1993). It has antioxidant, analgesic and anti-inflammatory effects (Ali and Blunden, 2003; Al-Gharably et al., 1997; Nagi et al., 1999). *N. sativa* has shown anti yeast activity against *C. albicans*, when used at 400 µg/disc or acid-ethyl alcohols at 0.20 ml/disc on agar plate. The dried seeds of *N. sativa* exhibited bactericidal activity against *Pseudomonas aeruginosa* (Hanafy and Hatem, 1991). Its hexane extract, water extract, ether extract and extract in 95% ethyl alcohol give weak activity against *Streptococcus pyogenes, S. aureus* and *Streptococcus viridians* (Morsi, 2000). Ajwain, *Trachyspermum ammi* (L.) used as a spice is a traditional herb widely used for curing various diseases in both humans and animals. Most utilizing part of Ajwain is its caraway like fruit, which is popular in cooking recipes, snacks, savory pastries and as spice. Aroma chemicals present in Ajwain; inhibit other undesirable changes in food affecting its nutritional quality, texture and flavor. Decoction of Ajwain seeds is used for treatments of abdominal discomfort, diarrhea, cough and stomach troubles (Anikumar et al., 2009).

Fruit of Ajwain is reported to have antiseptic, antifungal/ antibacterial and antithelminitic effects (Morsi, 2000). In Ajwain, the major phenolic compound Thymol is present and has been reported to be an antispasmodic, germicide and antifungal agent (Nagalakshmi et al., 2000). In the essential oil of *T. ammi*, the principle active constituents of the oil are phenols, mainly thymol (35 to 60%) and some carvacrol (Tsimidou and Boskou, 1994). Both the phenols Thymol and carvacrol are responsible for the antiseptic, antitussive and expectorant properties (Treas and Evans, 2002). Thymol also has antiseptic activity and carvacrol possesses antifungal properties (Menphini et al., 1995). The essential oil and chloroform, n- Hexane, ethyl acetate and methanol extracts of *T. ammi* were found to contain remarkable antibacterial effects against food borne and spoilage bacteria, *B. subtilis*, *Salmonella typhimurium*, *P. aeruginosa*, *Enterobactor aerogens* and *Staphylococcus aureus*. The scanning electron microscopic studies also investigated the inhibitory effect of essential oil on the morphology of *B. subtilis* at the minimum inhibitory concentration (MIC) (Paul et al., 2011). The objective of the present study is to evaluate the phytochemicals and nutritional value of Klongi and Ajwain to be used as a natural food additive and to compare the antimicrobial effects of their different fractions for medicinal use.

MATERIALS AND METHODS

Collection and identification of medicinal plant seeds

The seeds of *N. sativa* (Klonji) and *T. ammi* (Ajwain) were purchased from local market of Lahore, during January 2011. The dried plant seeds were grinded using pestle and mortar and packed in polythene bags and placed in a dried place for further extractions. The plant species were identified by The Flora of Pakistan (Nasir and Ali, 1978). The voucher specimen IAGS.HHC.633 and IAGS. HHC.634 were given to *N. sativa* (Klonji) and *T. ammi* (Ajwain), respectively and deposited in the Herbarium of Institute of Agricultural Sciences, IAGS, University of the Punjab (PU) Lahore, Pakistan.

Extract preparation

Soxhlet extraction assembly was used for this purpose. Each of 50 g dried and powdered seeds were mixed with 250 ml distilled water, methanol, ethanol and n-Hexane successively and continuous extraction was done for 5 to 6 h. After that extracts were filtered, aqueous extracts were dried by freeze drying technique, the organic extracts were dried in a rotary evaporator at reduced pressure. *T. ammi* (Ajwain) seeds were submitted to hydro distillation for 3 h. The extracted essential oil thus obtained was dried over anhydrous sodium sulphate and left overnight to remove traces of moisture. Finally powdered extracts and essential oil was weighed and transferred in clean and dried vial, then stored in refrigerator at 4°C until use.

Phytochemical screening

Chemical tests were carried out to evaluate the presence of the phytochemicals Alkaloids, Flavonoids, Saponins, Tannins and Sterols in the selected spices; using standard procedures described by Sofowora (1993); Trease and Evans (1989).

