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Antimicrobial activity of *Piper nigrum* L. and *Cassia didymobotyra* L. leaf extract on selected food borne pathogens

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ABSTRACT

Objective: To investigate the antimicrobial activity of leaf extract of *Piper nigrum* (*P. nigrum*) and *Cassia didymobotyra* (*C. didymobotyra*) (aqueous, methanol, ethanol and petroleum ether) against the food borne pathogenic bacteria [*Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Salmonella typhimurium* and *Pseudomonas aeruginosa*)] and fungi [*Aspergillus* spp. and *Candida albicans* (*C. albicans*)] and also to investigate the presence of various phytochemicals in the leaf extracts of tested plants.

Methods: The antimicrobial activity was determined by disc diffusion method. Minimum inhibitory concentration (MIC), minimum bactericidal and fungicidal concentration were determined by serial dilution method.

Results: Methanol leaf extract of test plants exhibited greater antimicrobial activity against the selected bacterial and fungal strains. The MIC results showed that ethanol, methanol and petroleum ether leaf extract of *P. nigrum* inhibited the growth of *S. aureus* and *E. coli* at concentration of 12.5 mg/mL. While, ethanol and methanol leaf extracts of *C. didymobotyra* inhibited the growth of *S. aureus* at concentration of 6.25 mg/mL. The MIC values for ethanol, methanol and petroleum ether leaf extract of *P. nigrum* inhibited the growth of *C. albicans* at concentration of 25.0 mg/mL. While, it was reported that at concentration of 12.5 mg/mL methanol leaf extract of *P. nigrum* was against the *Aspergillus* spp. The MIC values of methanol leaf extract of *C. didymobotyra* inhibited the growth of *C. albicans* and *Aspergillus* spp. at concentration of 12.5 mg/mL and 6.25 mg/mL, respectively. The minimum bactericidal concentration of ethanol, methanol leaf extract of *P. nigrum* for *E. coli* and ethanol, methanol leaf extract of *C. didymobotyra* for *S. aureus* was recorded at concentration 12.5 mg/mL. The minimum fungicidal concentration of ethanol and methanol leaf extract of *P. nigrum* and *C. didymobotyra* on *C. albicans* was recorded at concentration of 25.0 mg/mL, while, it was found at concentration of 50.0 mg/mL for petroleum ether and aqueous leaf extract of *P. nigrum* and *C. didymobotyra*. However, the MIC of methanol leaf extracts of *P. nigrum* and ethanol and methanol leaf extracts *P. nigrum* and *C. didymobotyra* for *Aspergillus* spp. was recorded at concentration of 12.5 mg/mL, while, the MIC concentration ranged from 25.0-50.0 mg/mL for other tested solvent leaf extract of *P. nigrum* and *C. didymobotyra*.

Conclusions: This study suggests that test plants could be potential candidates for developing the new antimicrobial drugs against the wide range of pathogenic bacteria and fungal strains.

1. Introduction

Plant can serve as a source of innovative therapeutic agents against the infectious diseases[1]. The natural products derived from medicinal plants have been used for years in traditional medicine to treat the different diseases and many of them possess antimicrobial activities[2,3]. According to an estimate, about 80% of the world population depends on plant derived products as medicine to meet the demands[4]. In general, the extracts of medicinal plant had antibacterial and antifungal activities due to presence of isoflavones, anthocyanins, and flavonoids compounds. Therefore, plant extracts or plant based products have been widely used for preservation of food against common food borne pathogens[5].

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In developing countries, food poisoning and food borne infection caused *Salmonella* spp., *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Aspergillus* and *Candida* spp. been recognized as serious problem for the deterioration of the quality of food products[6,7]. In this regard, use of medicinal as antimicrobial agents is cost-effective, environmentally safe and an alternative tool to control the microbial infections in the resistant cases[8]. Thus, the medicinal plants can serve as potential reservoir for the development of novel chemotherapeutic agents[9].

The genus *Piper nigrum* (*P. nigrum*) L. (Piperaceae) is used as spices in many countries of the world due to presence of piperine, which may contribute its value as a food additive. It has been also used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhea. Thakare found that ethanolic fruit extracts of *P. nigrum* showed the antibacterial activity against penicillin G resistant strain of *S. aureus*[10]. While, Sasidharan and Menon reported oil extract of *P. nigrum* was found effective against the *Bacillus subtilis* (*B. subtilis*) and *P. aeruginosa*[11]. Similarly, the genus *Cassia didymobotyra* (*C. didymobotyra*) L. (Fabaceae) has been grown as a wild crop or weed and unequivocally used in Ayurvedic medicine. The leaves and roots have been used in treatment of constipation common cold, fevers, intestinal disorders, skin disorders. Hossain *et al*. found that *Cassia senna* leaves had potential to control the growth of pathogenic microorganisms[12]. Recently, one study showed that several bioactive molecules had been extracted *Cassia* plants, and all these bioactive compounds exhibited strong antioxidant and antimicrobial activity against the pathogenic microorganisms[13]. Similarly, Panda and Ray found that the ethanolic leaf extracts of *Cassia* sp. had the antifungal activity against *Candida albicans* (*C. albicans*) and *Aspergillus niger* (*A. niger*)[14].

