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Research Article

# EFFICACY OF *EUPHORBIA HETEROPHYLLA* LATEX AGAINST PATHOGENIC BACTERIA AND FUNGI

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**ABSTRACT**

**Objective:** The objective of the study was to determine the antimicrobial activities of *Euphorbia heterophylla* latex.

**Methods:** The antibacterial and antifungal activities of acetone, chloroform, and diethyl ether extracts were assayed by disk diffusion method.

**Results:** The study of plant *E. heterophylla* latex revealed the presence of medically active metabolites. Bacterial strains such as *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris*, and *Pseudomonas aeruginosa* exhibited a strong zone of inhibition. Acetone extract exerts a potent zone of inhibition against *P. aeruginosa* compared to tetracycline. Fungi, *Aspergillus niger, Fusarium oxysporum,* and *Penicillium* sp., were used for the antifungal activity. It was observed that the highest zone of inhibition was noticed against *A. niger* in all the extracts. Interestingly, *F. oxysporum* and *Penicillium* sp. showed no zone of inhibition and were resistant to standard drug, fluconazole which was used as a control.

**Conclusion:** *E. heterophylla* latex extract was found to be more potent than the standard drugs which were used against both the bacterial and fungal strains.

**Keywords:** *Euphorbia heterophylla*, Latex, Antibacterial activity, Antifungal activity.

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## INTRODUCTION

Medicinal plants play a significant role in providing primary, health-care services to rural people and are used by about 80% of the marginal communities in the world [1-3]. Each medicinal plant has its own nutrient constituents along with the phytochemical constituents. For the physiological functions of the human body, the nutrients are essential. Such nutrients and biochemicals such as carbohydrates, fats, and proteins play an important role in satisfying human wants and need for energy and other life processes [4-6]. Over the past few decades, medicinal plants have widely been studied for the purpose of the mitigation and the treatment of various infectious diseases because microbial resistances against conventionally used synthetic antimicrobial agents are increasing at an alarming rate [7,8]. Each and every community has its own system of traditional medicine, and they utilize natural resources around their habitats for various medicinal purposes [9]. Many medicinal plants are used by marginal communities to cure various diseases [2] as diverse medicinal plant species are used either in the form of extract or decoction by the local people in different regions; therefore, evaluating their marginal significance can help to understand the worth of these plant species in different ecological conditions and few plants serve as both food and medicine. *Euphorbia heterophylla* is one of such plants [10]. It is commonly called Mexican fire plant, milk weed and spurge weed in English. Several chemical compounds such as friedelin, β-sitosterol, myricyl alcohol, ellagic acid, benzoic acid, diterpenes, stigmasterol, quercetin, and different amino acids are present in *E. heterophylla* [11]. This plant is native to Central and South America, but now observed in tropical and subtropical levels. *E. heterophylla* is herbaceous, erect and grows up to 20–200 cm in height. The morphological variation in the shape of the leaf has led to different species. The milky latex is acrid and the toxicity is neither alkaloid nor glycoside but probably a resin which can prove fatal [12]. Despite the toxicity hazard of this plant, it has various medicinal properties which include its use in the treatment of gonorrhea, respiratory tract infection, malaria, eczema, asthma, and wart cure [13]. In East Africa, *E. heterophylla* is used for the treatment of gonorrhea and to accelerate wound healing. It is also used as a purgative and lactogenic agent, as a cure for a migraine. The latex of the plant is also used as a fish poison and insecticide [14]. The latex is irritant to the skin and eyes and may be employed as a rubefacient and to remove warts and corns. *E. heterophylla* extract exhibited a wide spectrum of antibacterial action against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *P. aeruginosa* [15]. Antimicrobial drugs play a significant role on bacterial infections. Antimicrobial drugs have proved remarkably effective for the control of bacterial infections. However, there is an increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine due to the side effects and the resistance that pathogenic micro-organisms build against the antibiotics [16]. It was reported that infectious diseases caused by bacteria, fungi, viruses, and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [17].

## METHODS

### Collection and preparation of *E. heterophylla* latex extracts

The plant *E. heterophylla* was collected from Pandavapura, Mandya district, Karnataka, in the month of January 2017. The fresh latex of the plant was obtained from the stems and the leaves early in the morning by capillary action using the amber glass screw cap bottle.

