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Phytochemical Screening and Antibacterial Potential of *Piper nigrum* Seed Extract against the Bacterial Pathogens

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ABSTRACT

Medicinal plants have diverse therapeutic properties, due to the existence of phytochemical constituents. That are non-nutritive substances with the properties of disease prevention and protectives. The majority of the global population trust on therapeutic plants for their prime health concern desires. Bacterial infections are a universal civic health crisis, and their antibiotic drug resistance has augmented hastily. In this work, we evaluated the *Piper nigrum* fruit extract for antibacterial and phytochemical activity against the bacterial pathogens of the wound. Twenty-three significant pathogens were identified based on the microscopic and biochemical profiling viz *Pseudomonas aeruginosa* (6), *E. coli* (5), *Staphylococcus aureus* (5), and *Klebsiella pneumoniae* (5). Antibiotic sensitivity of these pathogens was executed. *P. aeruginosa*, *K. pneumonia,* and *E. coli* were resistant to Penicillin 10mcg and Streptomycin 10mcg. *P. aeruginosa* and *K. pneumonia* were resistant to chloramphenicol which shows a drug-resistant pattern. Antibacterial activity of *P. nigrum* aqueous and ethanolic extract exhibited a considerable zone of inhibition to all the isolates. The maximum zone of inhibition (17mm) was found at 60µg on *E. coli* and *P. aeruginosa.* The existence of phytochemical constituents are alkaloids, flavonoids, terpenoids, anthraquinone, and steroids. Our results disclosed that *P. nigrum* fruit extracts displayed good antimicrobial potential against pathogens and might be applied to treat wound pathogens in the future.

***Key words*:** *Piper nigrum,* Antibacterial activity, Phytochemical constituents

Drug resistance to the agents of antimicrobials has been frightening in modern years and stands a massive civic health risk universally endow to the World health organization. The augment in morbidity and fatality resultant from bacterial infections have been ascribed to the development of MDR (Multidrug resistance) pathogens. The raise in MDR is the be short of novel and efficient antimicrobial drugs [1]. There is emergent attention in medicinal plants [2-3], natural products [4-6], and conventional medicine with an aspiration to develop new plant-based pharmaceutical products. It might the alternate for synthetic antibiotic drugs and prevent the emergence of MDR pathogens. Hence to identify more effective and novel antimicrobial drugs from the plant, natural products [7-9], and plant derivatives that are eco-friendly and non-toxic. *Piper nigrum* L. (Fruit of Black pepper) is a solitary of the most well-known pungency and aroma spices in the globe, that is known as the spices of king owing to its spicy piperine [10] and belongs to the family Piperaceae. It is used conventionally for the therapy of various ailments. Pepper is a therapeutic and pungent liana was originated in India. Black pepper fruit is grown up in numerous humid regions like India, Indonesia, and Brazil. Dehydrated pepper is widely used in diverse types of foods for flavouring, naturally available food preservative in addition to conventional medication [11] that consists of alkaloid piperine is the chief pungent. It displayed various pharmacological properties viz antimicrobial, antioxidants, rheumatism arthritis, antihypertensive, chills, fever anti-diarrheal, anti-thyroids and antiplatelets, anti-inflammatory, pain management, antitumor, larvicidal, insecticidal, anti-asthmatics, influenza analgesic activities, etc., [12]. Peperine has been used for the augmentation of blood circulation, stimulation of appetite, and salvation [13]. Inhibition of free radicals, lipid peroxidation, and imprudent oxygen species owing to their phenolic and flavonoids contents of pepper [14]. Existing food preservation methods used synthetic preservatives that have undesirable health effects and may not completely purge microorganisms. Essential oil of pepper fruit can be used for the preservation of orange juice [15]. It is used as natural potential dietary supplements with the approaches of modern therapeutics. Hence in this

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research work, we investigated the Piper nigrum fruit extracts and examine their antibacterial property and phytochemicals study against the bacterial pathogens of the wound.

