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Antimicrobial activities of Methanolic and Aqueous extracts of *Nigella Sativa* and *Ficus Carica*

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ABSTRACT

New antimicrobials agents are needed to combat drugs resistance in microorganisms. The aqueous and methanolic extracts of *Nigella sativa* and *Ficus carica* seeds were used against bacteria (*Staphylococcus aureus* and *Escherichia coli*) and Fungi (*Candida albicans*, *Fusarium oxysporium* and *Microsporum canis*) to check their antimicrobial activities. For antibacterial assays, the volume and concentrations of extracts used were 0.5 µl (62.5 µg), 1 µl (125 µg), 5 µl (625 µg) and 10 µl (1250 µg). Specific laboratory protocols of antibacterial and antifungal assays were adopted. Absorbance of the broth bacterial culture was measured after 72 hours by using photo spectrometer at the wavelength of 600 nm (A_{600}). For antifungal assays, the volume and concentrations of extracts used were 2 µl (250µg), 4µl (500µg), 8 µl (1000 µg) and 10 µl (1250 µg). The methanolic extract of *Nigella sativa* (1µl, 125µg) and water extract of *Ficus carica* (1µl, 125µg) were found most effective against *E. coli* (A_{600} 0.003) whereas, water extract of *Ficus carica* (1µl, 125µg) was found most effective against *Staphylococcus aureus* (A_{600} 0.003). *Nigella sativa* methanolic extract (8 µl, 1000µg) was found most effective against *Candida albicans* and *Fusarium oxysporum* with inhibited fungal growth zone diameter of 15mm, while *Ficus carica* methanolic extract (10 µl, 1250 µg) was found most effective against *Fusarium oxysporum* and *Microsporum canis* with diameter of inhibited fungal growth zone 19 mm and 21 mm, respectively. These experiments showed that these extracts can be used as antibacterial agents.

Keywords: *Nigella sativa*; *Ficus carica*; Methanolic extracts; Water extracts; Antifungal; Antibacterial

INTRODUCTION

Resistance of bacteria to existing antibiotics has increased during past years. The use of natural medicinal plants in traditional medicines has been well-established (Kafaru 1994). Herbal drugs are less toxic and their active ingredients have the ability to being combined with other medicinal constituent (Manna & Abalaka 2000; Sheriff 2001). Several beneficial natural medicinal plants and their extracts have been identified with antiseptic antifungal activities in the human history with minimal side-effects (Tapsell *et al*., 2006; Lai & Roy, 2004; Sumner, 2000). For example, Thymol exhibits essential antifungal and antiseptic effect and is used in variety of products (Pierce, 1999). Bacterial, viral or fungal infections are common from the contaminated food or water, polluted air and unhygienic condition. To combat such infections the use of antimicrobial agents has

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increased and due to the extensive use of antimicrobial agents to bacteria and fungi to these agents have increased. Therefore, there is a need to search natural alternative medicines for antimicrobial activity. Research has been done on antibiotic susceptibility and resistant microorganisms to evaluate antimicrobial activities of plants' phytochemicals along with their synergistic effects as well (Compean & Ynalvez 2014).

Nigella sativa (black cumin) is "an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia". The natural extract of *Nigella sativa*is has been used to cure various infective diseases and ailments like asthma, hypertension, diabetes and cough, because of its medicinal properties (Ali & Blunden, 2003). *Nigella sativa* is also recommended for use as diuretic, diaphoretic, stomachic, liver ionic and digestive. With other ingredients, it can be used in migraine and headache, diarrhoea, indigestion, dyspepsia, obesity and sour belching. They are administrated internally for its antibilious activity in intermittent fever (Nadkarni, 1996; Usmanghani *et al*., 1997). The *Nigella sativa* has been described as useful in mercury poisoning, leprosy and sores. The seeds of *Nigella sativa* are used as anthelmintic and antibacterial (Kapoor & Saraf, 2011). Since ancient times, the herbal plants and their extracts have shown the antimicrobial effect and attempts have been made to illustrate these qualities in laboratories (Dorman *et al*., 1999). The additional attempts have been made to find out new therapeutic antimicrobial agent to eradicate infections and to overcome resistance due to most used antibiotics (Morsi, 2000; Hannan *et al*., 2008). The antimicrobial property of *Nigella sativa* could be from its active components particularly melanin and TQ (Bakathir & Abbas, 2011). The effective results as antifungal agent have been found in the essential oils and their constituents (Daferera *et al*., 2000).The antimicrobial effect of *Nigella sativa* oil extract (*in-vivo*) has been shown against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albican s*(Hanafy & Hatem, 1991). The inhibitory effects of aqueous extract of seed of *Nigella sativa* against *C. albicans* have also been shown (*in-vivo*) by Khan *et al*., 2003.