According to an estimate, approximately 800 medicinally important plants have been frequently used in the traditional healthcare system, of which about 80% of Ethiopian populations were due to their therapeutic properties[15,16], and these medicinally important plants also played a significant role in the management of various human and livestock diseases[17]. There is no published report or any work done in the past two decades to explore the potentials of *P. nigrum* and *C. didymobotyra* against the infectious food borne pathogens in Ethiopia. The aim of this study is to investigate the antimicrobial activity of *P. nigrum* and *C. didymobotyra* leaf extract on selected food borne bacterial [*S. aureus*, *Salmonella typhimurium* (*S. typhimurium*), *E. coli* and *P. aeruginosa*] and fungal (*C. albicans* and *Aspergillus* sp.) pathogens and to investigate the presence of various phytochemicals in the leaf extracts of tested plants.

2. Material and methods

2.1. Collection and preparation of plant materials

Fresh leaves of *P. nigrum* and *C. didymobotyra* were collected in plastic bag from Jimma Agricultural Research Station, and surrounding area of Jimma University, Jimma town, respectively. The collected plant samples were identified by the plant taxonomist belonging to Department of Biology and further the collected plant materials were then transported to Research and Post Graduate Laboratory for the further processing. After transporting the collected leaves, samples were washed gently with tap water followed by sterilized distilled water to remove the adhering dust and soil particles and dried in shaded place at room temperature for 10 d in order to prevent the decomposition of active compounds. After drying, the leaves were chopped into small pieces and grinded into fine powdery form using mechanical grinder (Hamburg 76, West Germany).

2.2. Extraction procedure and preparation of plant extracts

The plant materials were extracted by maceration process. Four types of extracts *viz.* aqueous, ethanol, methanol and petroleum ether extracted from both the plants, separately. About 50 g powder of each plant was soaked into 250 mL of distilled water, ethanol, methanol and petroleum ether, separately. The flasks were properly labeled and incubated at room temperature in a rotator shaker at 150 r/min for 72 h. The crude extracts of each plant with their respective solvents were filtered into another flask of 250 mL capacity with Whatman No.1 filter paper and the supernatant were discarded. The ethanol, methanol and petroleum ether extracts of each plant were concentrated at 40 °C, while the aqueous extract at 80 °C under reduced pressure in Rota Vapour (Heidolph, Germany).

2.3. Microorganisms used for study

The tested bacterial pathogens include *S. aureus* (ATCC 25923), *S. typhimurium* (ATCC 13311), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) and fungal pathogens (*C. albicans*

(ATCC 90028), and *Aspergillus* sp. (JUAS 27031). The pure cultures of all the tested microorganisms were obtained from Ethiopian Nutritional Health Research Laboratory, Addis Ababa, Ethiopia. While, the *Aspergillus* sp. was obtained from Research and Post Graduate Laboratory, Department of Biology, Jimma University, Ethiopia.

2.3.1. Standardization of inocula

Active cultures for bacterial pathogens were prepared by transferring a loop-full of bacterial cells from nutrient agar slants into test tubes containing Mueller-Hinton broth. After that, the test tubes were incubated without agitation for 24 h at 37 °C. The suspension turbidity was adjusted to a 0.5 McFarland turbidity standard (1.2×10^8 CFU/mL). Similarly, the fungal pathogens were prepared by transferring a loop-full of fungal cells from Sabourauds dextrose agar slants into test tubes containing Sabouraud dextrose broth followed by the incubation of the test tubes without agitation for 72 h at 28 °C. The suspension turbidity was adjusted to a 0.5 McFarland turbidity standard (1.0×10^6 CFU/mL).

2.4. Antimicrobial activity

2.4.1. Preparation of disk for antimicrobial activity

The disk of about 6 mm diameter was prepared from Whatman No. 1 filter paper using paper puncher.

2.4.2. Antibacterial test of plant extracts

Antibacterial activity of plant extracts were tested by agar disc diffusion method[18]. About 100 mg/mL extracts of both plants (aqueous/ethanol/methanol/petroleum ether) were poured onto the Whatman filter paper disc placed on the Petri plates pre-inoculated with bacterial pathogens. Dimethyl sulfoxide (DMSO) (10 μg/μL) was used as negative control against all the tested bacterial pathogens. While, vancomycin (5 μg/disc) was used as positive control for *S. aureus* and gentamycin (10 μg/disc) for the positive control against *E. coli*, *S. typhimurium*, and *P. aeruginosa*. The experiment was performed in triplet and all the Petri plates were allowed for 10 min at room temperature for the diffusion of the extract and then incubated 37 °C for 24 h for bacterial growth. After incubation, the antibacterial activity was indicated by a clear zone of inhibition and measured with the help of ruler.

