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Antibacterial activity of *Echinacea pallida* against some human pathogenic bacteria

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Abstract
Traditional medicine and treatments that use plants, animals, or their byproducts in traditional ways can provide relief from suffering for people. More susceptible than other illnesses are microbial infections. Antimicrobial agent from natural sources may be the best remedy in these cases. So, the aim of this study was to examine the antimicrobial activity of methanolic extract of *Echinacea pallida* to human pathogenic bacteria. *Echinacea pallida* was collected from the local market then dried in herb drier and mechanically powdered for methanolic extraction by maceration method. Disk diffusion method was used to evaluate the antimicrobial activity of *Echinacea pallida* extract to *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus spp.* by demonstrating zone of inhibition. The extract of *Echinacea pallida* containing test disk inhibited growth of pathogenic bacteria. Test disk containing 25, 50, 75 and 100 µg showed 8, 10, 11 and 15 mm zone of inhibition against *E. coli*. 7, 9, 10 and 12 mm zone of inhibition against *Staphylococcus aureus*. 8, 11, 14 and 16 mm zone of inhibition against *Klebsiella pneumoniae*. 7, 10, 12 and 14 mm zone of inhibition against *Pseudomonas aeruginosa.* 100 µg *E. pallida* containing disk inhibit 9 mm zone whereas 25, 50 and 75 µg *E. pallida* containing disk were resistance against *Proteus spp.* Whereas 30 µg Amikacin containing standard antibiotic disk showed 12, 16, 10 and 8 mm zone of inhibition against *E. coli, S. aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* on the other hand standard 30 µg Amikacin containing disk showed resistance against *Proteus spp.* respectively. This research concluded that methanolic extract of *Echinacea pallida* contains antibacterial properties.

Keywords: Antimicrobial, *Echinacea pallida*, methanolic extract, zone of inhibition

Introduction
The quest to overcome microbial resistance has spurred interest in the discovery and development of novel antimicrobial agents derived from diverse sources in recent years. As a result, screening and evaluating techniques for antimicrobial activity has received more attention. Even though they can yield quick results about the effects of the antimicrobial agent and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism, some bioassays, like disk-diffusion, well diffusion, broth or agar dilution, are well known and frequently used. However, others, like flow cytofluorometric and bioluminescent methods, are not widely used because they require specific equipment and further evaluation for reproducibility and standardization [1]. The word “echinacea” comes from the Greek word “echinos”, which means sea urchin or hedgehog. It has probably got this name from the prickly spikes found on the flower head. *E. pallida* are the species most often used medicinally [2]. Echinacea has long been used traditionally to treat a variety of illnesses. Scientific research has validated several of the traditional uses. With more therapeutic value in herbs, this is the most significant species. A typical herbal supplement is called Echinacea, which is taken either regularly as a prophylactic measure in people who are ill often or at the first signs of illness in an effort to speed up the healing process (in hopes of reducing the frequency of which they get sick). The term "Echinacea" refers to a few plant genera that include some regularly used species, including purpurea, angustifolia, and pallida. Echinacea is a highly popular "immune stimulant" herb used for the common cold [3]. The therapeutic qualities of echinacea are attributed to three classes of phytochemicals: lipophilic alkamides, polysaccharides, and derivatives of caffeic acid. Polysaccharides, flavonoids, chicoric acid, alkyl amides, polyacetylenes, and essential oils are the active ingredients in echinacea. Polysaccharides and chicoric acid glycosides have the ability to stimulate the immune system [4].

In our study to investigate the antimicrobial activity of *Echinacea pallida*, methanolic extract of *Echinacea pallida* was used to observe the zone of inhibition against *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus spp.* in Mueller Hinton agar media.

Materials and Methods

Plants extraction preparation

The leaves of *Echinacea pallida* plant used in this study were obtained from the local market Dhaka, Bangladesh. The plants were thoroughly cleaned and rinsed with distilled water. *Echinacea pallida* were grounded into fine powder to pass 100 mm sieve. 100 g of the fine powder was soaked in 400 ml of methanol with stirring for 72 hours, filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (1) to attain a clear filtration. The filtrate was evaporated and dried at 45ºC temperature, using water bath. The extract yield was weighted, stored in small bottle in fridge at 5ºC and their yield percentages were calculated using the following formula: Extract yield % = R/S×100 (Where R; weight of extracted plant residues and S; weight of plant raw sample).

Antibacterial activity of the plant extracts

E. coli, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus spp.* were provided from the diagnostic centers of Hamdard General Hospital, Gazaria, Munshigonj. Mueller Hinton agar media, and blank disk was purchased from Tradesworth Ltd. & Technoworth Associates Ltd. Dhaka, Bangladesh.

The inoculum of bacterial stain was prepared by sub-culture of each bacterial stain at the temperature of 35ºC incubated overnight in Mueller-Hinton agar SLANTs. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer.

The disk diffusion method is used to evaluate antimicrobial activity of *Echinacea pallida* extract. The plant extract residues (50 mg) were re-dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22 mm) then loaded over sterile blank disks to obtain final concentration of 10 mg/disc. Ten ml of Mueller-Hinton agar medium was poured into sterile petri dishes followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 10^5 CFU/ml of medium. Sterile blank discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hinton agar plates. Blank disk loaded with 30 µg of Amikacin was used as positive control. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 35 °C for 24 h. The presence of inhibition zones was measured by Vernier caliper, recorded, and considered as indication for antibacterial activity [5].

Result

Table 1: Zone of inhibition produced by methanolic extract of *Echinacea pallida* 25 µg, 50 µg, 75 µg and 100 µg containing paper disk and 30 µg Amikacin containing antibiotic disk against *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *and Proteus spp.*

Sample Name	Concentration (µg/ml)	Zone of inhibition (mm)				
		E. coli	*S. aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus spp.*
MEEP	25	8	7	8	7	Resistance
MEEP	50	10	9	11	10	Resistance
MEEP	75	11	10	14	12	Resistance
MEEP	100	15	12	16	14	9
Amikacin	30	12	16	10	8	Resistance

Note: Methanolic extract of *Echinacea pallida*

Discussion

Echinacea preparations have long been used to treat bacterial infection-related respiratory symptoms, boost the immune system, and promote wound healing. Antibacterial properties of echinacea extracts have been shown, and they are harmless.[6] In our study *Echinacea pallida* extract used for the antimicrobial activities assessed by disk diffusion method. The result showed good zone of inhibition by against *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *and Pseudomonas aeruginosa*. Mostly *E. pallida* containing disk were resistance against *Proteus spp.*

Conclusion:

Echinacea pallida extract obtained by maceration method, the antimicrobial activities assessed by disk diffusion method. The result showed good zone of inhibition by this crude extract against pathogenic microorganisms. *Echinacea pallida* got primary scientific validity as an antibacterial medicinal plant, further studies on this medicinal plant will give the effectivity as an antimicrobial plant for using in the formulation of natural medicine as well as pharmaceutical raw material for modern medicine.

Conflicts of interests

No conflicting interests are stated by the authors.

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