F. carica contains numerous active ingredients i.e. "phytosterols, organic acids, phenolic compounds phytosterols, anthocyanin composition, triterpenoids, coumarins, and volatile compounds" (Oliveira *et al*., 2009; Gibernau *et al*., 1997). Various parts of *F. carica* such as leaves, roots and fruits have been used traditionally for various medicinal purposes for respiratory, cardiovascular disorders, inflammatory disorders, gastrointestinal disorders (Ody, 1997). The phenolic compounds of *F. carica* exhibit antioxidant activities (Solomon *et al*., 2006). An *in-vitro* study suggested that *F. carica* fruit exhibits a highest antioxidant effect, because it contains flavonoids, polyphenols and anthocyanins. *F. carica* also showed cytotoxic and anticancer activities on various cancer cell lines (Yancheva *et al*., 2005). *T. rubrum, T. soudanense* and *S. brevicans* -the most resistant bacteria were tested against some extracts of *F. carica*. The *F. carica* has a strong antifungal activity against the opportunistic pathogenic yeasts. Its methanolic extract inhibits *C. albicans* totally. *M. canis* was strongly inhibited with methanolic and hexane extracts of *F. carica* and totally inhibited with ethyl acetate extract of *F. carica*. *A. fumigatus* was totally inhibited with ethyl acetate fraction, while the hexane fraction had a moderate inhibition against tested bacteria (Aref *et al*., 2010). "The ethyl acetate extract of *F. carica* had an inhibitory effect on the growth of five bacterial species: *E*. *fecalis*, *C*. *freundei, P*. *aeruginosa, E*. *coli* and *P*.*mirabilis*", as reported by Aref *et al*., (2010). The hexane and chloroform extracts were found most effective. *F. carica* extract also showed their effect against infectious bacteria such as "*Staphylococcus aureus*, *Enterococcus Fecalis*, *Citobacter freundei*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Echerchia coli* (Aref *et al*., 2010).

MATERIALS AND METHODS

Bacterial and Fungal species

In the current research, the methanolic and water extracts of *Nigella sativa* and *Ficus carica* were evaluated against bacterial species *Staphylococcus aureus* (pharmaceutical origin), *Escherichia coli* (Food origin) and fungal species (*Aspergillus niger, Fusarium oxysporum, Candida albicans*). The fungal and bacterial cultures were

obtained from First Fungal Culture Bank, University of the Punjab, Lahore.

Sampling of plant material

The plant samples *Nigella sativa* (black cumin**)** seeds and *Ficus carica* (Figs) were purchased from Nishtar colony, Lahore Cantonment. The samples were devoid of any contamination.

Preparation of *Nigella sativa* extract

The Methanolic and water extracts of *Nigella sativa* (black cumin) were made by following method. The 0.5 g of the seeds of *Nigella sativa* were weighed on electric balance, the crushed in pre sterilized pastel and mortal in 4 ml of methanol and water as solvent. Transferred this solution to the Eppendorf tube. In each experiment 0.5 µl (62.5 µg), 1 µl (125 µg), 5 µl (625 µg) and 10 µl (1250 µg) volumes and amounts of extracts were evaluated for antibacterial and antifungal assays (Rand et. 2011; Jennifer, 2012).

Preparation of *Ficus carica* extract

Methanolic and water extracts of *Ficus carica* (Figs) were made by following method. First measured 0.5 g of the fruits of *Ficus carica* were weight on electric balance, then crushed in pre-sterilized pastel and mortal in 4 ml of methanol and water as solvent. Transferred this solution to the eppendorf tube. In each experiment 0.5 µl (62.5 µg), 1 µl (125 µg), 5 µl (625 µg) and 10 µl (1250 µg) volumes and amounts of extracts were evaluated for antibacterial and antifungal assays.

Microfiltration of the extracts

The crushed extracts were micro-filtered by 2 micron size syringe filter to avoid any microbial contamination under aseptic condition in laminar flow. Transferred the micro-filtered extracts in eppendorf tubes and placed in freezer for subsequent analysis.

Media preparation and propagation of bacterial strains

For culturing bacteria that were used in experiment, prepared the “Luria Brittani” (LB) broth medium. The components of the medium are described in Table (I). Prepared the LB (Luria Brittani) broth in liquid and solid media. Inoculated the solid media with the inoculum of *staphylococcus aureus* (pharmaceutical origin) and *Escherichia coli* (Food origin) and incubated it in the incubator at 37 degree Celsius for 24 hours. A clear growth was obtained after 24 hours. Inoculated the LB broth medium with the inoculum of *Escherichia coli* and *Staphylococcus aureus* by picking up the colony from the solid medium with the sterilized inoculating loop under aseptic condition in a laminar flow. Incubated this LB broth medium in incubator at 37 degree Celsius for 24 hours, after that observed the bacterial growth in the LB broth medium by taking OD at 600 nm (Jennifer, 2012).