Based on the solubility, the stock solution was prepared. Thereafter, serial dilution of the stock solution was prepared to get various concentrations (5%, 10%, and 15%) of which were used for the antibacterial sensitivity test.

### Preliminary phytochemical analysis

The plant extracts were assayed for the presence of alkaloids, flavonoids, tannins, resins, saponins, cardiac glycosides, and steroids [18].

**Antimicrobial activity**

*Collection of bacterial cultures*

All the bacteria, i.e., *Staphylococcus aureus* (MTCC 7443), *B. subtilis* (MTCC 121), *P. vulgaris,* and *Pseudomonas aeruginosa* (NCIM 2200) used for the detection of antibacterial activity were collected from the Department of Microbiology, Yuvaraja's College, Mysuru, and subcultured on nutrient agar slants. The nutrient agar high medium was prepared as per the requirement according to the number of plates, and the pH was adjusted to 7. The media were sterilized by autoclave at 121°C for 15 min. After sterilization, the nutrient agar medium was poured into sterile Petri plates under aseptic conditions and allowed for solidification.

*Preparation of bacterial inoculum*

A 2 ml of double-distilled water was taken in a clean test tube and a loop full bacterial culture was suspended. Form this, 1 ml of the sample was inoculated into the agar medium and kept for culturing.

*Antibacterial activity*

The bacterial subculture was spread uniformly over the medium using an L-shaped glass rod. Different concentrations of various extracts (acetone, chloroform, and diethyl ether) were impregnated onto the sterile discs of 0.1 mm diameter and were placed onto the plates. The latex extract along with standard drug tetracycline was also prepared (50 mg/ml) and impregnated onto separate discs which would serve as the control in determining the antibacterial activity. The plates were then incubated at 37°C overnight [19].

*Antifungal assay*

The fungal cultures such as *Aspergillus niger, Penicillium* sp., and *Fusarium oxysporum* were obtained from the Department of Microbiology of Yuvaraja's College, Mysuru. It was subcultured in the potato dextrose broth solution for further uses. Potato dextrose agar high medium was prepared according to the requirements and autoclaved at 121°C for 15 min. After sterilization, the potato dextrose agar medium was poured into the sterile Petri plates under aseptic condition and allowed for solidification.

*Preparation of inoculum*

From subcultured broth solution, 1 ml of the inoculum was taken in a clean sterile test tube and was made up to 2 ml with sterile distilled water and shaken well. From this, 1 ml of inoculum was inoculated into the plates.

*Antifungal activity*

The fungal subculture was spread uniformly over the medium using an L-shaped glass rod. Different concentrations of various extracts (acetone, chloroform, and diethyl ether) were impregnated onto the sterile discs of 0.1 mm diameter and were placed onto the plates. The latex extract along with standard drug fluconazole was prepared (50 mg/ml) and impregnated onto separate discs which would serve as the control in determining the antifungal activity. The treated plates were then incubated at 30°C for 24–48 h.

Each experiment was carried out in triplicate. The standard deviation± mean of the inhibition zone was taken for evaluating the antimicrobial activity of the plant extracts.

**RESULTS**

**Phytochemical analysis of *E. heterophylla***

Preliminary phytochemical studies revealed that the plant is rich in the compound such as carbohydrates, alkaloids, and flavonoids. Secondary metabolite identified in the plant material has been reported as having an inhibitory action against pathogenic microorganisms [20,21]. The result of phytochemical screening in *E. heterophylla* revealed the presence of secondary metabolites such as saponins, tannins, flavonoids, phenols, alkaloids, and steroids in different solvent extractions (Table 1). The primary metabolite like proteins was detected in diethyl ether, whereas the negligible amount of carbohydrates was found. In the present experimental work, the different extracts of *E. heterophylla* were tested

**Table 1: The phytochemical screening of *E. heterophylla* latex extracts**

| S. No. | Biochemical test | Acetone latex extract | Chloroform latex extract | Diethyl ether latex extract |
|---|---|---|---|---|
| 1. | Benedicts test | – | – | – |
| 2. | Froth test | + | + | + |
| 3. | Xanthoproteic test | – | – | + |
| 4. | Gelatin test | + | + | + |
| 5. | Ferric chloride test | + | + | – |
| 6. | Salkowski's test | + | – | – |
| 7. | Ferric chloride test | – | + | – |
| 8. | Hanger's test | + | + | + |
| 9. | Test for steroids | + | + | + |
| 10. | Test for cardioglycosidic acid | – | – | – |