2.4.3. Antifungal test of plant extracts

The antifungal activity of the plant extract (*Aspergillus* spp. and *C. albicans*) was tested by disc diffusion method as described by Hasan *et al*[19]. Similar to antibacterial activity, antifungal activity was also performed in triplets. DMSO (10 μg/μL) was used as negative control and antibiotics ketoconazole (10 μg/disc) was used as positive control for both the fungal strains. All the Petri plates were allowed for 10 min at room temperature for the diffusion of the extract and then incubated 28 °C for 72 h for fungal growth. The antifungal activity was indicated by a clear zone of inhibition and measured with the help of ruler.

2.4.4. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The MIC of the tested plants against the bacterial pathogens was determined by method of Makut *et al*. with some modifications[20]. To test the MIC, the leaf extracts of both plants exhibited antibacterial activity were diluted with nutrient broth in a series of six test tubes with concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/mL prepared from stock solution (500 mg/mL). The control test tubes were also prepared simultaneously and all the tubes were incubated at 37 °C for 24 h. The lowest concentration of leaf extracts of each plant that produced no visible bacterial growth (no turbidity) compared to control was considered as MIC. The MBC was determined from MIC, according to standard protocol of National Committee for Clinical Laboratory Standards (NCCLS)[21]. For MBC, about 1 mL of mixture of plant extract and bacterial culture was pipetted from the MIC tubes and one step higher concentration than MIC, and the pipetted mixtures were streaked on Mueller-Hinton agar medium on Petri plates and incubated for 24 h at 37 °C. The least concentration of the extract with no visible bacterial growth after incubation was taken as MBC.

Similarly, the MIC of both the tested plants against the fungal pathogens was determined using the serial dilution method[22]. To determine the MIC, Sabourauds dextrose broth (5 mL) was poured in the set of six pre-sterilized test tubes for each tested fungal strain with concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/mL prepared from the stock solution. The control test tubes were also prepared simultaneously and all the tubes were incubated at 28 °C for 72 h. The lowest concentration of leaf extracts of each plant that produced no visible fungal growth (no turbidity) compared to control was considered as MIC for fungal strains. The MFC was determined from MIC, according to standard protocol of NCCLS[21]. About 1 mL of mixture of plant extract and fungal culture was pipetted from the MIC tubes and one step higher concentration than MIC, and the pipetted mixtures were streaked on Sabourauds dextrose agar medium on Petri plates and incubated for 72 h at 28 °C.

The least concentration of the extract with no visible fungal growth after incubation was taken as MFC.

2.5. Phytochemical screening methods of plant materials

Phytochemical screening was performed by the method of Ayoola *et al*[23].

2.5.1. Test for reducing sugars (Fehling's test)

The extracts (0.5 g in 5 mL of water) were added to boiling Fehling's solution in a test tube. The solution was observed for a colour reaction. The presence of reddish colour indicates the positive test for reducing sugars.

2.5.2. Test for anthraquinone

About 0.5 g of each plant extract were boiled with 10 mL of H_2SO_4 and filtered. The filtrate was shaken with 5 mL of $CHCl_3$. The layer of chloroform was pipette into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for colour changes. The presence of pink colour indicates the positive test for anthraquinone.

2.5.3. Test for terpenoids (Salkowski's test)

About 0.5 g of each plant extract were added to 2 mL of $CHCl_3$ followed by addition of concentrated H_2SO_4 (3 mL) carefully to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

2.5.4. Test for flavonoids

To test the presence of flavonoids, first diluted ammonia (5 mL) was added to a portion of filtrate of the respective plant extracts followed by addition of concentrated H_2SO_4 (1 mL). The appearances of yellow colour, which disappear on standing, indicate the presence of flavonoids, and secondly, a few drops of 1% aluminum solutions were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids.

2.5.5. Test for saponins

About 0.5 g of each plant extract was added to 5 mL of distilled water in a test tube and the solution was shaken vigorously to observe a stable persistent froth. The frothing was obtained by mixing with 3 drops of olive oil. It was again shaken vigorously to observe the formation of an emulsion, which indicates the presence of saponins.

2.5.6. Test for tannins

About 0.5 g of each plant extract was boiled in 10 mL of distilled water in a test tube and filtered. A few drops of 0.1% $FeCl_3$ were added to the filtrate. The presence of brownish green or blue black color indicates the presence of tannins.

2.5.7. Test for alkaloids

About 0.5 g of extract was diluted to 10 mL with acid alcohol, boiled and filtered. About 2 mL of dilute ammonia followed was added to 5 mL of the filtrate followed by the addition of 5 mL of $CHCl_3$ and shaken gently to extract the alkaloidal bases. The $CHCl_3$ layer was extracted with 10 mL of acetic acid. In the 5 mL of the resulting extracts, about 3 drops of Mayer's reagent were added. The formation of a cream precipitate indicates the presence of alkaloids.

2.6. Statistical analysis

The entire data were statistically analyzed by SPSS version 16.0 (Chicago, Inc., USA) and Tukey's test was employed to denote the significant difference between the treatments at P=0.05.