Nutrition analysis

The proximate analysis (Carbohydrate, Fats, Protein, Moisture and Ash) of *N. sativa* and *T. ammi* was determined by using the Association of Official Analytical Chemists (AOAC) methods 1990. Carbohydrate value was determined by difference (100– (moisture + ash + protein + fat)). The nitrogen content was determined by kjeldahl method and multiplied to factor 6.25 to find the protein content. The weight difference method was used to find moisture and ash content. Soxhlet apparatus was used and solvent extraction method used for fat content determination, n-Hexane was used as solvent. Nutrient contents were valued in percentage.

Investigation of antimicrobial activity

Micro well dilution assay

Micro well dilution methods are used to examine antifungal activity of the spices fractions against growth of three pathogenic fungi and

Table 1. Percentage yield of different fractions of *N. sativa* (Klonji) and *T. ammi* (Ajwain).

Extract fraction	% Yield	
	Klonji	Ajwain
Aqueous	5	3.5
Methanol	4.4	3.7
Ethanol	3.9	4.94
n-Hexane	9.6	2.92
Essential oil	-	4

Table 2. Qualitative analysis of the phytochemicals of seeds of *N. sativa* and *T. ammi.*

Spice	Alkaloids	Tannins	Saponins	Flavanoids	Sterols
N. sativa	+	+++	_	++	+++
T. ammi	+	++++	_	++++	++++

Presence of constituent = +; Absence of constituent = -.

antibacterial activity for four bacteria, respectively (Okeke et al., 2001). Each fungal and bacterial concentration was made containing 10 CFU/ml. Stock solution was prepared by dissolving the powdered extracts and essential oil 0.4 g/ml of dimethyl sulfoxide (DMSO) solution. 60 µl of each fungal and bacterial suspension was spread on MEA/NA with L shaped glass spreader. Single well (8 mm in diameter) was made at the center of the plate using sterilized cork borer. About 70 µl of the aqueous, methanol, ethanol, n-Hexane extract and essential oil of Ajwain was poured into the well with the help of micropipette (10 to 100 µl) and then left on a clean place. Controls of DMSO and flucanozone were prepared for antifungal activity and streptomycin sulphate for antibacterial activity. The inoculated plates were incubated at 27°C for 72 h for fungal and bacterial isolates. Antimicrobial activity was estimated by measuring the zone of inhibition against the test microorganisms in comparison to control. Studies were performed in triplicate and mean value was calculated.

RESULTS AND DISCUSSION

Klonji that is, *N. sativa* and Ajwain *T. ammi* are frequently used spices and an active part of certain medicines and reported to treat different human ailments Paarakh et al., 2010; Bonjar, 2004).

Percentage yield

Percentage yield of different extracts of *N. sativa* showed maximum amount of n-hexane extract 9.6% and minimum of ethanol extract 3.9%. In contrast *T. ammi* yielded highest concentration of ethanol extract 4.94% and lowest by aqueous extract 3.5% (Table 1).

Phytochemical investigation

All the test phytochemicals alkaloids, tannins, flavonoids and sterols were detected in *N. sativa* and *T. ammi* seeds except saponins. In Klonji, tannins and sterols were detected in considerable amounts >75% (Table 2). Flavonoids and alkaloids were present in decreasing order 50 and 25%, respectively. Tannins, flavonoids and sterols were found maximum while alkaloids were minimum 25%. The presence of these phytochemicals has contributed to medicinal value of spices. Flavonoids have been reported to have antibacterial, antineoplastic activity, anti inflammatory, anti allergic and antiviral activity (Alan and Miller, 1996). Steroids are of much importance in pharmacy as sex hormones (Okwu, 2001). Alkaloids play significant role for fitness of survival to plant species. These have insecticidal properties and are an integral part of certain medicines and recreational drugs (Roger and Wink, 1998).

