Research Article

Determination of Antimicrobial Potential of Economically Important Persimmon (*Diospyros kaki*) and Date Plum (*Diospyros lotus*)

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Abstract | This study presents a preliminary investigation of the antimicrobial properties of various solvent extracts including aqueous, methanolic crude, ethyl acetate, hexane, and butanol from the dried leaves of *Diospyros kaki* and *Diospyros lotus*. The antimicrobial efficacy was assessed against four bacterial species and one fungal strain using a disc diffusion assay. The results indicated that the ethyl acetate and n-butanol extracts from the leaves of both *Diospyros* species exhibited significant inhibitory activities against all the tested microorganisms. Notably, the crude methanol extract of *Diospyros lotus* demonstrated the highest zone of inhibition (57%) against *Escherichia coli*. The ethyl acetate extract fraction of *Diospyros kaki* showed a strong inhibitory effect on *E. coli* (54%), while *Diospyros lotus* exhibited a slightly higher inhibition rate of 55%. Additionally, n-butanol extracts of both species displayed notable inhibition (52%) against *E. coli* and *Pseudomonas aeruginosa*. However, both *E. coli* and *Staphylococcus aureus* showed resistance to the n-hexane fraction from the leaves of *Diospyros kaki*. Moreover, *P. aeruginosa* was completely resistant to both aqueous and n-hexane fractions from both plant species. All leaf-extracted fractions from *Diospyros kaki* and *Diospyros lotus* exhibited considerable activity against *Candida albicans*, except for the n-hexane fraction of *Diospyros kaki*, which showed no activity. In conclusion, the leaf extracts of *Diospyros lotus* demonstrated greater antimicrobial potency compared to those of *Diospyros kaki*.

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Introduction

Traditional medicines, particularly those derived from medicinal plants, have been utilized across various cultures to address a multitude of health conditions (Partel *et al*., 2005). These plants contain specific bioactive compounds that may offer protection against a diverse array of microbial species. Currently, the increasing prevalence of antibiotic resistance poses a significant public health challenge and represents a major obstacle within the pharmaceutical industry. Medicinal plants are crucial in the development of new pharmaceuticals, and to fully harness their antimicrobial potential, it is essential to conduct experimental

Links Researchers

assessments of plants from various agro-climatic regions for their possible antimicrobial applications. According to Shinwari *et al.* (2011), approximately 12% of the more than 6,000 flowering plant species identified in Pakistan possess potential medicinal properties. *Diospyros kaki* (persimmon) and *Diospyros lotus* (date plum) are two prominent and widely cultivated members of the *Ebenaceae* family (Bibi *et al*., 2007). This family encompasses four genera and roughly 500 species. Generally, *Diospyros* species are deciduous trees that can reach heights of approximately 9 meters and widths of 6 meters, predominantly found in tropical and subtropical regions worldwide (Uddin *et al*., 2011; Singh and Joshi, 2011; Kim and Lee, 2014). The genus *Diospyros* is well-regarded for its multiple benefits in traditional medicine, being valued for its therapeutic properties across various cultures. Different parts of *Diospyros* plants are utilized for their medicinal properties; for instance, the fruits are known to alleviate flatulence and biliousness, while the leaves are employed to relieve back pain. Additionally, the bark exhibits astringent and bitter qualities, with sedative effects attributed to its seeds (Pant and Samant, 2010). Numerous studies have successfully isolated various bioactive compounds from different parts of *Diospyros* plants (Rice-Evans *et al*., 1997; Yamada *et al*., 1999; Prakash *et al*., 2000; Ahn *et al*., 2002; An *et al*., 2005; Jung *et al*., 2005; Chen *et al*., 2007; Gu *et al*., 2008; Igual *et al*., 2008; Sun *et al*., 2011; Takashi *et al*., 2011; Lee *et al*., 2012; Rashed *et al*., 2013; Ueda *et al*., 2013; Abozaid *et al*., 2014; Butt *et al*., 2015). The fruits of *Diospyros kaki* are recognized for their nutritional, laxative, stomachic, and astringent properties. The leaves possess antimicrobial, antithrombotic, and anti-wrinkle effects. The calyx and peduncle are traditionally used to treat hiccups and coughs. Conversely, the fruit of *Diospyros lotus* is utilized for treating constipation and acts as both a central and peripheral analgesic (Said *et al*., 2009). Furthermore, it exhibits antidiabetic, antitumor, antioxidant, antiseptic, laxative, sedative, nutritive, and astringent properties (Azadbakht *et al*., 2011). The fruit of *Diospyros lotus* may also be beneficial in treating dry coughs, hypertension, and diarrhoea. This study aims to conduct an antimicrobial assay on the leaf extracts from both *Diospyros kaki* and *Diospyros lotus*.

