Research Article

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Antimicrobial activity of aqueous, ethanolic extracts and crude extracted phytoconstituents of *Nigella sativa* seeds.

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Nigella sativa seeds (black seed;Kalonji) have been used in traditional medicine for the treatment of a variety of diseases. In this study, the antibacterial and antifungal effects of ethanol and aqueous extracts of seeds as well as crude extracted phytoconstituents against two gram positive (*Bacillus subtilis ,Staphylococcus aureus*) and two gram negative strains of bacteria (*Escherichia coli ,Pasturella multocida*) and one strain of fungi (*Aspergillus* niger) have been investigated and compared with standard drug i.e chloramphenicol. Percentage of phytoconstituents in the seed was also determined. The inhibitory effects of extracts and phytoconstituents were assessed using disk diffusion method at three different concentrations i.e 50mg/mL, 100mg/mL and 15omg/mL. Results showed that the aqueous extract did not show any effect against *Pasturella multocida* and against *Aspergillus niger* of fungi, However against all bacterial strains *Nigella sativa* showed inhibition zone between the range of 20 and 23mm.Ethanolic extract showed inhibition zone between the range of 15 and 28mm against all the four bacterial strains. Against fungi 15mm inhibition zone was observed. Phytoconstituents showed varying degree of inhibition against all the four bacteria and fungi. Alkaloids showed no inhibition against *Escherichia coli* and *Pasturella multocida.* Steriods and triterpenoids against *Staphylococcus aureus*, Tannic acids against *Staphylococcus aureus* and *Bacillus subtilis* also showed no inhibition. All the extracts and phytoconstituents showed varying degree of inhibition.

Key words: Antimicrobial activity, medicinal plants, *Nigella sativam,* kalonji, black cumin, phytochemicals

Antimicrobial activities of many bioactive components of plants and their derivatives have been reported by many researchers. (Ozcan and Erkman 2001, Sagdic and Ozcan 2003). Due to the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Plants have been known to synthesize a variety of compounds to protect themselves against a variety of their own pathogens and therefore can be considered as potential source of different classes of antimicrobial substances. (Nimri *et al.,1999;*Grayer and Harbone, 1994). *Nigella sativa* (black cumin; kalonji) is an annual herbaceous plant grown in Western Asia and the Mediterranean region for its seeds. The seeds contain fixed and essential oils, proteins, alkaloids. Much of the biological activity of the seeds has been shown due to thymoquinone, the major component of the essential oil, but which is also present in the fixed oil.(Ali and Blunden 2003). The Study on garlic (*Allium sativum*) and black cumin (*Nigella sativa)* confirmed the fact that allicin of freshly crushed garlic (*Allium sativum*) and thymoquinone of black cumin *Nigella sativa* seeds are the major components, Which are responsible for therapeutic activity and also act as antimicrobial agents against different pathogens. (Roy *et al.,*2006)

The goal of this study was to investigate the antimicrobial activity of extracts as well as phytochemicals of *Nigella sativa.*

MATERIALS AND METHODS

Nigella sativa seeds have been collected from

local market. All Chemicals used were of analytical grade obtained from Sigma, Merk and Aldrich Chemical Co. Commercially available antibiotic chloramphanicol was purchased from Remingtom pharmaceutical industries (pvt)Ltd. Pakistan.

Detection and extraction of Phytochemicals: All phytochemicals like alkaloids, flavonoids, steroids and triterpenoids, were detected and extracted from the sample by using standard methods.

Alkaloids: The extract for the detection of alkaloids has been prepared by taking 3-4g plant material and added 30mL chloroform. It has been boiled for 15 minutes, and then filtered. Test has been performed for its alkaloidal contents with Dragondroff's reagent.

Flavonoids: Flavonoids were detected by the method described by (Siddique and Ali ,1997). In this method a filter paper dipped in alcoholic solution of plant material was exposed to vapors of ammonia solution.

Glycosides: The glycosides were identified by Stat-Otto procedure (Brain and Turner 1975). Alcoholic extract of plant material was prepared by boiling the plant material with alcohol in reflux apparatus, then this extract was treated with Benedict's and Fehling's solution separately.

Steriods and triterpenoids: The method used for the detection of Steriods and triterpenoids was developed by (Segal, 1960), and Harbone(1973) and later modified by (Siddique and Ali,1997). The solution of plant material was prepared in choloroform and then 2mL concentrated H_2SO_4 was added.