Media preparation and propagation of Fungal strains

Prepared the 100 ml media Potato Dextrose Agar (PDA) medium by adding 3.9 g of pre-mix PDA powder in 100 ml of water. Then let the media solidify and inoculated it with the inoculum of fungi *Aspergillus Niger, Fusarium oxysporum, Candida albicans*. Further, incubated this media in an incubator at 37 degree Celsius for 1 week. To analyse the antifungal assay of natural extracts, we used the filter paper disc diffusion method. Cut the filter paper in disc shape and socked it in natural extract of *Nigella sativa* plant and black cumin in following concentrations: 2 µl, 4 µl, 8 µl, and 10 µl and then placed it on fungal culture plate of *candida albicans, Fusarium oxysporum* & *Microsporum canis* at the central position and incubated the plates at 25 degree Celsius for 48 hours. After that, we analysed the antifungal activity of natural extracts by observing the growth inhibition zone.

Analysis of antibacterial assay of natural extract

Designed the 21 Eppendorf tubes to analyse the antibacterial assay in accordance with the protocol as followed. Autoclaved the blue tips, yellow tips, falcon tubes, eppendorf and LB broth media for one hour at 121 degree Celsius temperature and pressure of 18 Pascal. In eppendorf tube of 1.5 ml capacity, 0.5µl (62.5µg) and 1µl (125µg) of extract of *Ficus carica* and *Nigella sativa* (each) were added, and 5µl of bacterial culture as well as 800µl (LB) media were also transferred to each eppendorf tube. The

experiment was repeated by using the different concentration e.g. 5 µl (625 µg) and 10 µl (1250 µg) of natural extracts. The samples were incubated in the eppendorf tubes at 37 degree centigrade for 72 hours. Absorbance of the broth bacterial culture was measured after 72 hours by using photo spectrometer at the wavelength of 600 nm.

RESULTS

Antibacterial assays of *N. sativa & F. carica extracts* with *E. coli*

In the antibacterial assay, *Nigella sativa* and *Ficus carica* methanolic and water extracts were evaluated against *Escherichia coli* to explore their antibacterial potential. The volume and concentrations of extracts used were 0.5 µl (62.5 µg) and 1 µl (125 µg). In order to analyse the antibacterial assays of *Nigella sativa* and *Ficus carica* extracts, the experiment was divided into eight phases and each phase was then subdivided into two phases (Table II). To conduct an antibacterial assay, 0.5 µl (62.5 µg) of methanolic *Nigella sativa* extract was used in the first phase (A) of first experiment and resulting bacterial absorbance was found 0.004 (Table II). In the second phase (B) of first experiment 1µl (125 µg) of methanolic *Nigella sativa* extract was used and resulting bacterial absorbance was found 0.003.*Ficus carica* methanolic extract 0.5 µl (62.5µg) was used in the first part (A) of the second experiment and resulting bacterial absorbance was found 0.010 (Table II). *Ficus carica* methanolic 1µl (125µg) extract was used in the second part (B) of the second experiment and resulting bacterial absorbance was found 0.009 (Table II).

To conduct the antibacterial assay, 0.5 µl (62.5µg) of *Nigella sativa* aqueous extract was used in the first phase (A) of third experiment and resulting bacterial absorbance was found 0.005 (Table II). In the second phase (B) of third experiment 1µl (125 µg) of *Nigella sativa* aqueous extract was used resulting bacterial absorbance was found 0.004. *Ficus carica* aqueous extract 0.5 µl (62.5 µg) was used in the first part (A) of the forth experiment and in result bacterial absorbance was found 0.005 (Table II). *Ficus carica* 1µl (125µg) aqueous extract was used in the second part (B) of the forth experiment and resulting bacterial absorbance was found 0.003 (Table II). In control, containing only *E. coli* culture with no plant extract the absorbance was 0.11.

Antibacterial assays of *N. sativa & F. carica extracts* with *S. aureus*

To conduct the antibacterial assay, 0.5µl (62.5 µg) of methanolic *Nigella sativa* extract was used in first phase (A) of fifth experiment and resulting bacterial absorbance was found 0.006 (Table II). In the second phase (B) of fifth experiment 1µl (125 µg) of methanolic *Nigella sativa* extract was used and resulting bacterial absorbance was found 0.005. *Ficus carica* methanolic extract of 0.5 µl (62.5µg) was used in the first part (A) of the sixth experiment and in result bacterial absorbance noted was 0.009 (Table II). *Ficus carica* methanolic extract 1µl (125µg) was used in the second part (B) of the sixth experiment and resulting bacterial absorbance was found 0.008.