3. Results

3.1. Antimicrobial activity

3.1.1. Antibacterial activity of plant extracts

All the tested leaf extract (aqueous, ethanol, methanol and petroleum ether) of both plants showed the antibacterial activity against the tested bacterial strains. Minimum antibacterial activity was observed in aqueous leaf extract of both plants. However, maximum antibactial activity was observed in methanol leaf extract of both plants followed by ethanol and petroleum ether leaf extracts. Aqueous, ethanol, methanol and petroleum ether leaf extract of *P. nigrum* showed 8.00, 12.33. 16.33, 10.67 mm zone of inhibition against the *S. aureus*, 9.33, 14.67, 20.00, 11.33 mm zone of inhibition against *E. coli*, 8.67, 10.00, 12.00, 9.33 mm zone of inhibition against *S. typhimurium*, and 8.00, 10.33, 11.67, 9.67 mm zone of inhibition against *P. aeruginosa*, respectively (Table 1). Similarly, aqueous, ethanol, methanol and petroleum ether leaf extract of *C. didymobotyra* showed 8.67, 18.33, 20.67, 12.67 mm zone of inhibition against *S. aureus*, 10.67, 19.33, 18.00, 13.33 mm zone of inhibition against *E. coil*, 9.67, 16.33, 18.00, 13.33 mm zone of inhibition against *S. typhimurium*, 8.67, 13.33, 16.67, 11.67 mm zone of inhibition against *P. aeruginosa*, respectively (Table 1). However, the methanol leaf extract of both the plants showed the greater inhibition zone against all the tested bacterial strains compared to other tested solvent leaf extracts (Table 1). Vancomycin

used as positive control against *S. aureus* showed zone of inhibition of 27.33 mm, while, gentamycin used as positive control against *E. coli*, *S. typhimurium* and *P. aeruginosa* showed zone of inhibition of 28.00, 26.00 and 26.67 mm, respectively. DMSO used as negative control against all the tested bacterial strains showed no zone of inhibition against the tested bacterial strains (Table 1).

Table 1

Antibacterial activity of different solvent leaf extracts of *P. nigrum* and *C. didymobotyra* against tested bacterial strains.

Extracts	Zone of inhibition (mm±SD)			
	S. aureus (ATCC 25923)	*E. coli* (ATCC 25922)	*S. typhimurium* (ATCC 13311)	*P. aeruginosa* (ATCC 27853)
PNET	12.33±0.58^{cde}	14.67±0.58^{c}	10.00±1.73^{de}	10.33±2.08^{cd}
PNMT	16.33±2.08^{bc}	20.00±1.73^{b}	12.00±1.73^{de}	11.67±1.16^{cd}
PNPE	10.67±1.53^{de}	11.33±1.16^{cd}	9.33±0.58^{e}	9.67 ±1.53^{cd}
PNAQ	8.00±1.00^{e}	9.33±0.58^{d}	8.67±1.16^{e}	8.00±1.00^{d}
CDET	18.33±2.31^{b}	19.33±2.08^{b}	16.33±1.16^{bc}	13.33±1.53^{bc}
CDMT	20.67±2.52^{b}	21.67±1.53^{b}	18.00±2.00^{b}	16.67±2.31^{b}
CDPE	12.67±1.53^{cd}	14.00±1.73^{c}	13.33±1.53^{cd}	11.67±2.08^{cd}
CDAQ	8.67±0.58^{de}	10.67±2.08^{cd}	9.67±1.53^{de}	8.67±0.58^{d}
Positive control	27.33±1.16^{a}	28.00±1.00 ^{a}	26.00±1.00^{a}	26.67±1.16^{a}
Negative control	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{e}

Values within each column followed by same letter are not significantly different at $P<0.05$ by Tukey's test. Vancomycine was used as positive control against *S. aureus*, while, gentamycin was used as positive control against the *E. coli*, *S. typhimurium* and *P. aeruginosa*. DMSO was used as negative control against all the tested bacterial strains.

PNET=*P. nigrum* ethanol extract; PNMT=*P. nigrum* methanol extract; PNPE=*P. nigrum* petroleum ether extract; PNAQ=*P. nigrum* aqueous extract; CDET=*C. didymobotyra* ethanol extract; CDMT=*C. didymobotyra* methanol extract; CDPE=*C. didymobotyra* petroleum ether extract; CDAQ=*C. didymobotyra* aqueous extract.

3.1.2. Antifungal activity of plant extracts

All the tested leaf extract (aqueous, ethanol, methanol and petroleum ether) of both plants showed the antifungal activity against the tested fungal strains. Minimum antifungal activity was observed in aqueous leaf extract of both plants. However, maximum antifungal activity was found in methanol leaf extracts of both the plants followed by ethanol and petroleum ether leaf extract. Aqueous, ethanol, methanol and petroleum ether leaf extract of *P. nigrum* showed 8.33, 10.00, 12.67, 9.67 mm zone of inhibition against *C. albicans*, while, 11.33, 17.33, 19.67, 15.33 mm zone of inhibition against *Aspergillus* sp. (Table 2). Similarly, aqueous, ethanol, methanol and petroleum ether leaf extract of *C. didymobotyra* showed 9.33, 11.33, 12.00, 10.00 mm zone of inhibition against *C. albicans* and 12.00, 18.67, 21.33, 16.33 mm zone of inhibition against *Aspergillus* sp. Ketoconazole used as positive control against both the tested fungal strains showed 17.33 and 24.67 mm zone of inhibition respectively, while, DMSO used as negative control against both the tested fungal strains showed no zone of inhibition against the tested fungal strains (Table 2).