Materials and Methods

Plant material

Leaves of *Diospyros kaki* were collected from the Horticulture Research Farm at the University of Agriculture, Peshawar, while leaves of *Diospyros lotus* were sourced from the Swat district. Further analyses were conducted at the Nanobiotechnology Laboratory of the Institute of Biotechnology and Genetic Engineering.

Extraction and fractionation

The leaves from both plant species were shade-dried and subsequently ground into a homogeneous powder using an Infinigen™ Tissue Mixer Mill. A crude extract was prepared by soaking the powdered leaves in 97% methanol within a flask. This mixture was allowed to stand at room temperature for one week, with regular stirring to facilitate extraction (Chen *et al*., 2003). The initial methanol was filtered from the crude extract using Whatman filter paper, after which additional methanol was added to enhance the extraction of bioactive compounds. A rotary evaporator was employed to remove methanol from the crude extract at 45°C (Sasidharan *et al*., 2011). The resulting crude extract for each plant was divided into two portions: one portion (10 g) was retained as the methanol crude extract, while the other portion (100 g) underwent further fractionation. For fractionation, the larger portion (100 g) of crude extracts from both *Diospyros kaki* and *Diospyros lotus* was dissolved in distilled water and transferred into a separatory funnel. The solvent-solvent partitioning was performed sequentially, starting with less polar solvents and progressing to more polar ones, specifically using n-hexane, ethyl acetate, and n-butanol to obtain n-hexane soluble, ethyl acetate soluble, and n-butanol soluble fractions, respectively. The fractions were recovered using a rotary evaporator (Rota vapor R-R 210/R215; BUCHI Labortechnik AG) and dried to yield semi-solid materials. These semi-solid extracts were further dried in a water bath at 45°C (Kousar *et al*., 2023).

Culture media and microorganisms

Nutrient agar-modified media supplied by HiMedia Laboratories Pvt. Ltd. was utilized for culturing and growing microorganisms. Broth-modified media were used for incubating selected microbial strains (Supriatin *et al*., 2021). The bacterial strains *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*, while the fungal strain *Candida albicans* were used in this study.

Disc diffusion method

The disc diffusion method (DDM), also known as the agar diffusion method (ADM), was employed to evaluate the sensitivity or resistance of microorganisms to antibiotics, plant extracts, and other tested samples. In this method, the plant extract diffuses over an agar medium inoculated with the test microorganism. A filter paper disc serves as a reservoir for the tested sample and is placed on the agar surface. If the plant extracts or isolated compounds exhibit antimicrobial activity, an inhibition zone will form around the disc following incubation (Horvath *et al*., 2016; Sharma, 2022). The diameter of this inhibition zone is indicative of the antimicrobial potency of the plant extracts or individual compounds. For testing the solvent extracts of *Diospyros kaki* and *Diospyros lotus* leaves, the disc diffusion method was applied to establish zones of inhibition in microbial cultures (Bakht *et al*., 2014a). Ciprofloxacin at a concentration of 50 µg/6 µl was used as a control for Gram-negative bacteria while erythromycin at 50 µg/6 µl served as a control for Gram-positive bacteria. Fluconazole at 50 µg/6 µl was used as a control for the fungal strain.