To conduct the antibacterial assay, 0.5 µl (62.5 µg) of aqueous *Nigella sativa* extract was used in the first phase (A) of seventh experiment and resulting bacterial absorbance was found 0.007 (Table II). In the second phase (B) of seventh experiment 1µl (125 µg) of aqueous *Nigella sativa* extract was used and resulting bacterial absorbance was found 0.005. *Ficus carica* aqueous extract of 0.5 µl (62.5 µg) was used in the first part (A) of the eighth experiment and in result bacterial absorbance was found 0.006 (Table II). *Ficus carica* aqueous extract of 1µl (125 µg) was used in the second part (B) of the eighth experiment and resulting bacterial absorbance was found 0.003. In control containing only *S. aureus* culture with no plant extract the absorbance was 0.13.

In Table II, more effective extracts are highlighted based on the lowest absorbance values. Hence, methanolic extract (0.5 µl, 62.5 µg) of *Nigella sativa* and aqueous extract (1 µl, 125 µg) of *Ficus carica* with A_{600} value 0.003 were found most effective against *E. coli*. Whereas, aqueous extract (1 µl, 125 µg) of *Ficus carica* with A_{600} value 0.003 was found most effective against *S. aureus*. Moreover, both methanolic as well as aqueous extracts of *Ficus carica* (1 µl, 125 µg) were effective against both *E .coli* and *S. aureus.*

Antibacterial activity (*E. coli) of N. sativa & F. carica* extracts with different high concentrations

The experiment was repeated with the different high concentrations of *Nigella sativa* and *Ficus carica* extracts to examine maximum inhibition of bacterial growth. *Nigella sativa* methanolic extract of 5 µl (625 µg) was used in the first part (A) of first experiment and resulting bacterial absorbance was found 0.002 (Table III). *Nigella sativa* methanolic extract of 10 µl (1250 µg) was used in the second part (B) of first experiment and resulting bacterial absorbance was found 0.003 (Table III). *Ficus carica* methanolic extract of 5 µl (625 µg) was used in the first phase (A) of second experiment and resulting bacterial absorbance was found 0.003 (Table III). *Ficus carica* methanolic extract of 10µl (1250µg) was used in the second phase (B) of second experiment and resulting bacterial absorbance was found 0.002 (Table III).

Nigella sativa aqueous extract of 5 µl (625 µg) were used in the first phase (A) of third experiment and resulting bacterial absorbance noted was 0.002. *Nigella sativa* aqueous extract of 10µl (1250µg) were used in the second part (B) of third experiment and resulting bacterial absorbance was found 0.003 (Table III). *Ficus carica* aqueous extract of 5 µl (625 µg) was used in the first phase (A) of forth experiment and in result bacterial absorbance was found 0.001. *Ficus carica* aqueous extract of 10 µl (1250 µg) was used in the second part (B) of forth experiment and resulting bacterial absorbance was found0.000 (Table III).

Antibacterial activity *(S. aureus) of N. sativa & F. carica* extracts with different high concentrations

The experiment was repeated with the different high concentrations of *Nigella sativa* and *Ficus carica* extracts to examine the maximum inhibition of bacterial growth. *Nigella sativa* methanolic extract of 5 µl (625 µg) as used in the first phase (A) of fifth experiment and resulting bacterial absorbance was found 0.003. *Nigella sativa* methanolic extract 10 µl (1250 µg) was used in the second part (B) of fifth experiment and in result bacterial absorbance noted was 0.000 (Table III). *Ficus carica* methanolic extract of 5µl (625 µg) was used in the first phase (A) of sixth experiment and resulting bacterial absorbance was found 0.001 (Table III). *Nigella sativa* methanolic extract of 10 µl (1250 µg) was used in the second phase (B) of the sixth experiment and resulting bacterial absorbance was found 0.000 (Table III).

Nigella sativa aqueous extract of 5 µl (625 µg) was used in the first phase (A) of seventh experiment and resulting bacterial absorbance was found 0.003.*Nigella sativa* aqueous extract of 10 µl (1250 µg) was used in the second phase (B) of seventh experiment and resulting bacterial absorbance was found 0.002 (Table III). *Ficus carica* aqueous extract 5 µl (625 µg) was used in first phase (A) of eighth experiment and resulting bacterial absorbance was found 0.002. *Nigella sativa* aqueous extract of 10 µl (1250 µg) was used in the second phase (B) of eighth experiment and resulting bacterial absorbance was found 0.001 (Table III).

