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Antibacterial Potential of Geastrum triplex Jungh. Against Plant and Human Pathogens

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Abstract: The *in vitro* antibacterial property of various solvent extractsusing fourdifferent solvent systems (Petroleum ether, Chloroform and Methanol) of *Geastrum triplex* were evaluated by agar well diffusion method against plant and human pathogenic bacteria. The crude extracts of *G. triplex* have relatively high antibacterial activity. Among the three xtracts, Chloroform extract was found to be effective against tested plant pathogenic bacteria compared to methanol and petroleum ether, whereas, Petroleum ether extract, was found to be effective against tested human pathogenic bacteria compared to chloroform and methanol.
Key words: *Geastrum triplex*, Antibacterial activity, Wild mushrooms, Pathogens, Western Ghats.

Introduction

Whole world is frantically in search of new antibiotics because of an alarmingly increase in bacterial resistance to existing antibiotics due to their inappropriate and indiscriminate use. In search for new antibiotics, herbs, plants and mushrooms are being used [1]. It has been known that macro fungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity [2]. Dioscorides, first century Greek physician, knew that *Laricifomes* (*Fomitopsis*) *officinalis* (Vill.) Kotl. & Pouzar (Fomitopsidaceae) can be used for treatment of "consumption", a disease now known as tuberculosis [3]. It is believed that mushrooms need antibacterial compounds to survive in their natural environment. Antimicrobial compounds could be isolated from many mushroom species and some proved to be of benefit for humans [4]. Man has been hunting for the wild mushrooms since antiquity [5]. Antimicrobial activities of basidiomycete strains from different countries were screened in submerged culture [6, 7-8]. Similarly, 14 mushroom isolates were detected for significant activity against one or more of the target microorganisms [6], 75% of polypore fungi that have been tested showed strong antimicrobial activity [9]. The practice of using mushrooms in Chinese herbal medicines has been recorded in early records of the *Materia Medica*. The earliest book on medicinal compounds in China, the *Shen Noug's Herbal* (100-200AD), recorded the medicinal effects of several mushrooms, including *Ganoderma lucidum, Poriacocos, Tremella fuciformis* and others [10].

Geastrum triplex is an inedible fungus which is found in the detritus and leaf litter of hardwood forests in many parts of the world. It is commonly known as the collared earthstar, the saucered earthstar, or the triple earthstar and less commonly by the alternative species name *G. indicum*. Several authors have

regarded *G. indicum* as the correct name for *G. triplex* [11]. This is because *G. indicum* species described as *Cycloderma indicum* [12] and then moved to *Geastrum* in may be the same species as *G. triplex* [13-14]. If it is in fact the same species, the first published name (i.e., *G. indicum*) has nomenclatorial priority according to the rules of the International Code of Botanical Nomenclature. More recently, several authors argue that *G. indicum* should be rejected as a nomendubium and *G. triplex* maintained as the correct name for the species [15, 16-18].The specific epithet triplex means "threefold", and refers to the three layered peridium [19]. *G. triplex* has acquired several vernacular names, including the collared earthstar [20-21] the saucered earthstar [22] and the triple earthstar [23].

Materials and Methods

Collection of samples

The *Geastrum triplex* was collected from Kuvempu University campus, Shankaraghatta, Shimoga district, Karnataka during the month of September and October 2012. The *G. triplex* of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and kept for shade drying. The shade dried mushroom materials were powdered mechanically for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual [24, 32] and also through the electronic data on identification keys of mushrooms [25]. The specimen was deposited at the herbarium of mycology laboratory, Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shimoga district, Karnataka, India.

Extraction of Mushroom materials

The extracts were prepared according to the methodology of Indian Pharmacopoeia [26]. The powdered materials were subjected Soxhlet extraction by using various solvents namely petroleum ether, chloroform and methanol. Each extraction was carried out for 48 hours at suitable temperature. The yield of each extracts were recorded and preserved at 4° C for further experiments.

Antibacterial activity

The antibacterial activities of crude extracts were tested against three plant and six human pathogenic bacteria namely *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604), *Agrobacterium tumefaciens* (MTCC-431), *Klebsiella pneumonia* (MTCC-7028), *Staphylococcus aureus* (MTCC-902), *Salmonella parathyphi* (MTCC-1088), *Salmonella typhi* (MTCC-968), *Pseudomonas aeroginosa* (MTCC-1934) and *Escherichia coli* (MTCC-1698). These organisms were received and authenticated from IMTECH, Chandigrah, India and the cultures were maintained at 4° C for further use.