MATERIALS AND METHODS

Collection of plant materials

The clean healthy dried *Piper nigrum* seeds were collected from Kolli Hills, Namakkal District Tamil Nadu. The seeds of black pepper were washed, dried at 55°C under a hot air oven for 3 hours, milled, and stored at 4°C in an air-tight container. The seed powder was used for the preparation of the extract.

Piper nigrum seed extract preparation

Black pepper seed powder (20g) was soaked with 200 ml aqueous and ethanol with microwave-assisted extraction [16]. The seed extract was used to get ready the antibacterial activity by disc diffusion- Kirby Bauer method.

Collection of specimen

Twenty wound and skin specimens are were collected aseptically from the hospital in and around Namakkal District via sterile cotton swabs. The collected swabs were labeled and brought to the research laboratory and processed instantly [17].

Microscopic and biochemical characterization

In the lab, the collected swabs were inoculated on the plates containing blood agar and McConkey agar [18]. The plates were maintained overnight at 37°C [19]. After that, the growth was observed on the plates [20] and the secluded colonies were branded by phenotypic such as Gram reaction, motility, and biochemical profilings [21-22].

Kirby Bauer antibiotic sensitivity method

Kirby Bauer antibiotic disc diffusion assessment was executed with Chloramphenicol (30µg), penicillin (30µg), and streptomycin (30µg). Inoculums (100µl) were spreaded over the surface of Muller Hinton agar (MHA) to made a lawn and dried prior to applying the antibiotic disc. After that, antibiotic discs were located decisively on the MHA agar plates, after that, the inoculated plates were maintained for 18 hours at 37°C. The inhibition zone was deliberated with an electronic scale in millimeters [23].

Antibacterial activity

The antibacterial potential of aqueous and alcoholic seed extract was evaluated against the isolated bacterial pathogens by the inhibition zone. The lawn of the inoculums was spreaded on the plate then 6mm size wells were punched. The aqueous and alcoholic seed extract (20, 40, and 60 µg) were encumbered individually to the wells and maintained for 24 h at 35°C. Finally, plates were investigated for inhibition of zones [24-26]. Zones of inhibition were recorded in diameters.

Phytochemical profiling

The aqueous and ethanolic extract of *P. nigrum* seeds was subjected for qualitative phytochemical screening such as alkaloids, flavonoids, glycosides, terpenoids, coumarins, anthraquinone, steroids, phytosteroids, Phylobatannins, and carbohydrates. The intensity colour was created by the precipitate used as analytic test controls [27].

RESULTS AND DISCUSSION

Microscopic and biochemical profiling

The pathogens from skin and wound specimens were together from hospitals and laboratories in and around Namakkal district, Tamilnadu is inimitable based on phenotypic and biochemical characterization. The phenotypic characterization of these isolates was small, circular, shiny, convex, large, opaque, swarming growth, smooth colony, dome-shaped, translucent, tiny, and mucoid colonies. Under microscopic evaluation, gram-negative and gram-positive bacterial pathogens were identified. In this research work, twenty-one significant pathogens from 20 samples, were detected, that were four phenotypically diverse bacterial pathogens (Table 1, Fig 1). Among them *E. coli* and *P. aeruginosa* are motile and *S. aureus* and *K. pneumonia* are non-motile. All the pathogens were gram-negative except *S. aureus*, that is gram-positive. These present research findings were dependable with the results of Vakayil *et al*. [28].