In control containing only *E. coli* culture with no plant extract the absorbance (A_{600}) value was 0.11 while in second control containing only *S. aureus* culture with no plant extract the absorbance (A_{600}) value was 0.13.

In Table III, more effective extracts at higher concentrations are highlighted based on the lowest absorbance values. Hence, aqueous extract (10 µl, 1250 µg) of *Ficus carica* with A_{600} value 0.000 was found most effective against *E. coli*. Whereas, methanolic extracts (10 µl, 1250 µg) of *Nigella sativa* and *Ficus carica* with A_{600} value 0.003 were found most effective against *S. aureus*.

Antifungal activity of the *N. sativa & F. carica* extracts

The antifungal activity of the natural methanolic extracts of *Nigella Sativa* and *F. Carica* were tested by using following fungal cells: *Candida albicans, Fusarium oxysporum*and *Microsporum canis* (Table IV). With methanolic extract of *F. carica* (2 µl, 250 µg) the fungal cell-death zone was measured 8 mm, 10 mm & 11 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively. With methanolic extracts of *N. sativa* (2 µl, 250 µg), the fungal cell-death zone was measured 7 mm, 9 mm & 10 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively. With methanolic extract of *F. Carica* (4 µl, 500 µg), the fungal cell-death zone was measured 10 mm, 12 mm & 13 mm in *Candida*

albicans, Fusarium oxysporum and *Microsporum canis* respectively. With methanolic extract of *N. sativa* (4 µl, 500 µg), the fungal cell-death zone was measured 8 mm, 10 mm & 14 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively. With methanolic extract of *F.Carica* (8 µl, 1000 µg), the fungal cell-death zone was measured 12 mm, 13 mm & 14 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively. With methanolic extract of *N. sativa* (8 µl, 1000 µg), the fungal cell-death zone was measured 15 mm, 15 mm & 13 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively. With methanolic extract of *F.Carica* (10 µl, 1250 µg), the fungal cell-death zone was measured 17 mm, 19 mm & 21 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively. With methanolic extract of *N. sativa* (10 µl, 1250 µg), the fungal cell-death zone was measured 18 mm, 17 mm & 18 mm in *Candida albicans, Fusarium oxysporum* and *Microsporum canis* respectively.

In Table IV, more effective extracts for antifungal activities have been highlighted against three types of mentioned fungal cells. The maximum fungal cell-death zone (15 mm) was measured in fungal cells *Candida albicans* & *Fusarium oxysporum* with methanolic extract of *N. sativa* (8 µl, 1000 µg). Whereas, the maximum fungal cell-death zones i.e., 19 mm and 21 mm were measured in fungal cells *Fusarium oxysporum &Microsporum canis* respectively with methanolic extract of *F. carica* (10 µl, 1250 µg) (Table IV). The methanolic extracts of both *Nigella sativa* and *Ficus carica* both have antifungal activities. However, the *Nigella sativa* (8 µl, 1000 µg) extract was more effective than the *Ficus carica* extract (10 µl, 1250 µg).

DISCUSSIONS

Resistance by bacteria to existing antibiotics has increased, whereas, the medicinal plants are helpful against various ailments. Natural plant-derived based drugs are usually less toxic and their potential compounds have been verified and implemented for medicinal constituent. In current research, *Nigella sativa* and *Ficus carica* extracts were tested for their antibacterial and antifungal potentials. The methanolic extract (0.5 µl, 62.5 µg) of *Nigella sativa* and aqueous extract (1 µl, 125 µg) of *Ficus carica* with A_{600} value 0.003 were found most effective against *E. coli*. Whereas, aqueous extract (1 µl, 125 µg) of *Ficus carica* with A_{600} value 0.003 was found most effective against *S. aureus*. Moreover, both methanolic as well as aqueous extracts of *Ficus carica* (1 µl, 125 µg) were effective against both *E .coli* and *S. aureus.* Regarding high concentrations, the aqueous extract (10 µl, 1250 µg) of *Ficus carica* with A_{600} value 0.000 was found most effective against *E. coli*. Whereas, methanolic extracts (10 µl, 1250 µg) of *Nigella sativa* and *Ficus carica* with A_{600} value 0.003 were found most effective against *S. aureus*. These experiments showed that these extracts can be used as antibacterial agents. The maximum fungal cell-death zone (15 mm) was measured in fungal cells *Candida albicans* & *Fusarium oxysporum* with methanolic extract of *N. sativa* (8 µl, 1000 µg). Whereas, the maximum fungal cell-death zones i.e., 19 mm and 21 mm were measured in fungal cells *Fusarium oxysporum* & *Microsporum canis* respectively with methanolic extract of *F. carica* (10 µl, 1250 µg). The methanolic extracts of both *Nigella sativa* and *Ficus carica* both found with antifungal activities. However, the *Nigella sativa* (8 µl, 1000 µg) extract was more effective than the *Ficus carica* extract (10 µl, 1250 µg). More effectiveness was found in the case of *Nigella sativa* extract and can be exploited as antifungal agent because it showed inhibition at lower concentration as compared to *F. carica* extract. Currently, various microorganisms have developed a “resistance” as a result of overuse of diverse antimicrobial drugs against infectious diseases. Moreover, different adverse side effects of antibiotics have convinced scientists to discover new natural plant-based antimicrobial agents (Al Yousaf *et al*., 2012). Numerous studies have been confirmed the broad pharmacological spectrum of *N. sativa* including antimicrobial, anticancerous, anti-oxidant and anti-inflammatory actions (Ahmad *et al*., 2013). Now current pharmaceutical industry is considering medicinal plants as an effective resource (Cavero *et al*., 2013; Barolo *et al*., 2014).