Proximate analysis

The nutrition analysis (carbohydrate, fats, fibre, protein, moisture, energy and ash) of *N. sativa and T. ammi* were determined by using AOAC (1990) methods. The (Klonji) *N. sativa* seeds possess high energy content viz. 459.29%. The decreasing order of different food contents are given in order of carbohydrates> protein> fats which are in the range of 39.04 to 21.67%. Moisture, fibre and ash contents were found in the same range of 5.5 to 4.34%, fibre content being maximum and ash contents were minimum Table 3. These findings are in line with that of Sultan et al. (2009), which reported 6.46% moisture, ash 4.20%, protein 22.80%, fiber 6.03% and fat 31.16% Table 3. Similarly, (Ajwain) *T. ammi* seeds possess higher energy value that is, 314.55%. Also, Ajwain is rich source of carbohydrates 47.54%. Protein, moisture, ash and fibre contents were evaluated in the

Table 3. Proximate analysis of dried seeds of *N. sativa* and *T. ammi.*

Nutrient	Percentage ±S.D	
	Nigella sativa	***Trachyspermum ammi***
Energy	459.29%±0.05	314.55±0.89
Carbohydrates	39.04%±0.21	47.54±1.05
Fat	21.67±1.55	4.83±0.16
Protein	24.05±1.76	20.23±1.79
Moisture	5.4±0.15	11.6±0.77
Fiber	5.5±0.15	4.3±0.1
Ash	4.34±0.73	11.5±0

range of 20.23 to 4.3%, proteins being highest and fiber was the least (Table 3); very less fat content that is, 4.83% were detected (Anonymous, 2003). In contrast depicted different results from present proximate analysis of Ajwain where carbohydrates 24.6%, protein 17.1%, fat 21.1% and moisture 7.4% are studied. From the present nutritional analysis, one can deduce that, these spices when added in human diet supply the body with carbohydrates, proteins and energy. The presence of higher energy content, carbohydrates, proteins and less fat contents contribute to nutritive value of selected spices and thus can be rich sources of useful foods.

Antimicrobial activity

In this study antifungal and antibacterial effects of aqueous, methanol, ethanol and n-Hexane extracts of *N. sativa* and *T. ammi* seeds oil were investigated. In the present study, polar solvent (water), solvents of intermediate polarity (like methanol and ethanol) and solvent of low polarity (hexane) were used to extract plant secondary metabolites that differ in polarity and structure and thus different solvent extracts showed distinct biological properties. During extraction process, solvents diffuse into the solid plant material and solubilise compounds of similar polarity (Green, 2004). The polarity of solvent affects quantity and composition of secondary metabolite of an extract. Secondary metabolites having antimicrobial activity are phenols, quinones, flavones, limonoids, essential oils, alkaloids, saponins, glycosides, flavonoids, tannins and coumarins, etc. Traditional healers primarily use water for extract preparation from plant extracts but organic solvents have been found to give more consistent antimicrobial activity compared to water extracts (Parekh et al., 2005). Most other bioactive compounds including phenols are generally soluble in polar solvents such as methanol. Many bioactive components are not water soluble and thus organic solvent extracts of plants have been found to be more effective (Parekh et al., 2006). Methanol, ethanol and water are the most commonly used solvents for antimicrobial investigations. (Bisignino et al., 1999; Lourens et al., 2004; Salie et al., 1996; Rojas et al., 2006; Parekh et al., 2005). Micro well dilution assay was adopted against three pathogenic fungal strains *A. flavus, A. niger, C. albicans* and four bacterial strains *P. syringica, B. subtilis, E. coli and Staphylococcus* sp.

The results pertaining to the antifungal potential of the Klonji were given in (Table 4). Among different extracts of Klonji screened, the methanol extract showed highest antifungal potential against all the test fungi with 3.95, 1.95 and 2.3 cm zone of inhibition, even marked significant from the flucanonozone where 0.5 and 0.66 cm inhibition zone was recorded, in case of *A. flavus* and *A. niger*. The largest zone of inhibition (3.95 cm) was obtained with *A. flavus* and least (1.95 cm) with *A. niger*. Maximum bactericidal effect (2.43 cm) was observed with *B. subtilis and* minimum (2.36 cm) with *P. syringica.* Ethanol and n-hexane extracts of *N. sativa* were ineffective against all fungal pathogens except n-hexane extract in case of *A. flavus* (0.03 cm). Followed by methanol extract, aqueous extract showed marked antifungal activity where 1.0; 1.5 and 1.63 cm inhibition zone were recorded. Ethanol extract depicted no antifungal potential against all the test fungi. N-Hexane extracts showed slight antifungal potential (0.03 cm inhibition zone) against *A. flavus* where no inhibition zone was observed in case of *A. niger* and *C. albicans.* Similar to the antifungal results of *N. sativa* extracts, showed maximum inhibition zones were recorded by methanol extract, 2.36 cm *P. syringica*, 2.43 cm (*B. subtilis)*, 2.7 cm (*E. coli)* and 2.86 cm (*Staphylococcus* sp.) (Table 5). Streptomycin sulphate was taken as positive control. Minimum antibacterial activity was exhibited by n-Hexane extract where no inhibition zone was recorded in case of *B. subtilis.* Aqueous extract depicted marked bactericidal activity against all tested bacteria maximum for *P. syringica* (2.06 cm) and minimum for *Staphylococcus sp.* (2.1 cm). Similar is the case with ethanol extract showing significant decrease in bacterial growth. Mashhadian and Rakhshandeh (2005) investigated the antibacterial and antifungal effects of the aqueous, chloroform and methanol extracts of the Klonji seeds against pathogenic *C. albicans, coagulase*-positive *S. aureus* and *P. aeroginosa* isolated from wound, blood, urine and critical