Agar well diffusion method

The agar well diffusion method has been employed for testing antibacterial activity of mushroom extracts. Test microorganisms were activated in Mueller Hinton Broth (37°C, 24 h). 20 ml of sterilized Mueller Hinton Agar Media was poured uniformly in a sterilized petriplates and allowed to solidify and then 100µl of suspension of the test organisms was spread evenly on medium with sterilized L-shaped glass spreader to get a uniform lawn of bacteria. Later four wells were punched at the four corners of the plate [27] with the help of sterilized cork borer of 6 mm diameter. The 100µl crude extracts preparation were loaded to the each well by micropipette in four different concentrations *viz.*, 12.5 %, 25 %, 50 % and 100 % respectively which are prepared with DMSO [28].The standard drug tetracycline and ciprofloxacin were used as positive control and DMSO as negative control. The test was carried out by triplicates for each solvent extract against test organisms. All the plates were incubated at 37°C for 24 hours in the bacteriological incubator to favor the complete growth of the test organisms. Antimicrobial activity was determined by measuring the radius of the clear inhibition zone around each well [29].

Table: 1. Antibacterial activities of *Geastrum triplex* extract against Plant Pathogenic bacteria

Sl. No	Organisms	Diameter of zone of inhibition (in mm)													
		Petroleum ether Extract (Conc.mg/ml)				Chloroform Extract (Conc.mg/ml)				Methanol Extract (Conc.mg/ml)				Control	Standard
		100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %	DMSO	Tetracycline
1	*X. campestris*	13	8	-	-	11	10	9	8	13	-	-	-	-	40
2	*P. syringae*	7	-	-	-	8	7	7	-	12	-	-	-	-	38
3	*A. tumefaciens*	12	9	6	7	8	7	-	-	15	7	-	-	-	30

'-' No activity.

Table: 2. Antibacterial activities of *Geastrum triplex* extract against Human Pathogenic bacteria

Sl. No	Organisms	Diameter of zone of inhibition (in mm)													
		Petroleum ether Extract (Conc.mg/ml)				Chloroform Extract (Conc.mg/ml)				Methanol Extract (Conc.mg/ml)				Control	Standard
		100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %	DMSO	Ciproflaxacin
1	*S. parathyphi*	10	12	7	7	10	9	7	8	-	-	-	-	-	26
2	*S. typhi*	14	8	7	-	7	-	-	-	10	7	-	-	-	28
3	*P. aeroginosa*	11	8	-	-	7	-	-	-	12	-	-	-	-	32
4	*E. coli*	12	9	9	7	10	7	7	-	-	-	-	8	-	33
5	*K. pneumonia*	12	10	-	-	11	8	-	-	11	-	-	-	-	28
6	*S. aureus*	13	9	6	6	12	7	-	5	9	7	8	-	-	30

Result and Discussion

The antibacterial activities of petroleum ether, chloroform and methanol extract of mushroom *G. triplex* was analyzed *in vitro* by agar well diffusion method.The growth inhibitory effect of crude extracts of *G. triplex* were tested against three plants and six human pathogenic bacteria *viz., X. campestris, P. syringae, A. tumefaciens, K. pneumonia, S. aureus, S. parathyphi, S.typhi, P. aeroginosa* and *E. coli.* The antibacterial activity of all the solvent extracts were calculated by measuring inhibition zone formed around the well in millimeter (mm).

The antibacterial activities of *G. triplex* against plant pathogenic bacteria were presented in Table-1. Chloroform extract was found to be effective against tested plant pathogenic bacteria compared to methanol and petroleum ether. The maximum antibacterial activity of chloroform extracts of *G. triplex* was found against *X. campestris* (11mm) at 100% concentration and minimum against *P. syringae* (7mm) at 50% concentration. The methanol extract showed maximum activity against *A. tumefaciens* (15mm) and the minimum inhibition zone (7mm) was recorded against *P. syringae* (12mm) at 100% concentration. The extract at both 25% and 12.5% concentration does not show any inhibition zone against the tested plant pathogenic bacteria.The petroleum ether extract showed moderate degree of inhibition zone against *X. campestris* (13mm) at 100% concentration and mild activity against *A. tumefaciens* (6mm) at 25% concentration.

The antibacterial effects of different solvent extracts of *G. triplex* were tested against six human pathogenic bacteria and results were tabulated in Table 2. Among the three organic solvent extracts, petroleum ether extract showed more effective inhibitory activity against all the tested bacteria compared to chloroform and methanol extracts which showed considerable antibacterial activity.

The petroleum ether extracts were showed more active antibacterial proficiency against *S. typhi* (14mm) and *S. aureus* (13mm) at 100% concentration, moderate effect against *E. coli* (12mm) followed by *K. pneumonia* (12mm), *P. aeroginosa* (11mm) and *S. parathyphi* (10mm). The chloroform and methanol extract were highly active against *S. aureus* followed by *P. aeroginosa* and *E. coli* but fail to inhibit growth of test organisms at lower concentrations.