A Japanese study examined the antibacterial activity of *Ficus microcarpa*’s acetate extract. This study affirmed the role of *F. microcarpa* as effective antibacterial agent against Gram-negative and Gram-positive bacteria owing

high level of phenolic compounds in it. The inhibition zones of extracts were tested against "*Bacillus subtilis*, *Escherichia coli*, *Bacillus brevis*, *Bacillus cereus* and *Achromobacter polymorph"* (Ao *et al*., 2008). Aref *et al*., (2010) investigated antimicrobial proprieties through chloroform, hexane, methanolic and ethyl acetate extracts of *Ficus carica* against different bacteria and different strains of fungi via. minimal inhibition concentration for antibacterial activity and inhibition percentage method for antifungal activity. The ethyl acetate extract showed an inhibition in following five bacterial species: "*Enterococcus fecalis, Citobacter freundei, Pseudomonas aeruginosa, Echerchia coli* and *Proteus mirabilis"*. In pathogenic yeasts, ethyl acetate and chloroform fractions showed an effective inhibition. They found that the methanolic fraction had a total inhibition against *Candida albicans* at a concentration of 500 µg/ml (Aref *et al*., 2010). *F. carica* has been tested for the treatment of viral skin infections. A study tested five different extracts for *F. carica* for its antiviral properties against herpes simplex type 1, echovirus type 11 and adenovirus (Lazreg Aref *et al*., 2011). They concluded that hexane and hexane–ethyl acetate extracts were effective in inhibiting multiplication of viruses (Lazreg Aref *et al*., 2011). A study by Hashemi *et al*., (2011) evaluated *Ficus carica* role on stomach cancer. It was concluded that *Ficus carica* can inhibit proliferation of cancer cell line due to its proteolytic enzymes. Another study also evaluated the antibacterial potential of methanol extract of *Ficus carica*. This extract was found effective against oral bacteria (Jeong *et al*., 2009). A Korean study evaluated the antimicrobial activity of *Ficus carica* against methicillin- resistant *Staphylococcus aureus* isolate. It was found that methanolic extract of *Ficus carica* along with ampicillin induced an inhibition of more than 4-8 fold in all tested bacteria (Lee and Cha, 2010). Mahmoudi *et al*., (2016) mentioned that different extracts of *Ficus carica* exhibited antibacterial (*Bacillus cereus* and *Staphylococcus aureus*) activity and moderate antifungal activity. It was also reported Al Yousaf *et al*., (2012) regarding antioxidant and antibacterial potential of Ficus carica by disc diffusion and broth dilution technique against three different Gram positive ("*Bacillus subtilis, Staphylococcus aureus*, and *Bacillus megaterium*) and three different Gram negative bacterial strains (*Pseudomonas aeruginosa, Escherichiacoli* and *Proteus vulgaris"*).