Table 4. Effect of *N. sativa* on fungal isolates.

Extract	Mean diameter of zone of inhibition		
	Aspergillus flavus	***Aspergillus niger***	***Candida albicians***
Aqueous	1.5	1.5	1.63
Methanol	3.95	1.95	2.3
Ethanol	0	0	0
N-hexane	0.1	0	0
Flucanazone	1.5	2	3.3
Control	0	1.05	1.55

Table 5. Effect of *N. sativa* on bacterial isolates.

Extract	Mean diameter of zone of inhibition			
	Pseudomonas syringica	***Bacillus subtilis***	***Escherichia coil***	***Staphylococcus sp***
Aqueous	2.06	1.9	1.83	2.1
Methanol	2.36	2.43	2.7	2.86
Ethanol	1.16	2.26	0.1	1.63
N-hexane	0.1	0	0.1	0.1
Str. sulphate	2.1	1.43	2.53	2.4
Control	0	1.2	1.06	0

success factors (CSF), compared with standard drugs cloxacillin, clotrimazole and gentamicin.

Our antimicrobial results of *N. sativa* are in agreement with these previous findings, where the methanol extract of *N. sativa* seeds had shown the best antimicrobial activity using agar dilution, cylinder plate and disk diffusion methods. On contrary to present findings, in the previous study, aqueous extract did not show any antimicrobial activity against any test pathogen. Masood et al. (2008) investigated the antibacterial activity of aqueous infusions and decoctions of klonji (*N. sativa*) against 188 bacterial isolates of Gram-positive and Gram-negative belonging to 11 different genera isolated from oral cavity, using disc diffusion method. The aqueous decoctions of klonji showed (51%) antibacterial potential of the tested microorganisms. Preliminary clinical trials have investigated and reported the traditional therapeutic use of Klonji seeds for the treatment of diarrhoea, hypertension, asthma, diabetes, cough, bronchitis, inflammation, headache, eczema, influenza and dental caries (Ali et al., 2003; Gilani et al., 2001). The chemical constituents of seeds of kalonji have above one hundred and thymoquinone is regarded as active ingredient, responsible for antibacterial activity.

Other ingredients include dithymoquinone, nigellone, thymohydroquinone, ascorbic acid (vitamin C), tocopherol (vitamin E), linoeic acid, oleic acid, carvacrol, lipase, t-anethole and 4-terpineol, etc (Ali et al., 2003). In case of *T. ammi* extracts and essential oil, all the aqueous, organic extracts especially ethanol, n-hexane and essential oil significantly masked fungal growth (Table 6). Fungitoxicity of n-hexane extract was maximum in case of *A. flavus* where inhibition zone 3.2 cm was recorded followed by essential oil (2.9 cm),ethanol extract (2.4 cm),methanol extract (1.3 cm) and aqueous extract (1.2 cm), respectively. In case of *A. niger*, mycelia growth was suppressed by Ajwain ethanol extract (3.6 cm) and minimum by aqueous extract (1.6 cm). Essential oil of Ajwain proved to possess strong antifungal toxicity against all the three fungal strains being highest in *C. albicans* (3.66 cm), after that ethanol extract (3.53 cm), n-Hexane extract (3.16 cm), methanol extract (1.65c m)and aqueous extract (1.45 cm). Present antibacterial study of *T. ammi* is in agreement with its antifungal study as all the aqueous, organic extracts and essential oil represented marked inhibition against all bacteria under observation. n-hexane extract significantly inhibited bacterial growth (4.76 cm) *P. syringica*, (4.03 cm) *B. subtilis*, (3.73 cm) *E. coli* and (4.76 cm) *Staphylococcus* sp. Bactericidal action of different fractions of Ajwain decrease in the order of n-Hexane extract>essential oil>ethanol extract>methanol extract> and aqueous extract (Table 7). Amongst the different extracts of *N. sativa* investigated, methanol extract showed the most remarkable activity. This might have resulted from solubility of the active constituents in methanol and water also. The investigated plant *N. sativa* did not show strong antimicrobial activity of its ethanol and n-hexane extracts as compared to methanol extract, negative results do not mean absence of bioactive constituents. Active