This difference in response of mushroom extracts to test organisms might be due to a number of factors, as studies suggest that the antimicrobial activities of all mushroom extracts are changeable [30], depending upon the nature of environment and media in which it was grown. It also depends upon the genetic structure of mushroom species, physical and biochemical constituents, extraction solvents and test organisms. The sensitivity pattern of microorganisms also changes to chemotherapeutic agents depending on their strains, and susceptibility or resistance to antibiotic [31].

The antibacterial activity of mushroom sample varied according to the solvents.These combine activity of antibacterial increase the chance of the mushroom for medicinal purposes. The fact that the Basidiomycetes have been insufficiently investigated coupled with the broad range of structural types of antibiotics [11]. However, basidiomycetes may be a source of new and usefulbioactive compounds. Further studies onisolation and characterization of the active compounds may provide a better source for developing new therapeutic and pharmacological agents.

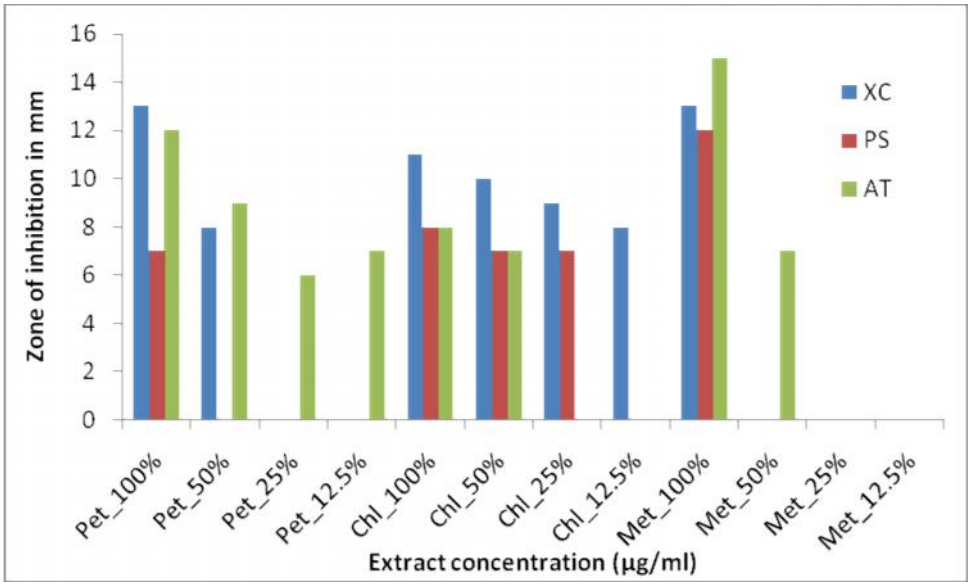


Figure: 1. Antibacterial activity of *Geastrum triplex* against three Plant Pathogenic strains