Table 1 Screening of pathogens from clinical specimens

Name of the organism	Considerable pathogens
E. coli	(6; 28%)
Staphylococcus aureus	(5;23%)
Pseudomonas aeruginosa	(5;23%)
Klebsiella pneumoniae	(5;23%)

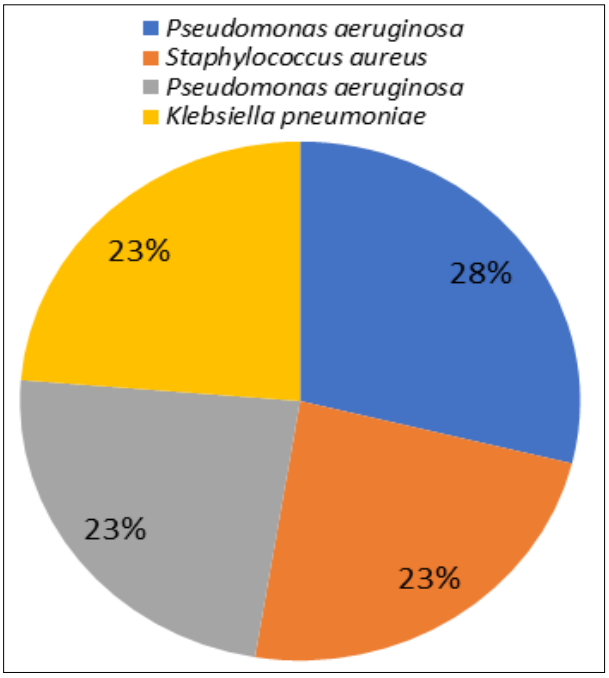

Fig 1 Significant pathogens in the clinical specimens

In the present study the rate of the wound and skin infection was found to be dominant over gram-negative isolates viz. *Escherichia coli* (6 isolates; 28%), *P. aeruginosa* (5 isolates; 23%), and *K. pneumonia* (5 isolates; 23%), pursued by gram-positive isolates *S. aureus* (5 isolates; 23%). Biochemical profiling of the isolated was depicted in (Table 2).

Antibiotic sensitivity test

According to the antibiotic sensitivity test result, the zone of inhibition for all three antibiotics was sensitive to *S. aureus*. Chloramphenicol (30mcg), was sensitive to *S.*

aureus (20mm) and *E. coli* (19mm), Penicillin (10mcg), and Streptomycin (10 mcg) was sensitive to *S. aureus* (18 mm and 20mm). *P. aeruginosa*, *K. pneumonia*, and *E. coli* were resistant to Penicillin 10mcg and Streptomycin10mcg. *P. aeruginosa* and *K. pneumonia* were resistant to chloramphenicol which shows a drug-resistant pattern (Table 3, Fig 2). Drug-resistant pathogens are a gratifying ruthless crisis in health and hospital care, mounting fatality, and morbidity [25], [29]. The profiles of drug resistance explored the fluctuations among hospitals around the globe. Multiple drug resistance was found in most bacterial infections.

Table 2 Biochemical characterization of the pathogenic isolates

Indole	Methyl red	Voges proskauer	Citrate utilization test	Pathogens
N	P	N	N	*Escherichia coli*
N	N	N	P	*Pseudomonas aeruginosa*
N	N	P	P	*Klebsiella pneumoniae*
N	P	N	N	*Staphylococcus aureus*

Table 3 Antibiotic sensitivity test against the isolates

Name of the organism	Zone of inhibition in mm					
	Chloramphenicol 30mcg		Penicillin 10 mcg		Streptomycin 10 mcg	
	ZOI	Inf	ZOI	Inf	ZOI	Inf
Escherichia coli	19±1	S	8±1	R	8±1	R
Staphylococcus aureus	20±1	S	18±1	S	20±1	S
Pseudomonas aeruginosa	14±1	I	12±1	R	14±1	R
Klebsiella pneumoniae	10±1	R	7±1	R	6±1	R