Tannic Acids: Detection of tannic acid was done by the method described by (Siddique and Ali, 1997). The solution of plant material was prepared in alcohol and then $FeCl_3$ solution was added slowly. After that dilute H_2SO_4 was added in it drop wise to check the presence of tannic acids.

Preparation of water and ethanol extract: Aqueous and ethanol extracts were prepared by soaking 50g of powdered plant material in ethanol and water respectively for one week. After one week it was filtered.

Microbiological assessment: Four bacterial and one fungal strain were used to investigate the antimicrobial activity of *Nigella sativa*. Two gram negative bacterial strains (*Escherichia coli ,Pasturella multocida*) and two gram positive bacterial strains (*Bacillus subtilis ,Staphylococcus aureus*) were used .One strain of fungi i.e.*Aspergillus niger* was used to test antifungal activity.

Disk diffusion method: Sterilized Petri dishes (9cm diameter) already poured with nutrient agar media were inoculated with 0.01 mL of nutrient agar media (10^5 - 10^6 bacteria per ml). Discs injected with extracts of different concentrations (50mg/ml, 100mg/ml, 150mg/ml and phytoconstituents were applied on the solid agar medium by pressing tightly. The treated Petri dishes were placed at 4 °C for 1-2 hours and then incubated at 37±0.1 °C for 18-24 hours. At the end of the period, the inhibition zones formed on the media were measured with inhibition zone reader in mm. The antimicrobial activity was expressed as the mean diameter of the inhibition zone (mm) produced by plant extracts. Chloramphenicol was used as a positive control.

RESULTS

The study was designed to investigate the antimicrobial activity of this magical seed. Antimicrobial activity of various crude fractions of phytoconstituents was also evaluated.

Phytochemical Analysis: First different phytoconstituents have been identified and then extracted by using various standard methods. The percentage of various phytoconstituents present in the seeds of *Nigella sativa* is presented in table 1-5.

The results of the study have shown that *Nigella sativa* contains all other phytochemicals except saponins. It contains almost 50% of tannins. Apart from that there is also sufficient quantity of alkaloids, flavonoids, glycosides, steroids and triterpenoids.

Antibacterial activity: The antibacterial activity has been assessed quantitatively by the presence or absence of inhibition zones (Figure 1 & 2). These results suggest that the aqueous extract did not show any activity against *Pasturella multocida (P.multocida).* However ethanolic extract showed some activity against this particular strain, whereas

Table 1: Percentage of phytoconstituents in *Nigella sativa*.

Sr. No	Phytochemicals	Qualitative analysis	Quantitative analysis
1	Alkaloides	+	9%
2	Flavonoids	+	4%
3	Glycosides	+	7%
4	Tannic acids	+	50%
5	Steriods&triterpenoids	+	12%
6	Saponins	-	-

+: Presence of Phytochemical, -: Absence of Phytochemical

Table: 2 Antibacterial activity of ethanol and aqueous extracts.

		Concentration	Tested Bacteria			
		mg/ml	*E.coli*	*P.multocida*	*S.aureus*	*B.subtilis*
Extracts	Eth	50mg/ml	16mm	15mm	N.I	13mm
		100mg/ml	25mm	19mm	13mm	15mm
		150mg/ml	28mm	21mm	15mm	17mm
	W	50mg/ml	13mm	N.I	13mm	17mm
		100mg/ml	17mm	N.I	17mm	21mm
		150mg/ml	20mm	N.I	21mm	23mm

