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

Shabnam Javed¹*, Ahmad Ali Shahid¹, Muhammad Saleem Haider¹, Aysha Umeera¹, Rauf Ahmad² and Sobia Mushtaq¹

¹Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590 Lahore, Pakistan.
²Pakistan 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, emmenagogue; 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; Hosseinazadeh et al., 2007). N. sativa seeds are reported to contain numerous chemical compounds. Active ingredients are nigelone, beta-sisterol, and thymoquinone. Other compounds include 2-methyl-4-isopropyl-p-quinones, anthraquinones, saponins, glycosoieds, 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 antihelminthic effects (Morsi, 2000). In Ajwain, the major phenolic compound Thymol is present and has been reported to be an antisapmodic, 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 (Tsidomidou 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, Enterobacter 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 Klonji 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
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 a 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 fluconazole 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 1. Percentage yield of different fractions of N. sativa (Klonji) and T. ammi (Ajwain).

<table>
<thead>
<tr>
<th>Extract fraction</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>5</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.9</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>9.6</td>
</tr>
<tr>
<td>Essential oil</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Spice</th>
<th>Alkaloids</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Flavanoids</th>
<th>Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. sativa</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>T. ammi</td>
<td>+</td>
<td>++++</td>
<td>-</td>
<td>++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Presence of constituent = +; Absence of constituent = -.
range of 20.23 to 4.3%, proteins being highest and fiber was the least (Table 3); very low 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 *S. aureus*. 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 Rakshshande (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. aeroginos* isolated from wound, blood, urine and critical

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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage ±S.D</th>
<th>Nigella sativa</th>
<th>Trachyspermum ammi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>459.29±0.05</td>
<td>314.55±0.89</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>39.04±0.21</td>
<td>47.54±1.05</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>21.67±1.55</td>
<td>4.83±0.16</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>24.05±1.76</td>
<td>20.23±1.79</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>5.4±0.15</td>
<td>11.6±0.77</td>
<td></td>
</tr>
<tr>
<td>Fiber</td>
<td>5.5±0.15</td>
<td>4.3±0.1</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>4.34±0.73</td>
<td>11.5±0</td>
<td></td>
</tr>
</tbody>
</table>
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,
thythymohydroquinone, ascorbic acid (vitamin C), tocopherol
(vitamin E), linoleic 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 (1.65 cm) 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 4. Effect of *N. sativa* on fungal isolates.

<table>
<thead>
<tr>
<th>Extract</th>
<th><em>Aspergillus flavus</em></th>
<th><em>Aspergillus niger</em></th>
<th><em>Candida albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>1.5</td>
<td>1.5</td>
<td>1.63</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.95</td>
<td>1.95</td>
<td>2.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-hexane</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flucanazone</td>
<td>1.5</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>1.05</td>
<td>1.55</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Extract</th>
<th><em>Pseudomonas syringica</em></th>
<th><em>Bacillus subtilis</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Staphylococcus sp</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>2.06</td>
<td>1.9</td>
<td>1.83</td>
<td>2.1</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.36</td>
<td>2.43</td>
<td>2.7</td>
<td>2.86</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.16</td>
<td>2.26</td>
<td>0.1</td>
<td>1.63</td>
</tr>
<tr>
<td>N-hexane</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Str. sulphate</td>
<td>2.1</td>
<td>1.43</td>
<td>2.53</td>
<td>2.4</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>1.2</td>
<td>1.06</td>
<td>0</td>
</tr>
</tbody>
</table>

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,
thythymohydroquinone, ascorbic acid (vitamin C), tocopherol
(vitamin E), linoleic 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 (1.65 cm) 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
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 moniliforme*, *Fusarium graminearum*, *Pencillium citrinum*, *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

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

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mean diameter of zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aspergillus flavus</em></td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.4</td>
</tr>
<tr>
<td>N-Hexane</td>
<td>3.2</td>
</tr>
<tr>
<td>Essential oil</td>
<td>2.9</td>
</tr>
<tr>
<td>Flucanazone</td>
<td>1.5</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mean diameter of zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Pseudomonas syringica</em></td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.9</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.23</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.33</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>4.76</td>
</tr>
<tr>
<td>Essential oil</td>
<td>2.83</td>
</tr>
<tr>
<td>Str. sulphate</td>
<td>2.1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>
bacteria in this regard should be more sensitive since they lack this outer layer (Arias et al., 2004). Flavonoids' action mechanism might be through cytotoxic 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 genisteen 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 neutrophilic point of view, selected spices Klöntji 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 antimicrobial potential of klöntji/ajwain especially N. sativa methanic extract and T. ammi organic extracts and essential oil as a potent antimicrobial agent.

**REFERENCES**