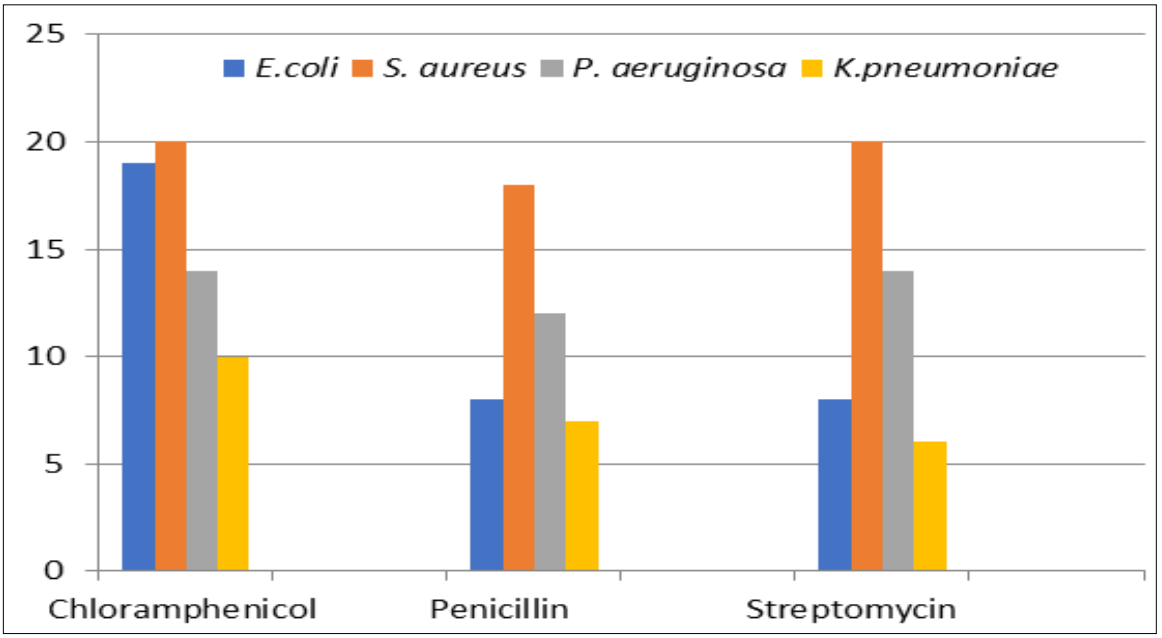

Fig 2 Antibiotic sensitivity test against the isolates

Antibacterial activity

Antibacterial activity of *P. nigrum* aqueous and ethanolic extract exhibited a considerable zone of inhibition 11mm to 17mm to all the isolates. The maximum zone of inhibition (17mm) was found at 60µg in aqueous extract on *E. coli* and ethanolic extract on *P. aeruginosa* (Table 4, Fig 3). It has abundant bioactive properties on the health of the human body. That has numerous benefits, and it possess obvious antibacterial potential, due to the presence of piperine. It has the impending to be used since natural nutritional supplements are in concert with current therapeutics. Recent scientific research has established the presence of many active compounds in these spices that are known to possess specific pharmacological properties. Many plant-derived molecules have shown a promising effect in therapeutics [30-31].

Table 4 Antibacterial activity of *P. nigrum* against the pathogens

Name of the organism	Zone of Inhibition in mm					
	Aqueous extract			Ethanolic extract		
	20µg	40µg	60µg	20µg	40µg	60µg
Escherichia coli	11 mm	12mm	17mm	11mm	12mm	14mm
Staphylococcus aureus	11mm	12mm	14mm	11mm	12mm	15mm
Pseudomonas aeruginosa	12mm	13mm	16mm	12mm	14mm	17mm
Klebsiella pneumoniae	11mm	13mm	16mm	11mm	12mm	16mm