Table 2

Antifungal activity of different solvent leaf extracts of *P. nigrum* and *C. didymobotyra* against tested fungal strains.

Extracts	Zone of inhibition (mm±SD)	
	C. albicans (ATCC 90028)	*Aspergillus* sp. (JUAS 27031)
PNET	10.00±1.00^{bc}	17.33±1.53^{cd}
PNMT	12.67±1.16^{b}	19.67±2.08^{bc}
PNPE	9.67±0.58^{bc}	15.33±1.16^{d}
PNAQ	8.33±0.58^{c}	11.33±1.53^{e}
CDET	11.33±1.53^{bc}	18.67±1.16^{bcd}
CDMT	12.00±1.73^{bc}	21.33±1.16^{b}
CDPE	10.00±1.73^{bc}	16.33±1.53^{cd}
CDAQ	9.33±1.16^{bc}	12.00±2.00^{e}
Positive control	17.33±2.31^{a}	24.67±1.16^{a}
Negative control	0.00±0.00^{d}	0.00±0.00^{f}

Values within each column followed by same letter are not significantly different at $P<0.05$ by Tukey's test. Ketoconazole was used as positive control against *C. albicans* and *Aspergillus* sp. DMSO was used as negative control against both the tested fungal strains.

PNET=*P. nigrum* ethanol extract; PNMT=*P. nigrum* methanol extract; PNPE=*P. nigrum* petroleum ether extract; PNAQ=*P. nigrum* aqueous extract; CDET=*C. didymobotyra* ethanol extract; CDMT=*C. didymobotyra* methanol extract; CDPE=*C. didymobotyra* petroleum ether extract, CDAQ=*C. didymobotyra* aqueous extract.

3.2. MIC, MBC and MFC

3.2.1. MIC

The MIC results showed that ethanol, methanol and petroleum ether leaf extract of *P. nigrum* inhibited the growth of *S. aureus* and *E. coli* at concentration of 12.5 mg/mL, while, the MIC was found in between concentration of 25.0-50.0 mg/mL for *S. typhimurium* and *P. aeruginosa*. Moreover, the aqueous leaf extract inhibited the growth of *S. aureus*, *S. typhimurium* and *P. aeruginosa* at concentration of 50.0 mg/mL, while, the MIC was recorded at concentration of 12.5 mg/mL for *E. coli* (Table 3). However, MIC was recorded at concentration of 25.0 mg/mL for *C. albicans* and it ranged in between the concentration of 12.5-25.0 mg/mL for *Aspergillus* sp. for ethanol, methanol and petroleum ether leaf extract of *P. nigrum*, while, MIC for aqueous leaf extract of *P. nigrum* was recorded at concentration of 50.0 mg/mL for *C. albicans* and *Aspergillus* sp. (Table 4).

Similarly, the MIC results for ethanol, methanol and petroleum ether leaf extract of *C. didymobotyra* inhibited the growth of *S. aureus* ranging in between the concentration of 6.25-12.50 mg/mL, while, it was recorded in between the concentration of 6.25-25.0 mg/mL for *S. typhimurium* and concentration of 12.5-25.0 mg/mL for *E. coli* and *P. aeruginosa* (Table 3). However, the MIC recorded for ethanol, methanol and petroleum ether leaf extract of *C. didymobotyra* against the *C. albicans* and *Aspergillus* sp., ranged in between the concentration of 12.5-25.0 mg/mL and

6.25–12.50 mg/mL, respectively, while, MIC for aqueous leaf extract of *C. didymobotyra* was recorded at concentration of 50.0 mg/mL for *C. albicans* and concentration of 25.0 mg/mL for *Aspergillus* sp. (Table 4).

Table 3

MIC and MBC value for different solvent leaf extracts of *P. nigrum* and *C. didymobotyra* against tested bacterial strains.

Extracts	*S. aureus* (ATCC 25923)		*E. coli* (ATCC 25922)		*S. typhimurium* (ATCC 13311)		*P. aeruginosa* (ATCC 27853)	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
PNET	12.5	25.0	12.5	12.5	25.0	50.0	50.0	50.0
PNMT	12.5	25.0	12.5	12.5	25.0	25.0	25.0	50.0
PNPE	12.5	25.0	12.5	25.0	50.0	50.0	50.0	50.0
PNAA	50.0	50.0	25.0	25.0	50.0	50.0	50.0	50.0
CDET	6.25	12.5	12.5	25.0	12.5	25.0	12.5	25.0
CDMT	6.25	12.5	12.5	25.0	6.25	12.5	12.5	12.5
CDPE	12.5	25.0	25.0	25.0	25.0	25.0	25.0	25.0
CDAQ	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0

PNET=*P. nigrum* ethanol extract; PNMT=*P. nigrum* methanol extract; PNPE=*P. nigrum* petroleum ether extract; PNAQ=*P. nigrum* aqueous extract; CDET=*C. didymobotyra* ethanol extract; CDMT=*C. didymobotyra* methanol extract; CDPE=*C. didymobotyra* petroleum ether extract, CDAQ=*C. didymobotyra* aqueous extract.