Results and Discussion

The antimicrobial potential of five different solvent extracts from the leaves of *Diospyros kaki* and *Diospyros lotus* was evaluated against four bacterial strains and one fungal strain in this study. The ethyl acetate fraction of *Diospyros kaki* and the methanol crude fraction of *Diospyros lotus* exhibited the highest zones of inhibition measuring 54% and 57%, respectively against *Escherichia coli* at a concentration of 3 mg disc^{-1} (Figure 1). Notably, the n-hexane fraction at all tested concentrations as well as the aqueous fraction at 1 mg disc^{-1} did not demonstrate any zone of inhibition (Figure 1A). The n-butanol and methanol fractions of *Diospyros lotus* showed significantly higher antimicrobial activity compared to the corresponding fractions of *Diospyros kaki* (Figure 1B). The aqueous fractions of *Diospyros kaki* and *Diospyros lotus* displayed zones of inhibition measuring 43% and 31%, respectively at a concentration of 3 mg disc^{-1} (Figure 1). For *Bacillus subtilis*, the ethyl acetate fraction of *Diospyros kaki* and the n-butanol fraction of *Diospyros lotus* revealed the highest zones of inhibition measuring 41% and 47%, respectively at a concentration of 3 mg disc^{-1} (Figure 2). The n-hexane fractions of *Diospyros kaki* exhibited no activity, while *Diospyros lotus* showed the lowest activity at all the tested fractions (Figure 2A, B). The methanol, aqueous, and n-butanol fractions from *Diospyros kaki* demonstrated an increasing trend in their antimicrobial activity yielding zones of inhibition measuring 34%, 35%, and 38% respectively (Figure 2A). In contrast for *Diospyros lotus*, aqueous, ethyl acetate and methanol fractions also exhibited a progressive increase in activity with zones measuring 34%, 35%, and 38% (Figure 2B). The ethyl acetate fraction from *Diospyros kaki and* n-butanol fraction from *Diospyros lotus* showed significant zone inhibition against *Pseudomonas aeruginosa* measuring 52% at a concentration of 3mg disc^{-1} (Figure 3). Notably, n-butanol fraction from *D. kaki* exhibited significant activity at 2mg disc^{-1} and 3mg disc^{-1} concentrations but showed no activity concentration at 1mg disc^{-1}. Conversely, the methanol and n-hexane, aqueous fractions did not display any activity at any concentrations (Figure 3A).

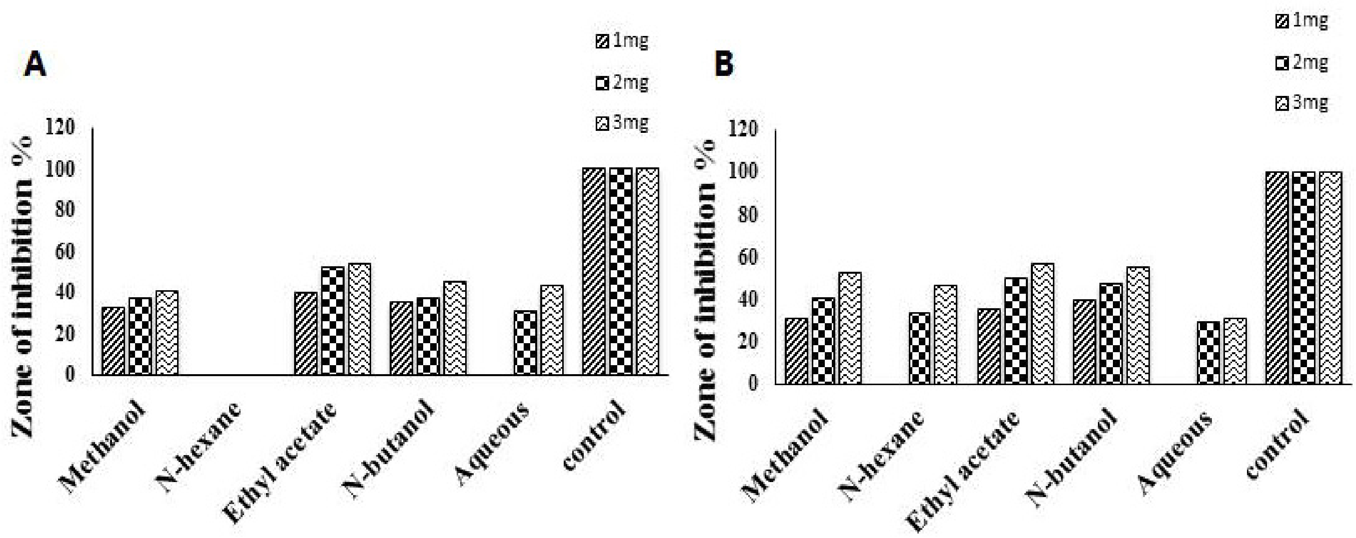

Figure 1: *Antibacterial activity of different leaves solvent extracts of (A) Persimmon and (B) Date plum against E. coli.*