**Table 2: Zone of inhibition area in mm of the plant extracts against selected bacteria**

| Bacterial strain | Acetone latex extract | | | Chloroform latex extract | | | Diethyl ether latex extract | | | +ve control (tetracycline) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5% | 10% | 15% | 5% | 10% | 15% | 5% | 10% | 15% | | | |
| *Staphylococcus aureus* | 6 | 8 | 9 | 4 | 8 | 9 | 6 | 7 | 9 | 11 | 13 | 11 |
| *Proteus vulgaris* | 3 | 4 | 5 | 0 | 4 | 6 | 6 | 7 | 11 | 10 | 10 | 10 |
| *Bacillus subtilis* | 5 | 7 | 10 | 4 | 5 | 9 | 6 | 7 | 8 | 5 | 11 | 8 |
| *Pseudomonas aeruginosa* | 5 | 7 | 14 | 0 | 3 | 5 | 4 | 6 | 10 | 9 | 7 | 10 |

**Table 3: Zone of inhibition area in mm of the plant extracts against selected fungi**

| Fungal strains | Acetone latex extract | | | Chloroform latex extract | | | Diethyl ether latex extract | | | +ve control (fluconazole) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10% | 20% | 30% | 10% | 20% | 30% | 10% | 20% | 30% | | | |
| *Aspergillus niger* | 5 | 12 | 16 | 7 | 11 | 13 | 15 | 17 | 2 | 3 | 5 | 3 |
| *Fusarium oxysporum* | 0 | 2 | 10 | 0 | 0 | 3 | 0 | 6 | 9 | 0 | 0 | 0 |
| *Penicillium sp.* | 1 | 7 | 11 | 2 | 6 | 9 | 4 | 10 | 15 | 6 | 0 | 0 |

against the four bacterial strains of *P. vulgaris*, *S. aureus*, *B. subtilis,* and *P. aeruginosa.* The study carried out on *E. heterophylla* revealed the presence of bacterial activity (Table 2). The antifungal activity of different latex extracts was tested against the fungal strains by the disk diffusion method. The different latex extracts showed a zone of inhibition against the fungal activity of *A. niger, F. oxysporum, and Penicillium* sp. (Table 3).

**Antibacterial activity of *E. heterophylla* against *S. aureus***

The experiment was carried out to determine the antibacterial activity of *E. heterophylla* latex extract against bacterial strain of *S. aureus.* It was observed that *S. aureus* exhibited similar to the zone of inhibition in acetone, chloroform, and diethyl ether extracts. It was observed that the zone of inhibition increased as the concentration of the extract increased (Fig. 1).

**Antibacterial activity of *E. heterophylla* against *B. subtilis***

In the present investigation, the three different solvent extracts were tested against *B. subtilis.* A high potential zone of inhibition was seen in acetone extract in comparison to diethyl ether and chloroform extracts. It was observed that tetracycline which was used as a control showed a significant zone of inhibition against *B. subtilis* (Fig. 2).

**Antibacterial activity of *E. heterophylla* against *P. vulgaris***

The result was recorded for the inhibitory activities against *P. vulgaris* in three different solvents. *P. vulgaris* showed a significant zone of inhibition in latex extract from diethyl ether. The result was far better than the standard drug tetracycline that was used as a control (Fig. 3).

**Antibacterial activity of *E. heterophylla* against *P. aeruginosa***

The experiment was carried out to determine the antibacterial activity of *E. heterophylla* latex extract against bacterial strain of *P. aeruginosa.* A high potential zone was observed in acetone latex extract against *P. aeruginosa* whereas in chloroform, and diethyl ether exhibited moderate activity (Fig. 4). The bacterial strains possessed a substantial zone of inhibition compared to tetracycline in acetone extract.

**Antifungal activity of *E. heterophylla* against *A. niger***

The studies were carried out to evaluate the antifungal activity of *E. heterophylla* latex extract against *A. niger.* A significant zone of inhibition was observed in acetone, chloroform, and diethyl ether extracts. When these extracts were compared with the standard drug fluconazole, the inhibition zone was excellent and can be placed on record (Fig. 5).