3.2.2. MBC/MFC

The MBC results showed that ethanol, methanol and petroleum ether leaf extract of *P. nigrum* and *C. didymobotyra* inhibited the growth of all tested bacterial strains ranging in between the concentration of 12.5–50.0 mg/mL (Table 3). The MBC of ethanol, methanol, and petroleum ether leaf extract of *P. nigrum* was recorded at concentration of 25.0 mg/mL for *S. aureus*. The MBC was recorded at concentration of 12.5 mg/mL for ethanol and methanol leaf extract of *P. nigrum* for *E. coli*, while it was recorded at concentration of 25.0 mg/mL for petroleum ether and aqueous leaf extracts of *P. nigrum* for *E. coli* and methanol extracts of *S. typhimurium*, whereas in other cases it was recorded at concentration of 50.0 mg/mL (Table 3). Similarly, MBC was recorded at concentration of 12.5 mg/mL for ethanol and methanol leaf extract of *C. didymobotyra* for *S. aureus* and petroleum ether extracts for *S. typhimurium* and *P. aeruginosa*. However, it ranged in between the concentration of 25.0–50.0 mg/mL for other solvent leaf extracts against the tested bacterial strains (Table 3).

The MFC results showed that ethanol, methanol and petroleum ether leaf extract of *P. nigrum* and *C. didymobotyra* inhibited the growth of all tested fungal strains ranging in between the concentration of 12.5–50.0 mg/mL (Table 4). The MFC was recorded for ethanol and methanol leaf extract of *P. nigrum* and *C. didymobotyra* at concentration of 25.0 mg/mL for *C. albicans*, while, it was recorded at concentration of 50.0 mg/mL for petroleum ether and aqueous leaf extract of *P. nigrum* and *C. didymobotyra*. The MFC was recorded at concentration of 12.5 mg/mL for methanol leaf extract of *P. nigrum* for *Aspergillus* sp., and ethanol and methanol leaf extract of *C. didymobotyra*. Moreover, MFC was recorded at concentration of 25.0 mg/mL for ethanol and petroleum ether leaf extract of *P. nigrum* for *Aspergillus* sp., and petroleum ether leaf extract of *C. didymobotyra*, while, it was recorded at concentration of 50.0 mg/mL for the aqueous leaf extract of *P. nigrum* and *C. didymobotyra* against the tested fungal strains (Table 4).

Table 4

MIC and MBC value for different solvent leaf extracts of *P. nigrum* and *C. didymobotyra* against the tested fungal strains.

Extracts	*C. albicans*		*Aspergillus* sp.	
	MIC (mg/mL)	MFC (mg/mL)	MIC (mg/mL)	MFC (mg/mL)
PNET	25.0	25.0	12.5	25.0
PNMT	25.0	25.0	12.5	12.5
PNPE	25.0	50.0	25.0	25.0
PNAA	50.0	50.0	50.0	50.0
CDET	25.0	25.0	12.5	12.5
CDMT	12.5	25.0	6.25	12.5
CDPE	25.0	50.0	12.5	25.0
CDAQ	50.0	50.0	25.0	50.0

PNET=*P. nigrum* ethanol extract; PNMT=*P. nigrum* methanol extract; PNPE=*P. nigrum* petroleum ether extract; PNAQ=*P. nigrum* aqueous extract; CDET=*C. didymobotyra* ethanol extract; CDMT=*C. didymobotyra* methanol extract; CDPE=*C. didymobotyra* petroleum ether extract; CDAQ=*C. didymobotyra* aqueous extract.

3.3. Phytochemical screening

The phytochemical screening of tested plants showed the presence of alkaloids, anthraquinone, flavonoids, reducing sugars, saponins, tannins and terpenoids (Table 5). *P. nigrum* petroleum ether extracts showed the absence of anthraquinone and terpenoids, while its aqueous extracts were tested negative for anthraquinones, tannins and terpenoids. *C. didymobotyra* petroleum ether extracts showed the absence of anthraquinones and terpenoids, while its aqueous extracts were tested negative for the presence of tannins (Table 5).

Table 5

Presence of various phytochemical constituents in the various solvent leaf extracts of *P. nigrum* and *C. didymobotyra*.

Extracts	Phytochemicals						
	Alkaloids	Anthraqui-none	Flavonoids	Reducing sugars	Saponins	Tannins	Terpenoids
PNET	+	+	+	+	+	+	+
PNMT	+	+	+	+	+	+	+
PNPE	+	–	+	+	+	+	–
PNAQ	+	–	+	+	+	–	–
CDET	+	+	+	+	+	+	+
CDMT	+	+	+	+	+	+	+
CDPE	+	–	+	+	+	+	–
CDAQ	+	+	+	+	+	–	+

+: Presence; –: Absence. PNET=*P. nigrum* ethanol extract; PNMT=*P. nigrum* methanol extract; PNPE=*P. nigrum* petroleum ether extract; PNAQ=*P. nigrum* aqueous extract; CDET=*C. didymobotyra* ethanol extract; CDMT=*C. didymobotyra* methanol extract; CDPE=*C. didymobotyra* petroleum ether extract; CDAQ=*C. didymobotyra* aqueous extract.