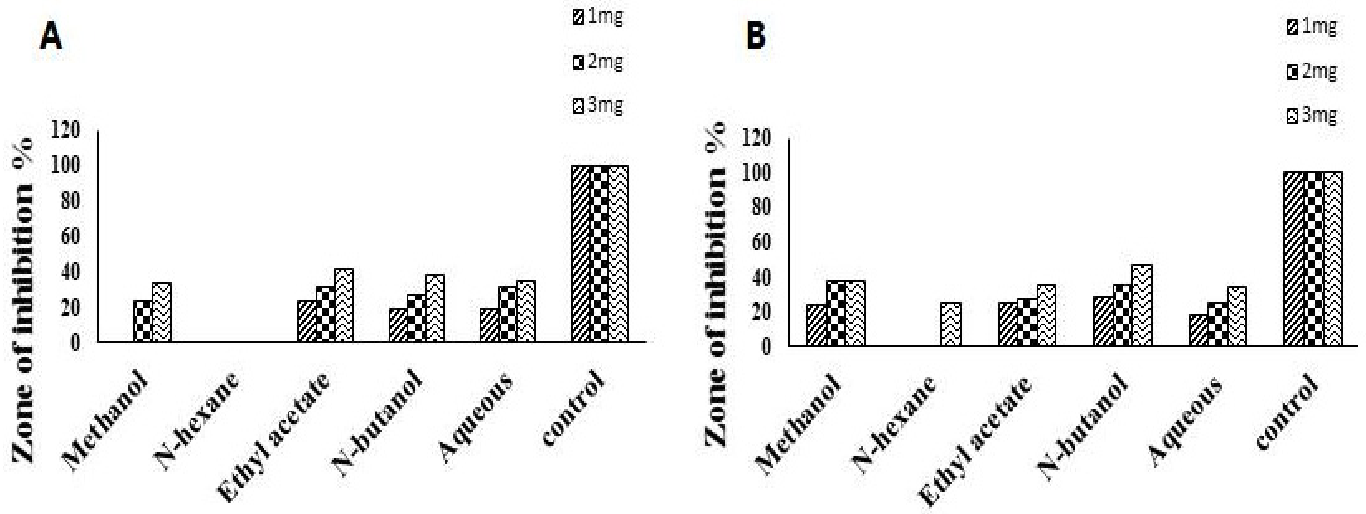

Figure 2: *Activity of 5 different solvents extracted samples from leaves of (A) Persimmon and (B) Date plum against B. subtilis.*