**Antifungal activity of *E. heterophylla* against *F. oxysporum***

The result was recorded for the inhibitory activity against *F. oxysporum*.Based on the concentration of three different solvents, a considerable zone of inhibition was documented. *F. oxysporum* is resistant to standard drug fluconazole and showed no zone of inhibition (Fig. 6). Diethyl ether and acetone extracts can be used in antifungal activity.

**Antifungal activity of *E. heterophylla* against *Penicillium* sp.**

During the investigation, the solvent extracts were tested against *Penicillium* sp. The zone of inhibition in *Penicillium* sp. was concentration dependent. Higher the concentration greater is the zone of inhibition. *Penicillium* sp. exhibited a high potential zone of inhibition in diethyl ether extract. Fungus was resistant to fluconazole which was used as

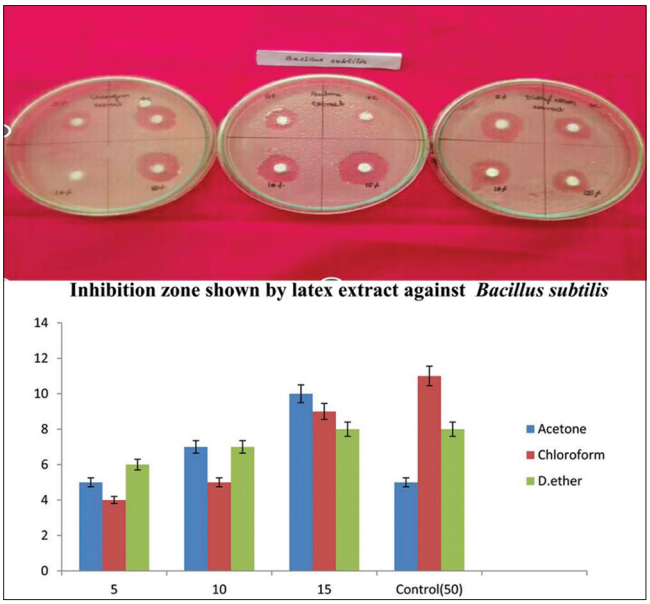


**Fig. 2: Graphical representation of inhibition zone antibacterial activity of latex extract against *Bacillus subtilis* (standard deviation±mean *p<0.05, **p<0.005)**

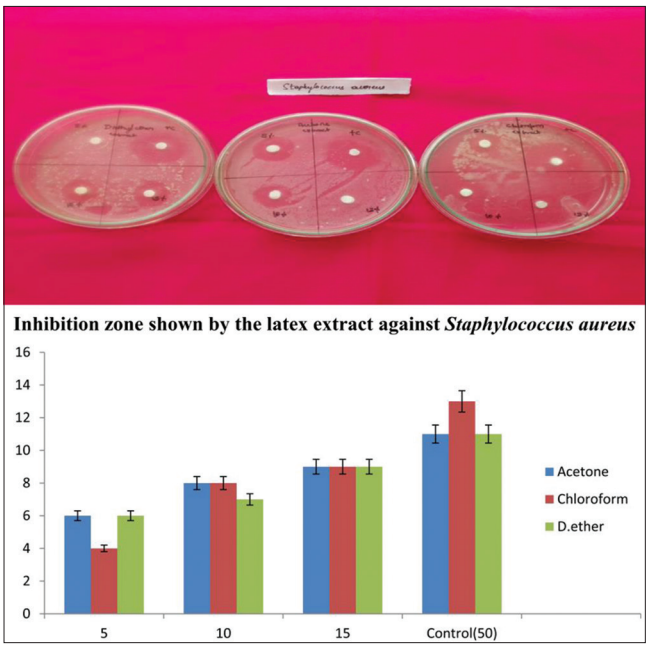


**Fig. 1: Graphical representation of inhibition zone antibacterial activity of latex extract against *Staphylococcus aureus* (standard deviation±mean *p<0.05, **p<0.005)**