N.I*: No Inhibition, W: water, Eth: ethanol

Table: 3 Antibacterial activities of phytoconstituents**.**

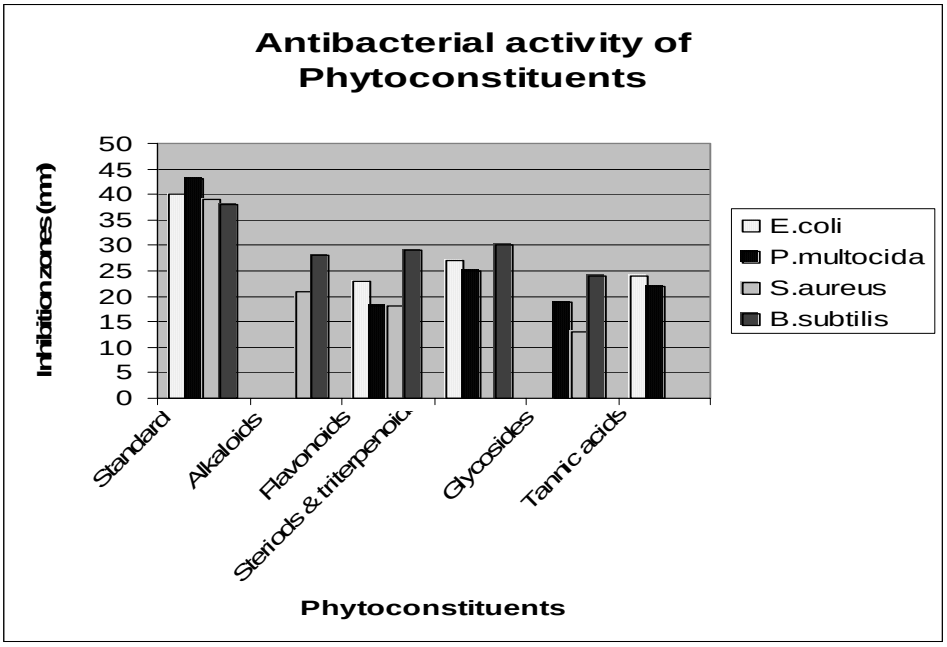
	Tested Bacteria			
Phytochemical	*E.coli*	*P.multocida*	*S.aureus*	*B.subtilis*
Alkaloids	N.I	N.I	21mm	28mm
Flavonoids	23mm	18mm	18mm	29mm
Steriods & triterpenoids	27mm	25mm	N.I	30mm
Glycosides	N.I	19mm	13mm	24mm
Tannic acid	24mm	22mm	N.I	N.I
Standard	40mm	43mm	39mm	38mm

Table: 4 Antifungal activities of ethanol and aqueous extracts against *Aspergillus niger*

	Inhibition zones		
Extract	50mg/ml	100ng/ml	150mg/ml
Ethanol extract	11mm	13mm	15mm
Water extract	N.I	N.I	N.I

Table: 5 Antifungal activities of phytoconstituents against *Aspergillus niger.*

Zones of Inhibition at 100mg/ml		
Flavonoids	Tannic acids	Glycosides
13mm	18mm	17mm



Graph 1: Antibacterial activity of phytoconstituents.

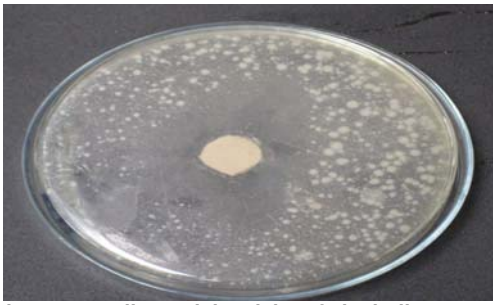


Figure 1: Antibacterial activity of alcoholic extract against *E.coli.*

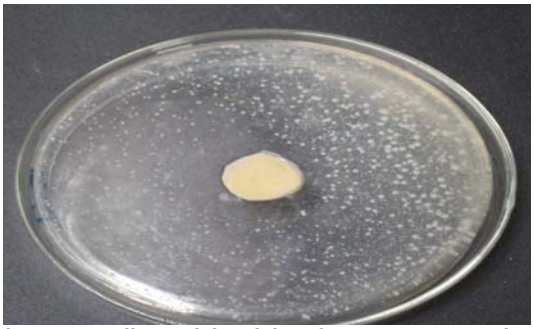


Figure 2: Antibacterial activity of water extract against *E.coli.*

against *Escherichia coli* (*E.coli), Pasturella multocida (P.multocida), Staphylococcus aureus (S.aureus)* and *Bacillus Subtilis (B.subtilis)* ethanolic extract gave considerable diameter of inhibition zones, having 23mm as the highest value against *B.subtilis* at 150mg/ml) (Graph 1). Ethanolic extract showed no inhibition against *S.aureus* only at lower concentration i.e 50mg/mL. Ethanolic extract gave higher inhibition zone than aqueous against *E.coli,* but against *S.aureus* and *B.subtilis* water extract showed higher inhibition zones. Among phytoconstituents alkaloids showed no inhibition against *E.coli* and *P.multocida* while steroids and triterpenoids showed no inhibition against *S. aureus,* whereas tannic acid showed no inhibition against *S.aureus* and *P.multocida.* Against fungal strain i.e *Aspergillus niger* aqueous extract did not show any inhibition. As far as the ethanolic extract is concerned, *Nigella sativa* showed considerable inhibition, it kept on rising with the increasing concentration. Among phytoconstituents, tannic acids showed considerable inhibition value i.e. 18mm.