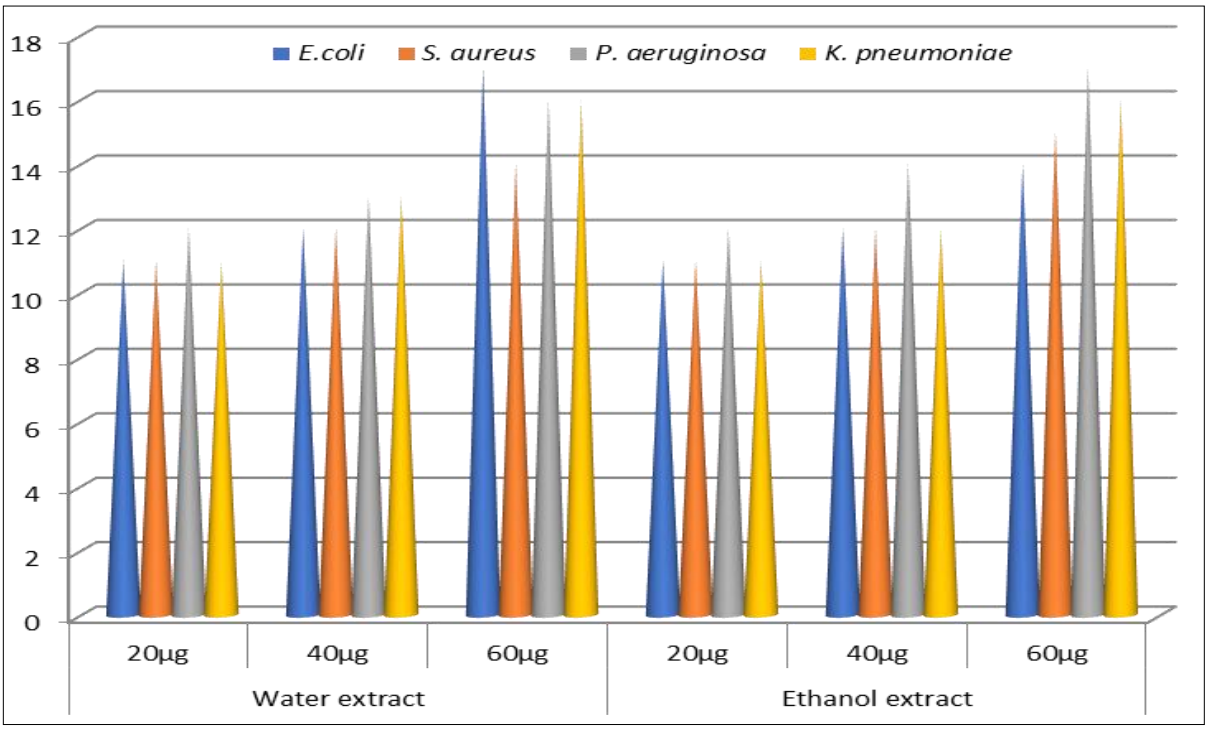

Fig 3 Antibacterial activity of *P. nigrum* against the pathogens

Table 5 Qualitative phytochemical analysis of *Piper nigrum* seeds

Phytochemicals constituents	*P. nigrum* seeds	
	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Flavonoids	-	+
Glycosides	-	-
Terpenoids	+	+
Coumarins	-	-
Anthraquinone	+	+
Steroids	+	-
Phytosteroids	-	-
Phylobatannins	-	-
Carbohydrate	-	-

Phytochemical screening

The existence of phytochemical constituents in *P. nigrum* seed aqueous and ethanolic extracts are alkaloids, terpenoids, and anthraquinone. The presence of steroids in aqueous and flavonoids in ethanolic extract. Absence of Glycosides, Coumarins, Phytosteroids, and Phylobatannins in both the extracts (Table 5). The result of the primary selection of phytochemical constituents laid the support for further works as it explored the existence of alkaloids, terpenoids, and anthraquinone, steroids, and flavonoids in P. nigrum seed aqueous and ethanolic extracts [27], [32].

CONCLUSION

Ancient time plants and their natural products as a source of traditional medicine. Spices such as pepper are used as food and also in therapeutics. Our results disclosed that *Piper nigrum* fruit extracts displayed good antimicrobial potential against pathogens and might be applied to treat wound pathogens in the future.

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