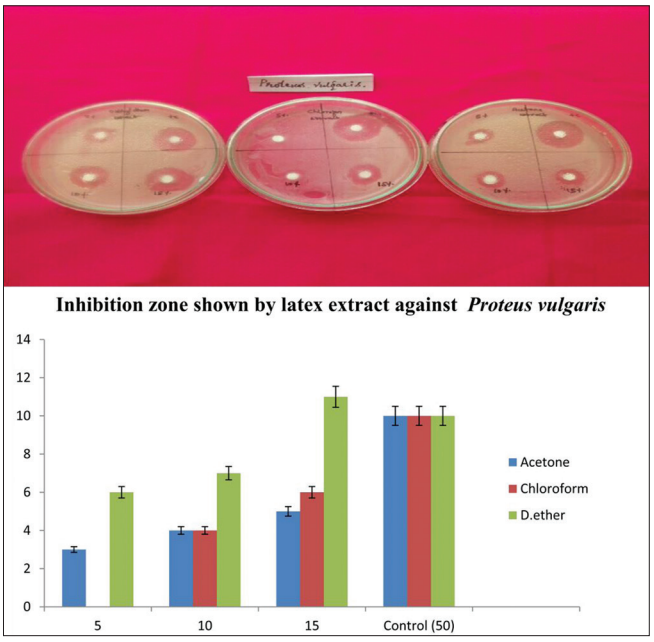


**Fig. 3: Graphical representation of inhibition zone antibacterial activity of latex extract against *Proteus vulgaris* (standard deviation±mean *p<0.05, **p<0.005)**

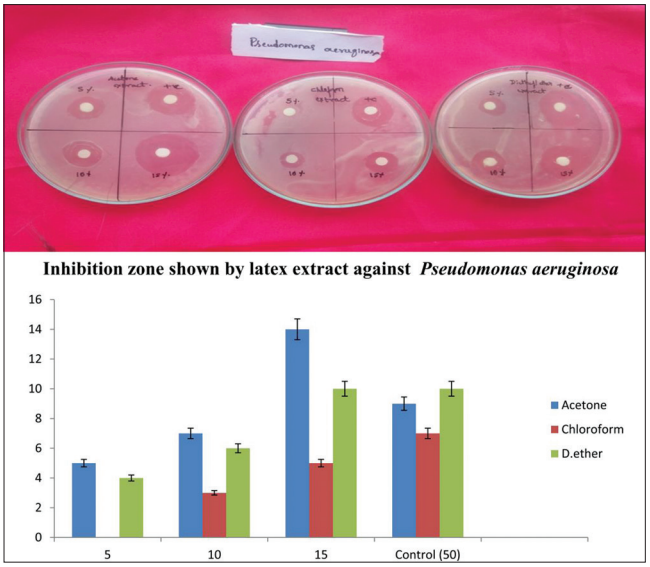


**Fig. 4: Graphical representation of inhibition zone antibacterial activity of latex extract against *Pseudomonas aeruginosa* (standard deviation±mean *p<0.05, **p<0.005)**

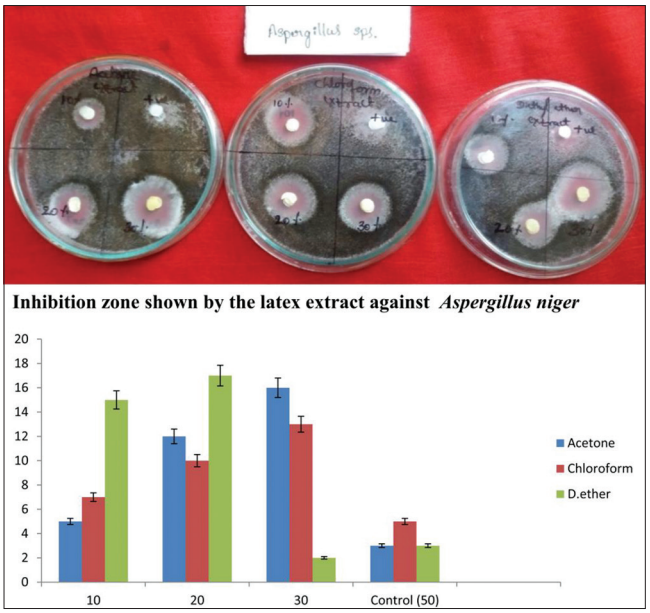


**Fig. 5: Graphical representation of inhibition zone antibacterial activity of latex extract against *Aspergillus niger* (standard deviation±mean *p<0.05, **p<0.005)**

a control (Fig. 7). Hence, diethyl ether extract of *E. heterophylla* can be used as a standard drug.