Table 6. Effect of *Trachyspermum ammi* on fungal isolates.

Extract	Mean diameter of zone of inhibition		
	Aspergillus flavus	***Aspergillus niger***	***Candida albicians***
Aqueous	1.2	1.6	1.45
Methanol	1.3	1.35	1.65
Ethanol	2.4	3.6	3.53
N-Hexane	3.2	2.96	3.16
Essential oil	2.9	2.4	3.66
Flucanazone	1.5	1.55	3.3
Control	0	1.1	1.55

Table 7. Effect of *Trachyspermum ammi* on bacterial isolates.

Extract	Mean diameter of zone of inhibition			
	Pseudomonas syringica	***Bacillus subtilis***	***Escherichia coil***	***Staphylococcus sp.***
Aqueous	1.9	0.9	0.8	2.2
Methanol	2.23	2.03	1.43	2.03
Ethanol	2.33	2.36	2.1	2.03
n-Hexane	4.76	4.03	3.73	4.76
Essential oil	2.83	3.33	3.21	3.53
Str. sulphate	2.1	1.7	2.56	2.23
Control	0	1.2	1.1	0

compounds may be in insufficient quantities to show activity with the applied concentration (Taylor et al., 2001). In case of *T. ammi*, organic extracts mainly essential oil and n-hexane showed significant antimicrobial activity. Aqueous extract depicted least activity; this might have resulted from the lack of solubility of the active constituents in water. The ajwain essential oil and acetone extract exhibited a broad spectrum of fungitoxicity against all tested fungi; *A. niger*, *A. flavus*, Aspergillus *oryzae*, *Aspergillus ochraceus*, *Fusarium monoliforme*, *Fusarium graminearum*, *Pencillium citrium*, *Pencillium viridicatum*, *Pencillium madriti* and *Curvularia lunata*. However, acetone extract was found less effective than the essential oil. Gas chromatography (GC) and Gas chromatograph and mass spectrometer (GC-MS) analysis of ajwain identified 26 components from essential oil and 18 components from acetone extract, Thymol (39.1%) was found as a major component in both, along with p-cymene, *ç*-terpinene, *â*-pinene, terpinene-4-ol and carvacrol 0.3% (Singh et al., 2004). The essential oil of Ajwain has proved as potential nematicide against the pinewood nematode. The nematicidal activity of ajwain oil was mainly attributed to the activity of thymol, major component of this oil and carvacrol (Park et al., 2007; Choi et al., 2007). Fungi toxicity of essential oil *T*. *ammi* has shown against *Epidermophyton floccsum*, *Microsporum canis* and *Trichophyton mentagrophytes* at 900 ppm concentration. Thymol has been identified as the fungitoxic chemical in Ajwain essential oil (Singh et al., 1986). The essential oil also extracted antibacterial activity against *S. aureus*, *E. coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholera* (Syed et al., 1986; Anonymous, 1995; Mayaud et al., 2008.