4. Discussion

It is evident from our results that aqueous, ethanol, methanol and petroleum leaf extract of *P. nigrum* and *C. didymobotyra* had the strong antimicrobial activity against the bacterial and fungal strains. Amongst all the tested solvent leaf extracts of both plants, methanol leaf extract had the maximum bacterial and antifungal activity against the tested bacterial and fungal strains. Pundir and Jain reported that aqueous, ethanolic and methanolic extracts of *P. nigrum* exhibited antimicrobial activity against *Bacillus megaterium*, *Bacillus sphaericus*, *Bacillus polymyxa*, *S. aureus* and *E. coli*, while, Hasan *et al*. found that chloroform root extract of *Polygonum hydropiper* showed significant antibacterial activities against the *B. subtilis*, *Bacillus megaterium*, *S. aureus*, *Enterobacter aerogenes*, *E. coli*, *P. aeruginosa*, *Salmonella typhi* and *Shigella sonnei*[19,24]. However, Shanmugapriya *et al*. reported that methanolic leaf extracts of *P. nigrum* had considerable antifungal activity, while, ethyl acetate extract was feeble antifungal activity even at higher concentration. Moreover, Rao and Suresh reported that the acetone and methanol leaf extract of *Cassia sophera* possessed inhibitory activity against the *Staphylococcus citreus*, and *S. aeures*, *E. coli*, *Klebsiella*, and *C. albicans*[25,26].

Bhalodia *et al*. reported that *Cassia fistula* extracts were effective against Gram positive (*S. aureus* and *Streptococcus pyogenes*), Gram negative (*E. coli* and *P. aeruginosa*) bacterial strains and fungal strains (*A. niger*, *Aspergillus clavatus* and *C. albicans*) with zone of inhibition ranging from 10.00-20.00 mm for bacterial strains and 12.00-21.00 mm for fungal strains. Similarly, Panda and Ray found that methanolic and ethanolic extracts of *Cassia fistula* L. had antifungal activity against *A. niger*, *Aspergillus flavus* and *Aspergillus fumigatus* with zone of inhibition greater than 12.00 mm[14]. Our results showed that the aqueous leaf extract in both plants exhibited the least antimicrobial activity with zone of inhibition ranging in between 8.67-10.67 mm for bacterial and 9.33-12.00 mm for fungal strains which was contradictory to the findings of Sharma *et al*[28]. They have reported that aqueous leaf extract of *Cassia tora* had maximum antibacterial activity against *S. aureus*, *Lactobacillus* and moderate activity against *P. aeruginosa*, *Proteus vulgaris* and *Enterobacter* compared to ethanolic extracts.

Amongst all the tested bacterial strains, *E. coli* exhibited the highest susceptibility towards all the solvents' leaf extract of *P. nigrum* with maximum inhibition zone (20.00 mm and 14.67 mm) for methanol and ethanol extracts. Similarly, *S. aureus* exhibited the highest susceptibility towards methanol and ethanol extract of *P. nigrum* with 16.33 mm and 12.33 mm inhibition zone, respectively. However, the results of leaf extract of *C. didymobotyra* for *S. aureus*, and *E. coli* exhibited the highest susceptibility towards methanol extract with 20.67 mm and 21.67 mm zone of inhibition, respectively. Similarly, Saganuwan and Lawal reported that *E. coli* was sensitive to methanol, hexane, and chloroform leaf extract of *Cassia occidentalis*[29]. The antifungal results of petroleum ether, ethanol, methanol and aqueous leaf extract of *P. nigrum* exhibited antifungal susceptibility towards the *Aspergillus* sp., with zone of inhibition 15.33, 17.33, 19.67 and 11.33 mm respectively, while, it was recorded 12.67 mm for *C. albicans* for methanol leaf extracts of *P. nigrum*. Our findings are in alignment with the reports of Shanmugapriya *et al*[25]. They have found that ethyl and acetone leaf extract of *P. nigrum* exhibit antifungal activity against the *C. albicans* and *A. niger* with zone of inhibition ranging from 8-18 mm. However, Timothy *et al*. reported that ethanol leaf extract of *Cassia alata* (*C. alata*) exhibited remarkable antimicrobial activity against *A. niger* and *C. albicans* compared to aqueous extract[30]. Thus, it is obvious from our results that both the plants showed the greater zone of inhibition for methanol leaf extract for *C. albicans* as reported by Ashok and Priscilla[22].