DISCUSSION

Due to the increased resistance of microorganisms against the currently used antibiotics and the high cost of production of synthetic drugs, pharmaceutical companies are now searching for alternatives. Medicinal plants could be one approach because most of them are safe with little side effects, if any, are of low cost and affect a wide range of antibiotic resistant microorganisms. (Maleki *et al.,2008*). The present findings demonstrated

that the plant material has been blessed with many of the natural phytoconstituents. These phytoconstituents as well as plant extracts are responsible for antimicrobial activity. By comparing aqueous and ethanolic extracts with each other it was clear that against gram negative bacteria, ethanolic extract showed higher inhibition zone than aqueous extract, whereas for gram positive bacteria aqueous extract showed more activity as compared to ethanolic extract.Against *Aspergillus niger* aqueous extract showed no inhibiton .Phytoconstituents also showed broad spectrum of activity against all of the bacterial and fungal strains. The results indicated that the standard antibiotic was stronger than the phytochemicals.

Previous study on *Nigella sativa* crude extracts and phytoconstituents also supports the fact that plant is active against various pathogens. In past many researchers investigated the antimicrobial potential of *Nigella sativa.* The drug *Nigella sativa* (*N. sativa* ethanolic extract) is found active against standard as well as multi drug resistant strains of tested bacteria. (Salman *et al.,*2005). Our results were in agreement with those reported by (Ani *et al.,* 2006) showing singnificant antibacterial activity of *Nigella sativa* against *Bacillus subtilis*, *Bacillus cereus* .Thus, *Nigella sativa* with an array of polyphenolic compounds possesses antibacterial activitiy. (Ani *et al.,* 2006).

Lycosides, tannins, saponins, flavonoids and alkaloids were detected in the plant material. These phytoconstituens were active against *B. subtilis, E. coli.* and *Salmonella typhi* (*S. typhi.* (Okoli *et al .,* 2002). Alkaloids and flavonoids showed considerable antibacterial activity against *S.aureus* and *B.subtilis* these results were also in accordance with the work done by (Nazrul Islam *et al.,* 2002) They reported that six alkaloids and seven flavonoids including the novel products were active against six clinical strains.The active compounds showed mild to moderate activities against *Bacillus subtilis, Staphylococcus aureus, Sarcina lutea*, exterotoxigenic *Escherichia coli, Salmonella typhi* and *Klebsiella* sp. The flavonoids were observed to have higher spectrum and magnitude of activity than those of the alkaloids.

Staph. aureus showed susceptibility only to the hot extract of Glycosides, Tannins, saponins ,flavonoids and alkaloids. (Okoli *et al.,* 2002). Flavonoids, the combination of antibiotic and flavonoids is a potential new strategy for developing therapies for infections caused by ESBL-producing bacteria in the future (Lino *et al*.,2005). Tannic acids have great potential of antibacterial activity (Okuda, 2005). All zones produced by extracts and phytoconstituents of *Nigella sativa* (Black cumin) were compared to the standard drug having inhibition zone 39mm, 38mm, 43mm, 40mm against *Staphylococcus aureus* ,*Bacillus subtilis, Pasturilla multosida* and *Escherichia coli* respectively. About 0.5% solution of standard drug was applied. The results of this research work indicated that it has effective antibacterial activity. Over all phytoconstituents showed varying degree of antibacterial activity against all the four strains. It showed activity against gram positive as well as gram negative bacteria. Antifungal activity of thymoquinone against *Aspergillus niger* (*A. niger)* and gave 100% inhibition at 2 mg/ml. (Al Jabre *et al*., 2003). Finally, our results are in agreement with others who showed that *Nigella sativa* extracts produce antimicrobial activity against the tested organisms. Negative results do not mean absence of bioactive constituents nor is that the plant is inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of activity can thus only be proven by using large doses .The results supported the use of this plant to treat infectious diseases. Further studies on the activity directed fractionation for the isolation of respective pure compounds may result in interesting results.

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