Moreover, seed extract at 1:20 dilution exhibited fungicidal action against *Rhizoctonia solani* (Ansari, 1995). Different aqueous and organic extracts *T*. *ammi* extracts prepared exhibited variable activity against *E. coli*, *P. aeruginosa*, *S. typhi* and *S. aureus* justifying its use to cure for various gastrointestinal disorders (Kaur and Arora, 2009; Ahmad et al., 1998; Patel et al., 2008). Methanol extracts of *T*. *ammi* significantly *in vitro* inhibited hepatitis C virus (HCV) protease at a concentration of 100 µg/ml (Hussein et al., 2000). Secondary metabolites mechanisms of antimicrobial action are not fully understood but many investigations have been conducted. Single compound may not be responsible for the bioactivity but rather a combination of compounds interacting in an additive or synergistic manner. Gram-positive and negative bacteria showed differential sensitivity to plant extracts, might be due to the morphological differences. An outer phospholipidic membrane carrying structural lipopolysaccharide is present in Gram-negative bacteria making the cell wall impermeable to organic solutes, while porins make a barrier to the aqueous solutes. The Gram-positive bacteria in this regard should be more sensitive since they lack this outer layer (Arias et al., 2004). Flavonoids action mechanism might be through cytoplasmic membrane, deoxyribonucleic acid (DNA) gyrase inhibition and β-hydroxyacyl-acyl carrier protein dehydratase activities (Cushnie and Lamb, 2005; Zhang et al., 2008). The cell morphology can be changed by isoflavone genistein through formation of filamentous cells and inhibiting the synthesis of DNA and ribonucleic acid (RNA) of *Vibrio harveyi* (Ulanowska et al., 2006). Terpenes abundantly present in essential oil promote membrane disruption, coumarins cause cell respiration reduction and tannins bind to polysaccharides or enzymes promoting inactivation and effect microorganism membrane (Ya, 1988; Cowan, 1999). Essential oils being lipophilic in nature make it permeable to cellular membrane (Bakkali et al., 2008).

Conclusion

From neutraceutical point of view, selected spices Klonji and Ajwain have served as important constituents of human diet supplying the body with sufficient amount of proteins, carbohydrates and energy. The presence of biologically active compounds also contributes to its nutritive value and thus proved to be potential sources of useful foods. The presence of phytochemicals justifies the biological properties of spices. The study supports the antibmicrobial potential of kalonji/ajwain especially *N. sativa* methanolic extract and *T. ammi* organic extracts and essential oil as a potent antibmicrobial agent.

REFERENCES

Ahmad I, Mehmood J, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol., 62: 183-193.

Akhtar MS (1988). Anthelmintic evaluation of indigenous medicinal plants for veterinary usage. Final Report of the PARC Research Project (1985-86), University of Agriculture, Faisalabad, Pakistan.

Alan L, Miller ND (1996). Antioxidant Flavonoids. structure, function and chemical usage. Alt. Med. Rev., 1: 103-111.

Ali BH, Blunden G (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res., 17(4): 299-305.

Al-Gharably NM, Badry O, Nagi M (1997). Protective effect of thymoquinone against CCl4-induced hepatotoxicity in mice. Res. Comm. Pharmacol. Toxicol., 2: 41-50.

Al-Shabanah OA, Badary OA, Nagi MN, Al-garably NM, Al-Rikabi AC,

Al-Bekairi AM (1998). Thymoquinone protects against doxorubicin-induced cardiotoxicity without compromising its Antitumor activity. J. Exp. Clin. Cancer Res., 17(2): 193-198.

Arias ME, Gomez JD, Cudmani NM, Vattuone MA, Isla MI (2004). Antibacterial activity of ethanolic and aqueous extracts of Acacia aroma Gill. Ex Hook et Arn. Life Sci., 75: 191-202.

Anilkumar KR, Saritha V, Khanum F, Bawa SA (2009). Ameliorative effect of ajwain extract on hexachlorocyclohexane-induced lipid peroxidation in rat liver. Food Chem Tox., 47: 279-282.

Anonymous (2003). The wealth of India. CSIR., New Delhi: 260-271.

Anonymous (1995). The wealth of India, A dictionary of Indian Raw Materials and Industrial Products. Publications and Information Directorate (CSIR), New Delhi, 13: 351.

Ansari MM (1995). Control of sheath blight of rice by plant extracts. Indian Phytopathol., 48: 268-270.

AOAC (1990). Offical methods of analysis. 15th edition Association of official analytical chemists, Washington DC, USA.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils – A review. Food Chem. Toxicol., 46: 446-475.

Bisignino G, Sanogo R, Marino A, Aquino R, D'angelo V, Germano MP, De Pasquale R, Pizza C (1999). Antimicrobial activities of Mitracarpus scaber extract and isolated constituents. Lett. Appl. Microbiol., 30: 105-108.