## DISCUSSION

The study carried out on the plant latex revealed the presence of active metabolites. The phytochemical analysis, in acetone latex extract of the plant, showed considerable results in saponins, tannins, flavonoids, phytosterols, alkaloids, and steroids. There was no result found in carbohydrates, proteins, phenols, and cardioglycosidic acid. Chloroform latex extract of the plant showed significant results in saponins, tannins, flavonoids, phenols, alkaloids, and steroids. It showed the insignificant result in carbohydrates, proteins, phytosterols, and cardioglycosidic acid. Diethyl ether latex extract of plant showed positive results in saponins, proteins, tannins, alkaloids, steroids, and cardioglycosidic

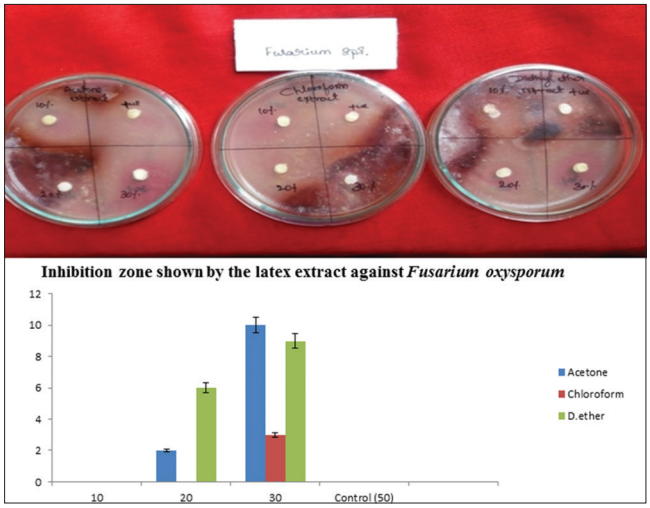


**Fig. 6: Graphical representation of inhibition zone antibacterial activity of latex extract against *Fusarium oxysporum* (standard deviation±mean *p<0.05, **p<0.005)**

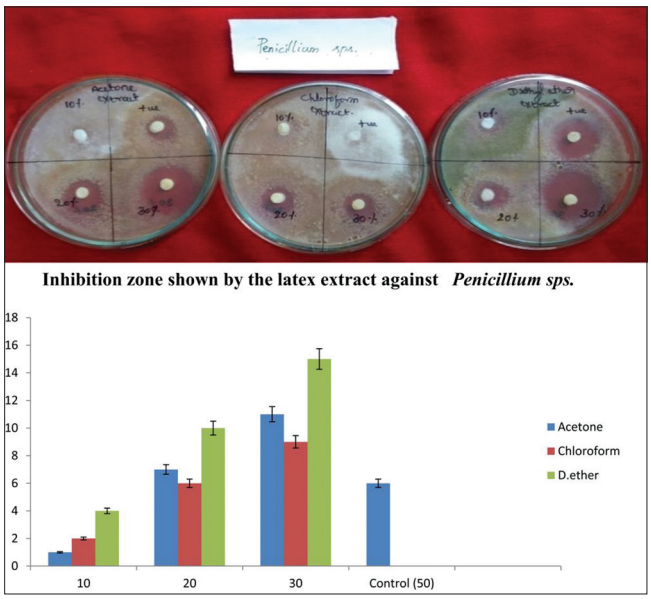


**Fig. 7: Graphical representation of inhibition zone antibacterial activity of latex extract against *Penicillium* sp. (standard deviation±mean *p<0.05, **p<0.005)**

acid, and no result was seen in carbohydrates, flavonoids, phytosterols, and phenols.

The latex extract showed good antibacterial activity against *P. vulgaris*, *S. aureus*, *B. subtilis,* and *P. aeruginosa* and exhibited good zone of inhibition against the fungal activity of *A. niger, Penicillium* sp., and *F. oxysporum.* In a study, it was reported that diethyl ether extract of latex of *E. heterophylla* exhibited significant antibacterial activity against *S. aureus* and *P. aeruginosa.*

## CONCLUSION

The presence of saponins and alkaloids has been reported to be responsible for various pharmacological properties, by exerting toxic effects against cells of foreign organisms. In general, *E. heterophylla* latex extract was found to be more potent than the standard drugs which were used against both the bacterial and fungal strains.

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**AUTHORS' CONTRIBUTIONS**

Pruthvi and Rohini have prepared the manuscript under the guidance of Dr. Mahesh.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

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