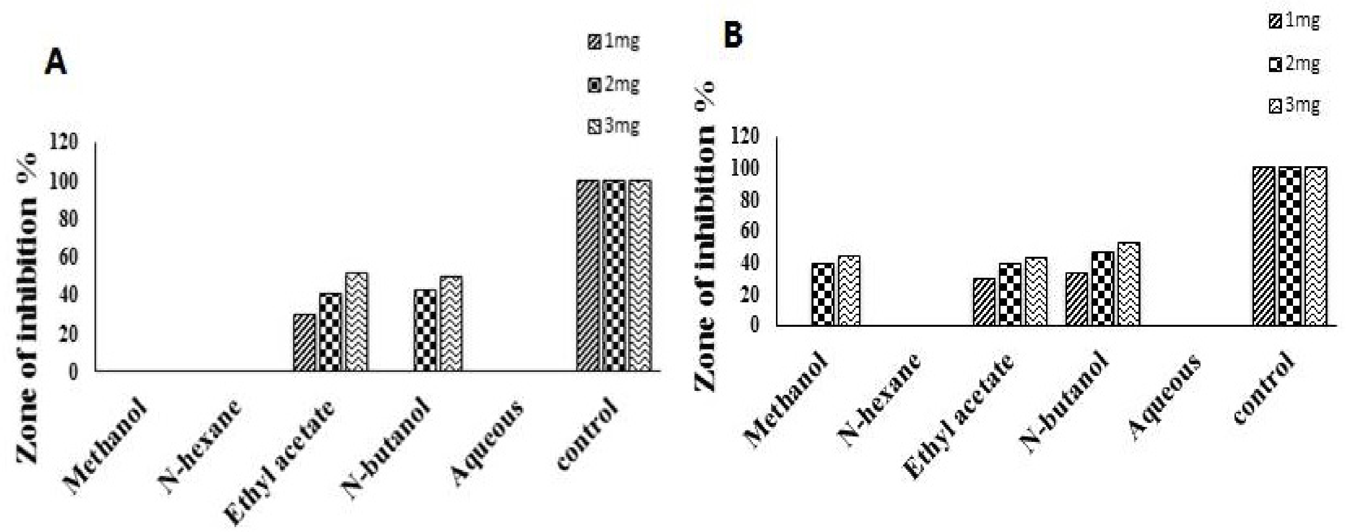

Figure 3: *Antibacterial potential of leaves extracted samples of (A) Persimmon and (B) Date plum against P. aeruginosa.*

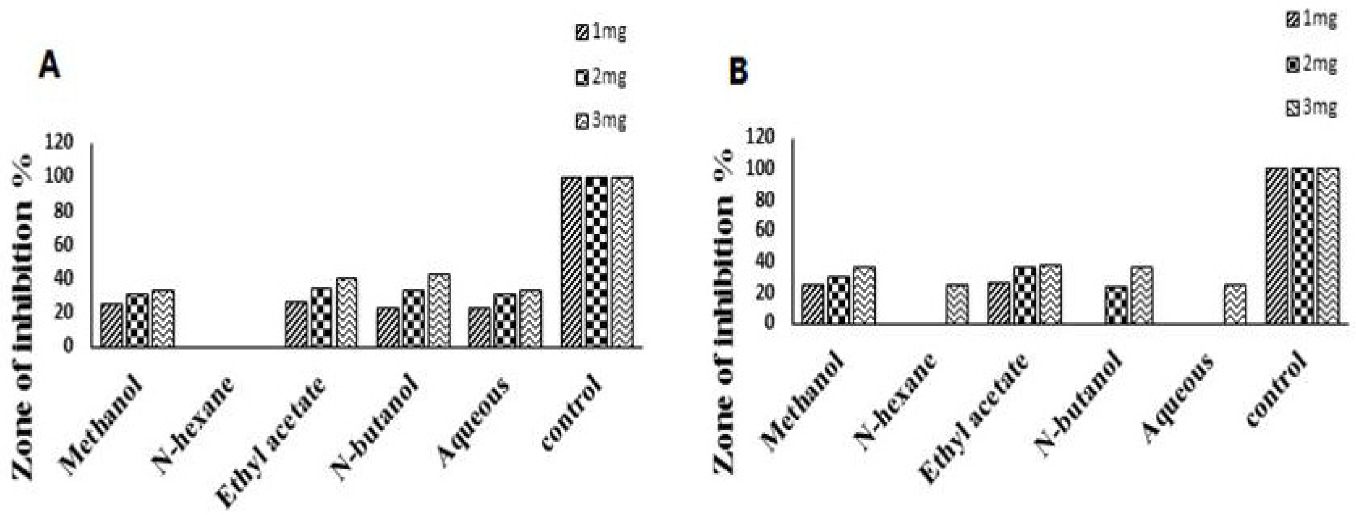

Figure 4: *Different leaf extracts of (A) Persimmon and (B) date plum showing the extent of zones of inhibition contrary to S. aureus.*