Bonjar GHS (2004). Screening for antibacterial properties of some Iranian plants against two strains of *E. coli*. Asian J. Plant Sci., 3(3): 310-314.

Choi IH, Shin SC, Park IK (2007). Nematicidal activity of onion (*Allium cepa*) oil and its components against the pine wood nematode (*Bursaphelenchus xylophilus*). Nematology, 9: 231–235.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Cushnie TP, Lamb AJ (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.

ElTahir KEH, Ashour MM, AlHarbi MM (1993). The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: Elucidation of the mechanism of action. Gen. Pharmacol., 24: 1123-1131.

ElTahir KEH, Ashour MM, Al-Harbi MM (1993). The respiratory effects of the volatile oil the black seed (*Nigella sativa*) in guinea-pigs; elucidation of the mechanism of action. Gen. Pharmacol., 24: 1115-1122.

Ghaznavi K (1988). Tibb-i-Nabvi (Peace Be Upon Him) aur jadeed Science.Faisal Publishers, Urdu Baza. Lahore, 1(228): 231-232.

Gilani A, Aziz N, Khurram I, Chaudhary K, Iqbal A (2001). Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): a traditional herbal product with multiple medicinal uses. J. Pak. Med. Assoc., 51(3): 115-120.

Green RJ (2004). Antioxidant Activity of Peanut Plant Tissues. Masters Thesis. North Carolina State University. USA.

Hanafy MSM, Hatem ME (1991). Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J. Ethn., 34: 275-278.

Hosseinzadeh H, Bazzaz BSF, Haghi MM (2007). Antibacterial activity of total extracts and essential oil of *Nigella sativa* L., seed in mice. Pharmacolgyonline, 2: 429-435.

Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N, Shimotohno K (2000). Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytother. Res., 14: 510-516.

Ikram M, Hussain SF (1978). Compendium of medicinal plants.Pakistan Council of Scientific and Industrial Research (PCSIR), Pakistan.

Kaur GJ, Arora DS (2009). Antibacterial and phytochemical screening of *Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi*. BMC Complement. Altern. Med., 9: 30.

Lourens ACU, Reddy D, Baser KHC, Viljoen AM, Van Vuuren SF (2004). *In vitro* biological activity and essential oil composition of four indigenous South African Helichrysum species. J. Ethnopharmacol., 9: 253-258.

Mashhadian NV, Rakhshandeh H (2005). Antibacterial and antifungal effects of Nigella sativa extracts against S.aureus,P.aeroginosa and C.albicans. Pak. J. Med. Sci., 21(1): 47-52.

Masood N, Chaudhry A, Tariq P (2008). *In vitro* antibacterial activities of kloji, cumin and poppy seed. Pak. J. Bot., 40(1): 461-467.

Mayaud L, Carricajo A, Zhiri A, Aubert G (2008). Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett. Appl. Microbiol., 47: 167-173.

Menphini A, Pagiotti R, Capuccella M (1995). Antifungal activity of carvacrol chemotypes of winter savory harvested in Italy. Riv. Italy Chem. Abstr., 123: 566-571.

Morsi NM (2000). Antimicrobial effect of crude extracts of Nigella sativa on multiple antibioticresistant bacteria. Acta Microbiol. Polonica, 49(1): 63-74.

Nagalakshmi G, Shankaracharya NB, Puranaik J (2000). Studies on chemical and technological aspects of Ajwain (*Trachyspermum ammi*) syn (Cerum copticum Hiren) seeds. J. Food Sci. Technol., 37:

277- 281.
Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, Al-Behairy AM (1999). Thymoquinone protects against CCl4-hepatotoxicity in mice via an antioxidant mechanism. Biochem. Mol. Biol. Int., 47: 153-159.
Nasir E, Ali SI (1978). Flora of Pakistan National Herbarium, Islamabad .National Research Council (NRC) (1984). Nutrient requirement for Beef Cattle, 6th rev. ed. Nutrient requirement of domestic animals. Natl. Acad. Sci. Wash., 4: 421-427.
Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO (2001). Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. J. Ethnopharmacol., 78: 119-127.
Okwu DE (2001). Evaluation of the chemical composition of indigenous spices and flavoring agents. Global J. Pure Applied Sci., 7: 455-459.
Paarakh MP (2010). *Nigella sativa* Linn. Ind. J. Nat Prod. Res., 1(4): 409-429.
Parekh J, Jadeja D, Chanda S (2005). Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk. J. Biol., 29: 203-210.
Parekh J, Karathia N, Chanda S (2006). Screening of some traditionally used medicinal plants for potential antibacterial activity. Indian J. Pharm. Sci., 68(6): 832-834.
Park K, Kim J, Lee SG, Shin SC (2007). Nematicidal Activity of Plant Essential Oils and Components From Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*) and Litsea (*Litsea cubeba*) Essential Oils Against Pine Wood Nematode *Bursaphelenchus Xylophilus*). J. Nemato., 39(3): 275-279.
Patel JD, Patel DK, Shrivastava A, Kumar V (2008). Screening of plant extracts used in traditional antidiarrhoeal medicines against pathogenic *Escherichia coli*. Sci. World, 6: 63-67.
Paul S, Dubey RC, Maheswari, DK, Kang SC (2011). Trachyspermum ammi (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens. J. Food Cont., 22: 725-731.
Rojas JJ, Ochoa VJ, Ocampo SA, Monoz JF (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in treatment of nonnosocomial infections. BMC Complement. Alternat. Med., 6: 2.
Roger MF, Wink M (1998). Alkaoids:Biochemistry, ecology and medicinal applications. Plennum press: 2-3.
Saeed M (1969). Hamdard Pharmacopoeia Of Eastern Medicine. Hamdard Foundation, Karachi, Pakistan.
Salie F, Eagles PFK, Lens HMJ (1996). Preliminary antimicrobial screening of four South African Asteraceae species. J. Ethnopharmacol., 52(1): 27-33.
Singh G, Maurya S, Catalan C, DE Lampasona MP (2004). Chemical Constituents, Antifungal and Antioxidative Effects of Ajwain Essential Oil and Its Acetone Extract. J. Agric. Food Chem., 52: 3292-3296.
Singh SP, Dubey P, Tripathi SC (1986). Fungitoxic properties of the essential oil of *Trachyspermum ammi* Sprague, Mykosen. India, 29: 37-40.
Sofowora A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nig., p. 289.
Sultan MT, Butt MS, Anjum FM, Jamil A, Akhtar S, Nasir M (2009). Nutritional profile of indigenous cultivar of Black cumin seeds and antioxidant potential of its fixed and essential oil. Pak. J. Bot., 41(3): 1321-1330.
Syed M, Sabir AW, Chaudhary FM, Bhatty MK (1986). Antimicrobial activity of essential oils of umbelliferae part II- *Trachyspermum ammi*, *Daucus carota*, *Anethum graveolens* and *Apium graveolens*. Pak. J. Sci. Ind. Res., 28: 189-192.
Taylor JLS, Rabe T, McGaw LJ, Jäger AK, van Staden J (2001). Towards the scientific validation of traditional medicinal plants. Plant Growth Regul., 34(1): 23-37.
Trease GE, Evans WC (1989). Pharmacognosy, 11th Edn. Brailliar Tiridel Can., Macmillian Publishers.
Treas and Evans (2002). Pharmacognosy, international 15th edition 2002, saunder Edinburgh, New York: 258.
Tsimidou M, Boskou D (1994). Antioxidant activity of essential oils from the plants of the Lamiaceae family, In Spices, Herbs and Edible Fungi, ed, G Charalambous, pp. 273-284.
Ulanowska K, Tkaczyk A, Konopa G, Węgrzyn G (2006). Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. Arch. Microbiol., 184: 271-278.
Ya C, Gaffney SH, Lilley TH, Haslam E (1988). Carbohydrate-polyphenol complexation. In: Hemingway RW, Karchesy JJ, eds.Chemistry and significance of condensed tannins. New York, NY: Plenum Press: 553 p.
Zhang L, Kong Y, Wu D, Zhang H, Wu J, Chen J, Ding J, Hu L, Jiang H, Shen X (2008). Three flavonoids targeting the β-hydroxyacyl-acyl carrier protein dehydratase from Helicobacter pylori: Crystal structure characterization with enzymatic inhibition assay. Protein Sci., 17: 1971-1978.