There is an extensive increase in resistant bacterial strains to several antimicrobial agents. The essential oil of *N. sativa* was studies for its antibacterial activity against different bacterial isolates to many antibiotics. Salman *et al*., (2008) used disc agar diffusion technique. This oil showed a dose-dependent antibacterial activity against Gram positive and Gram bacteria. Among 144 strains, 97 strains were inhibited by black cumin's essential oil. The role of *N. sativa* as antimicrobial has also been affirmed from various researches with its different crude extracts against Gram positive and Gram negative bacteria. The most effective extracts were the crude alkaloid (methanol) and water extracts. Moreover, Gram negative isolates were affected more than the gram positive isolates (Morsi, 2000). Ferdous *et al*., (1992) tested antibacterial activity of the volatile oil of *Nigella sativa* seeds against isolates of "*Shigella dysenteriae 1, Shigella flexneri, Shigella sonnei* and *Shigella boydii* and strains of *Vibrio cholerae* and *Escherichia coli".* Most of the strains were resistant to ampicillin, co-trimoxazole and tetracycline. All the strains tested showed promising sensitivity to the volatile oil (Ferdous *et al*., 1992). The antifungal activity of *N. sativa* was determined by Mahmoudv and *et al*., (2014) through disk diffusion procedure and from the evaluation of minimum inhibitory concentration through broth macrodilution method. Moreover, they tested cytotoxic activity of *N. sativa* through colorimetric assay. It was concluded that *N. sativa* seeds can be used in natural medicines for the treatment of dermatophytic infections (Mahmoudvand *et al*., 2014). The contents of N. sativa along with its active compounds such as thymoquinone has been tested for its potential against nephrotoxicity and hepatotoxicity and other diseases like dermatitis. The seeds or oils of *N. sativa* have been proved as cytoprotective, antioxidant, antipyretic, antimicrobial and analgesic agents with minimum adverse side effects on liver or kidneys (Ali and Blunden, 2003). Khan *et al*., (2013) has reported that a water extract of *N. sativa* seeds against *Candida albicans*. Therefore, such extracts can be used as antifungal

agents. Halawani *et al*., (2009) has investigated antibacterial two major components (i.e., thymoquinone and thymohydroquinone) of N. sativa against "*Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Salmonella* Typhimurium*, Salmonella Enteritidis* and *Staphylococcus aureus".* This study mentioned that both thymoquinone and thymohydroquinone components bear antibacterial activity especially in case of *S. aureus* (Halawani *et al*., 2009). A commonest Methicillin resistant *Staphylococcus aureus* is a major health problem in clinical practice. Therefore, new alternative plant-based antibacterial drugs are required to synthesize. *Nigella sativa* can be used as anti-*staphylococcal* activity (Hannan *et al*., 2008). Hannan *et al*., (2008) used disc diffusion and in agar dilution methods and observed inhibitory effects on Methicillin resistant *Staphylococcus aureus.*

A Saudian research by Al-Ghamdi (2001) explained the anti-inflammatory properties and pharmacological impacts on antibacterial activities through *N. sativa*. It was also proved by this study that the inhibition of an antibacterial activity was greater with *N. sativa* as compared to thymoquinone. Chanda and Kaneria (2011) mentioned that crude extracts of plants are more effective because synergistic effects are part of the anti-microbial inhibition activities. Through agar diffusion method, they showed that the leaves of such plants exhibit promising antimicrobial properties which can be used in the treatment of various infectious diseases including alternative of resistant antibiotics as a natural remedy. Erkan at al., (2008) studied anti-oxidant properties of pure compounds *N. sativa*'s essential oil through radical scavenging assays and ferric thiocyanate tests. A Pakistani review article by Gilani *et al*., (2004) mentioned *N. sativa*'s medicinal properties as digestive, antidiarrheal, analgesic, antibacterial, antihypertensive, anticancer, antimicrobial, antioxidant and immunomodulator. An Egyptian study mentioned the use of *N. sativa's* oil to treat skin diseases and as liver tonic. It was also mentioned that N. sativa would also be beneficial in using it as supplement (Ramadan, 2007).It has also been demonstrated that *N. sativa's* oil and seed components especially thymoquinone shows potential immunotherapeutic properties in immunomodulation (Salem, 2005). Salman *et al*., (2008) explained the remarkable role of *N. sativa* in dealing with various gram positive and gram negative bacteria resistant drugs

A review paper by Mawa *et al*., (2013) discussed phytochemicals of *F. carica* as traditional medicine for antimicrobial diseases and cancer. They also suggested more research should be conducted in the biological activities of such plant's phenolic, organic acids and volatile compounds to discover more natural therapeutical agents. A latest research by Mopuri *et al*., (2017) evaluated *F. carica's* extracts' ant diabetic, antioxidant and antiobesogenic effects in vitro. They mentioned that the ethanolic extract of the fig fruit contains a high amount of polyphenols and flavonoids. Therefore, they concluded that the effect by the ethanolic extract of this fruit was significantly greater than other extracts. The pastes of figs have been prescribed for patients with urinary stones and in asthma (Patil and Patil, 2011). Barolo *et al*., (2014) explained that *F. carica* is an ancient source of health and food as it has a lot of medicinal properties due to its chemistry and pharmacological properties. It has various antiviral, antibacterial properties and also it is a good inhibitor of proliferation of many cancer cell lines as an anti-carcinogenic agent. Similarly, the antibacterial effects of *F. carica* extracts have been tested on tomato plant's bacterial pathogens (Balestra *et al*., 2009). Ali *et al*., (2010) also explained the anti-inflammatory and antioxidant properties of *F. carica's* leaves in various ailments. It was mentioned that *F. carica's* leaves contain free-radical scavenging activities and thus it can be used as a drug safely.