The methanolic extract demonstrated significant activity at higher concentrations but showed no activity at the lowest concentration, similarly, both n-hexane aqueous fractions exhibited no activity across all concentrations (Figure 3B). The n-butanol fraction of *Diospyros kaki* leaves recorded the highest zone of inhibition against *Staphylococcus aureus* followed by ethyl acetate, methanolic crude, and aqueous fractions with zones measuring 43%, 40%, 34%, and 33% respectively at 3mg disc^{-1} concentration. In contrast tested strain *S. aureus* exhibited complete resistance to n-hexane fraction across all three concentrations (Figure 4A). The ethyl acetate extract of *Diospyros lotus* leaves showed 38% inhibitory activity against this bacterial strain. Both n-butanol and crude samples showed zone inhibition measuring 36% at the highest concentration however methanol extract exhibited zero percent inhibition at the lowest concentration (Figure 4B). The aqueous and n-hexane fractions were comparatively less effective for inhibiting the growth of *S. aureus,* achieving only 25 % inhibition at 3mg disc^{-1} concentration with zero percent inhibition observed at lower concentrations (Figure 4B). The antifungal activity of various extracts from leaves *Diospyros kaki* and *Diospyros lotus* against *Candida albicans* is illustrated in Figure 5. The crude methanol fraction of *D. kaki* leaves produced the highest zone of inhibition at 42%, closely followed by both ethyl acetate and n-butanol fractions, which showed similar zones of 41% at a concentration of 3 mg disc^{-1}. The n-hexane fraction was found ineffective against this fungal strain at any tested concentration while the aqueous fraction revealed a noticeable zone of inhibition (Figure 5A). The ethyl acetate fraction of *D. lotus* leaves again demonstrated significant inhibitory effects followed by methanol with an inhibition zone measuring up to 48%. Both aqueous and n-hexane fractions of *D. lotus* leaves displayed some level of inhibitory activity across all concentrations except the lowest concentration where no activity was noted (Figure 5B).

Diospyros kaki and *Diospyros lotus* are economically significant fruit crop species cultivated in various regions worldwide. This study employed a disc diffusion susceptibility assay to evaluate the antimicrobial activity of different solvent extracts of *D. kaki* and *D. lotus* leaves against four selected bacterial strains and one fungal strain used. *E. coli* strain exhibited a lower level of resistance in ethyl acetate, and n-butanol extracts of both *D. kaki* and *D. lotus*. These findings align with those reported by Nwaozuzu et al. (2016), and Rahman et al. (2015). At elevated concentrations (3mg disc^{-1}) crude extracts from *D. lotus* leaves demonstrated significant inhibitory efficacy consistent with previous studies. The maximum inhibitory activity (3 mg disc^{-1}) observed from crude extracts of D. lotus corresponds to earlier reports by Selvamohan et al. (2012). Notably a significant activity against *E. coli* was recorded for n-hexane-extracted leaf samples of *D. lotus* producing substantial zone inhibition (46%). In contrast, n-hexane fraction leaves of *D. kaki* showed no activity at any concentrations suggesting it lacks specific antimicrobial compounds that are effective against *E. coli.* These findings are supported by Ahmad et al. (2015). The highest inhibition against *Bacillus subtilis* growth was recorded for n-butanol extracted samples of *D. lotus* followed by an ethyl acetate fraction of *D. kaki* (3mg disc^{-1}). These results are consistent with earlier studies of Bakht et al. (2014b), Muthu et al. (2014), Ali et al. (2015). Crude extracts of both species exhibited significant antibacterial activity against *B. subtilis* at all tested concentrations compared to controls and other fractions corroborating previous findings of Chaudhary et al. (2012). Additionally, all three concentrations including aqueous extracts reduced the growth of *B. subtilis* indicating that except for n-hexane extracted samples, all other solvent extracts of *D. kaki* demonstrated inhibitory activity against this bacterium. Our results indicate that the aqueous fractions were less effective against *Staphylococcus aureus* however, the ethyl acetate, crude, and n-butanol