The MIC results showed that petroleum ether, methanol and ethanol leaf extract of *P. nigrum* were found better compared to aqueous leaf extract for *S. aureus* and *E. coli* than *S. typhimurium* and *P. aeruginosa*. The MIC for petroleum ether, methanol and ethanol leaf extract of *P. nigrum* inhibited the growth of *S. aureus* and *E. coli* at concentration of 12.5 mg/mL, while, the MIC for methanol and ethanol leaf extract of *C. didymobotyra* inhibited the growth of *S. aureus* and *E. coli* at concentration of 6.25 and 12.50 mg/mL, respectively. Chika *et al*. reported that the *n*-hexane and methanol extracts of *Buchholzia coriacea* inhibited the growth of *E. coli*, *Klebsiella pneumoniae*[31], *B. subtilis*, *Streptococcus pneumoniae*, and *Shigella* sp., at concentration of 12.5 mg/mL, while for *S. aureus* it was recorded at concentration of 6.25 mg/mL. Similarly, Rao and Suresh[26] reported that MIC for methanol leaf extract of *Cassia sophera* was more than the concentration of 80 mg/mL. Our study clearly indicated that the MIC for all the tested solvent leaf extract of *C. didymobotyra* inhibited the bacterial and fungal strains ranging in between the concentration of 6.25 to 50.00 mg/mL.

The MBC results of all the tested solvent leaf extract of both plants were found ranging in between the concentration of 12.5-50.0 mg/mL for all the tested bacterial strains. The MBC for petroleum ether, ethanol and methanol leaf extract of *P. nigrum* inhibited the growth of *S. aureus* at concentration

of 25.0 mg/mL, whereas, the MBC for methanol and ethanol leaf extract of *P. nigrum* inhibited the growth of *E. coli* at concentration of 12.5 mg/mL. Similarly, the MBC for methanol and ethanol leaf extract of *C. didymobotyra* for *S. aureus* and *E. coil* was recorded at concentration of 12.5 mg/mL, while, it was recorded at concentration of 12.5 mg/mL for *S. typhimurium* and *P. aeruginosa*. In our results, the MIC values are always lower than MBC values, which is in agreement with Doughari *et al*[32]. They have reported that MIC values were lower than the MBC values for acetone, hexane, dichloromethane and methanol extracts of *Senna obtusifolia* (L.), which indicated that the tested extracts had high efficiency with strong bactericidal action.

The MFC results for tested fungal pathogens for petroleum, ethanol, methanol and aqueous leaf extract of both plants were ranging in between the concentration of 12.5-50.0 mg/mL. *Aspergillus* sp. had the least concentration of 12.5 mg/mL for methanol leaf extract for *P. nigrum* and *C. didymobotyra*. However, MFC for *C. albicans* was found at concentration of 25.0 mg/mL for ethanol and methanol leaf extract of both the plants. According to the study of Timothy *et al*.[30], the MFC of aqueous and ethanolic leaf extract of *C. alata* to inhibit the growth of *C. albicans*, *A. niger*, *Penicillium notatum*, *Microsporium canis* and *Trichophyton mentagrophytes* ranged in between the concentration of 26.90-32.40 mg/mL. This clearly indicated that our study plants were highly potent against the tested fungal strains.

The phytochemicals present in the plants such as alkaloids, flavonoids, anthraquinone, reducing sugars, terpenoids, saponin, and tannins are of medicinal importance and pharmacological values. The presence of various phytochemicals in the plant extracts showed its efficacy against the wide pathogenic microorganisms[23,33]. Different solvents exhibited a range of solubility for different phytochemicals. Our results showed that the ethanol and methanol extract of *P. nigrum* and *C. didymobotyra* contained all types of tested phytochemicals. This is the reason why all the solvent leaf extracts of both plants had high degree of susceptibility towards tested bacterial and fungal strains. The leaf extract of *P. nigrum* contains reducing sugar, anthraquinone, terpenoids, flavonoids, saponin, tannins and alkaloids, which is in agreement with the study of previous researchers[23,25,33]. The present study showed that the leaf extract of *C. didymobotyra* contained reducing sugar, anthraquinone, terpenoids, flavonoids, saponin, tannins and alkaloids which was in line with the report of Mohammed *et al*[34]. However, Veerachari and Bopaiah reported that leaf extract of *Cassia tora*[35], *C. alata* and *Cassia surattensis* contained alkaloid, tannins, reducing sugar and terpenoids and the leaf extract of *Cassia occidentalis*, *Cassia auriculata* and *Cornus sericea* contained alkaloids, tannins, flavonoids, reducing sugar and terpenoids. Similarly, Makut *et al*. reported that the presence of phytochemicals *viz.* saponins, tannins, alkaloids, glycosides, steriods, terpenoids and flavonoids present as the active compounds in the leaves and bark of *Khaya senegalensis* had the high degree of susceptibility towards *S. aureus*, *Streptococcus faecalis* and *C. albicans*[20]. Thus, it has been concluded from our study that the methanol leaf extract of *P. nigrum* and *C. didymobotyra* exhibited highest antimicrobial activity and minimum MBC and MFC values on the tested bacterial and fungal strains. Furthermore, studies are required to quantify and purify the active components of plant extracts which inhibit the growth and metabolic activity of bacterial and fungal pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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