Table (1) Components of Luria Brittani (LB) Broth Medium

Sr. No	Ingredients	g/ml
1	Trypton	1g
2	Yeast extract	0.5g
3	Sodium chloride	1g
4	Water	100 ml

Table (II) Antibacterial Assays of Plant Extracts at Low Concentrations

Experiment No.	Part of Experiment	Plant	Solvent for Extract	Volume and Amount of Extract	Bacterial culture	Absorbance (600 nm)
1	**A**	*Nigella sativa*	Methanol	0.5 µl, 62.5 µg	*E. coli*	0.004
	B			1 µl, 125 µg		0.003
2	**A**	*Ficus carica*	Methanol	0.5 µl, 62.5 µg		0.010
	B			1 µl, 125 µg		0.009
3	**A**	*Nigella sativa*	Water	0.5 µl, 62.5 µg		0.005
	B			1 µl, 125 µg		0.004
4	**A**	*Ficus carica*	Water	0.5 µl, 62.5 µg		0.005
	B			1 µl, 125 µg		0.003
5	**A**	*Nigella sativa*	Methanol	0.5 µl, 62.5 µg	*S. aureus*	0.006
	B			1 µl, 125 µg		0.005
6	**A**	*Ficus carica*	Methanol	0.5 µl, 62.5 µg		0.009
	B			1 µl, 125 µg		0.008
7	**A**	*Nigella sativa*	Water	0.5 µl, 62.5 µg		0.007
	B			1 µl, 125 µg		0.005
8	**A**	*Ficus carica*	Water	0.5 µl, 62.5 µg		0.006
	B			1 µl, 125 µg		0.003

Key: Grey highlighted are most effective extracts.
In control containing only *E. coli* culture with no plant extract the absorbance (A_{600}) value was 0.11 while in second control containing only *S. aureus* culture with no plant extract the absorbance (A_{600}) value was 0.13.

Table (III) Antibacterial Assays of Plant Extracts at High Concentrations.

Experiment No.	Part of Experiment	Plant	Solvent for Extract	Volume and Amount of Extract	Bacterial Culture	Absorbance (600 nm)
1	**A**	*Nigella sativa*	Methanol	5 µl, 625 µg	*E. coli*	0.002
	B			10 µl, 1250 µg		0.003
2	**A**	*Ficus carica*	Methanol	5 µl, 625 µg		0.003
	B			10 µl, 1250 µg		0.002
3	**A**	*Nigella sativa*	Water	5 µl, 625 µg		0.002
	B			10 µl, 1250 µg		0.003
4	**A**	*Ficus carica*	Water	5 µl, 625 µg		0.001
	B			10 µl, 1250 µg		0.000
5	**A**	*Nigella sativa*	Methanol	5 µl, 625 µg	*S. aureus*	0.003
	B			10 µl, 1250 µg		0.000
6	**A**	*Ficus carica*	Methanol	5 µl, 625 µg		0.001
	B			10 µl, 1250 µg		0.000
7	**A**	*Nigella sativa*	Water	5 µl, 625 µg		0.003
	B			10 µl, 1250 µg		0.002
8	**A**	*Ficus carica*	Water	5 µl, 625 µg		0.002
	B			10 µl, 1250 µg		0.001

Key: Grey highlighted are most effective extracts. In control containing only *E. coli* culture with no plant extract the absorbance (A_{600}) value was 0.11 while in second control containing only *S. aureus* culture with no plant extract the absorbance (A_{600}) value was 0.13.

Table (IV). Antifungal Assays of Plant Extracts at High Concentrations

Plant	Solvent	Volume (µl) and Amount of Extract (µg)	Fungal Cells Death Zone Diameter (mm)		
			Candida albicans	***Fusarium oxysporum***	***Microsporum canis***
Ficus carica	Methanol	2 µl, 250 µg	8	10	11
Nigella sativa	Methanol	2 µl, 250 µg	7	9	10
Ficus carica	Methanol	4 µl, 500 µg	10	12	13
Nigella sativa	Methanol	4 µl, 500 µg	8	10	14
Ficus carica	Methanol	8 µl, 1000 µg	12	13	14
Nigella sativa	Methanol	8 µl, 1000 µg	15	15	13
Ficus carica	Methanol	10 µl, 1250 µg	17	19	21
Nigella sativa	Methanol	10 µl, 1250 µg	18	17	18

Key: Grey highlighted are more effective extracts. 1cm= 10mm.

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