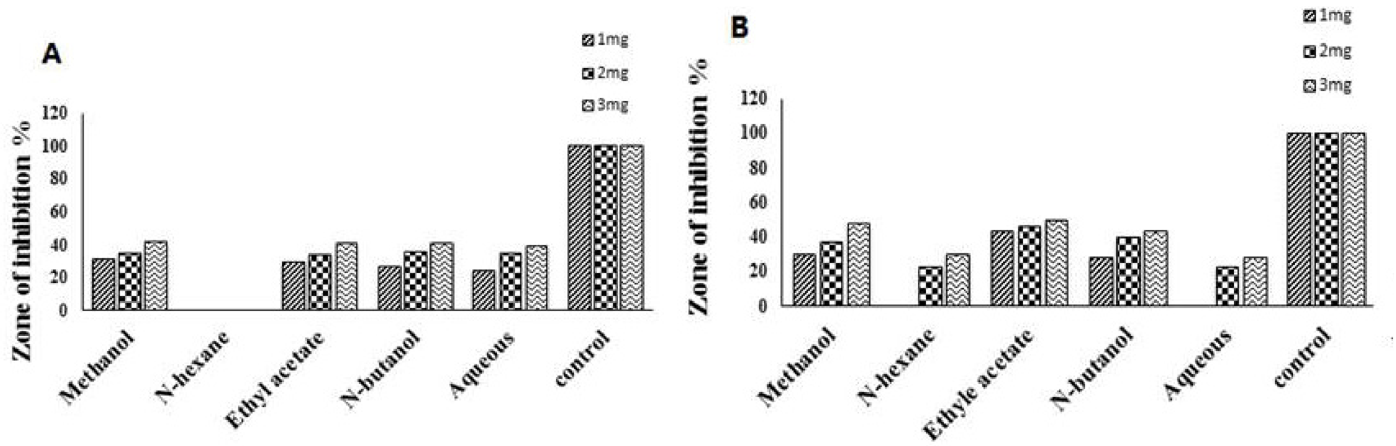

Figure 5: *The extent of zones of inhibition developed in C. albicans cultures using leaf extracts of (A) Persimmon and (B) date plum.*

extracted fractions of both species exhibited inhibitory effects against this pathogen. These observations supported previous reports (Javed *et al*., 2015; Ejaz *et al*., 2014). The n-butanol fraction from *D. kaki* demonstrated the strongest inhibitory effect against *S. aureus*, followed by the ethyl acetate extract, which is consistent with the findings of Machado *et al*. (2003) and Abozaid *et al*. (2014). Crude extracts of *D. lotus* leaves inhibited the growth of *S. aureus* at all tested concentrations. Neither n-hexane extracted fractions of either species exhibited activity against *Pseudomonas aeruginosa* at any concentration when compared to controls and other fractions. Conversely, ethyl acetate extracted fractions of both species displayed significant inhibitory activity against *P. aeruginosa* resulting in zones of inhibition measuring 52% and 43%, respectively. The n-butanol fractions of both plants also demonstrated notable activity against this pathogen aligning results reported by Mamta and Monica (2016). Aqueous extracted fractions of both species showed no activity against *P. aeruginosa* corroborating the findings of Philip *et al*. (2009). Crude-extracted fractions of both species exhibited significant antifungal activities against *Candida albicans*, yielding zones of inhibition measuring 42% and 48%, respectively. Similar results have been reported by Toledo *et al*. (2015). At the highest concentration (3mg disc^{-1}) n-hexane fraction from *D. lotus* showed a zone of inhibition measuring 30% while the same fraction of *D. kaki* leaves did not exhibit any inhibitory effect against the fungal strain at any concentration. The ethyl acetate fractions of both species resulted in zones of inhibition measuring 41% and 50%, respectively against *C. albicans*. The effectiveness of ethyl acetate extracts against fungal pathogens has been previously documentedby Bakht *et al*. (2013). The n-butanol fractions of both species showed inhibition zones of 43% and 41% at the highest concentration inhibiting the growth of *C. albicans*. Similar findings were reported by Oliveira *et al*. (2014), and More *et al*. (2008). Additionally, an aqueous extracted fraction from *D. kaki* yielded zone inhibition measuring 41% against *C. albicans* while a moderate inhibition zone (28%) was recorded by an aqueous fraction from *D. lotus*. Overall, all derived extracts demonstrated potential antimicrobial activity, which justifies further exploration into isolating bioactive compounds.

Conclusions and Recommendations

This study has revealed that the leaves of both *Diospyros kaki* and *Diospyros lotus* have antimicrobial potential through differential activities as shown by the various extracts of these two plant species. This potential can be exploited in the future for the determination of new plant-based antimicrobial drugs.

Novelty Statement

The antimicrobial potential of economically important persimmon (*Diospyros kaki*) and date plum (*Diospyros lotus*) leaves was assessed for the first time, making this a novel research endeavor.

Author's Contribution

Sundus Malik: Performed the experiment and wrote the article.
Dawood Ahmad and Muhammad Sayyar Khan: Designed the experiments.
Irfan Safdar Durrani: Carried out statistical analysis.

Conflict of interest
The authors have declared no conflict of interest.

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