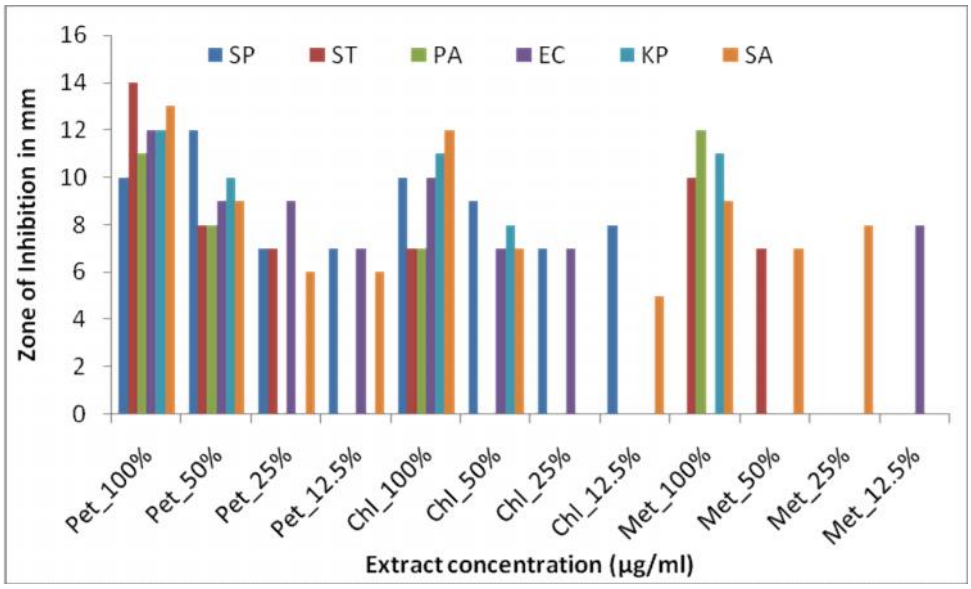
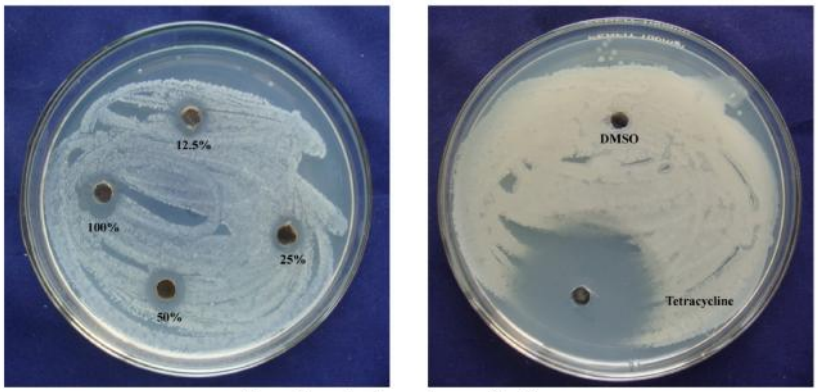
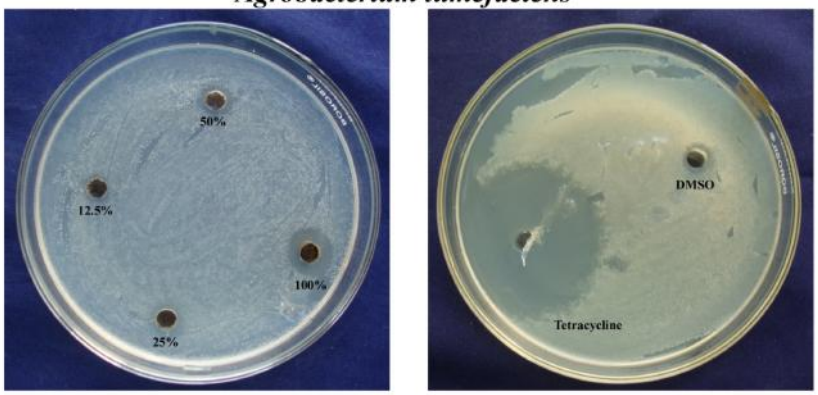


Figure: 2. Antibacterial activity of *Geastrum triplex* against six Human Pathogenic strains

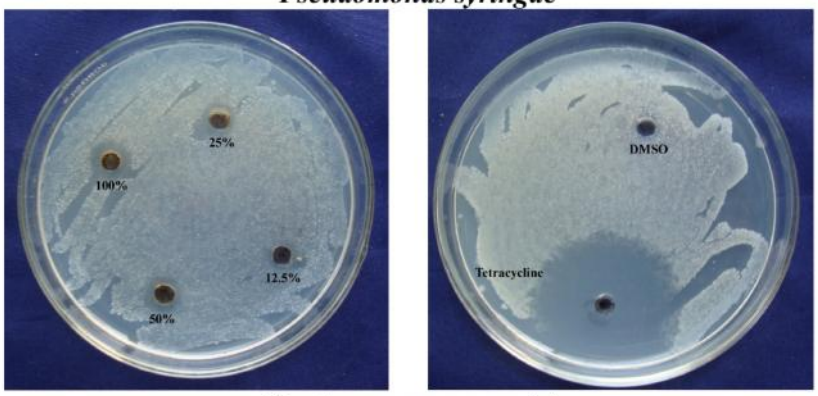
Plate: 1. Antibacterial activities of Chloroform Extract of *Geastrum triplex* against Plant Pathogens



Agrobacterium tumefaciens

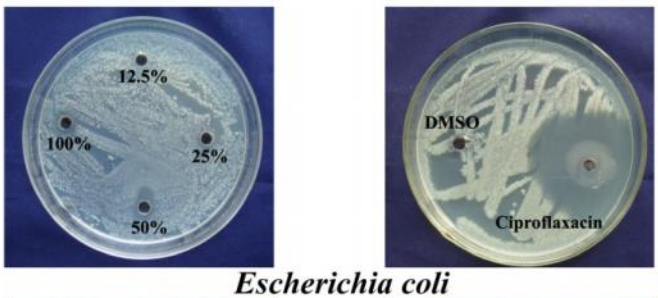


Pseudomonas syringae

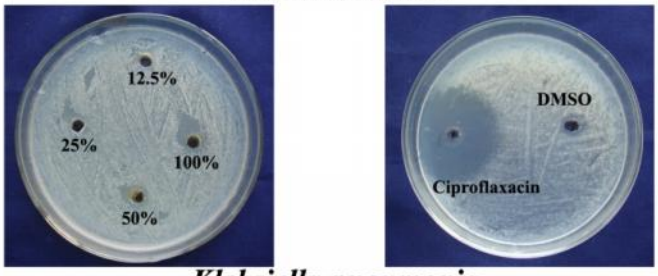


Xanthomonas compestris

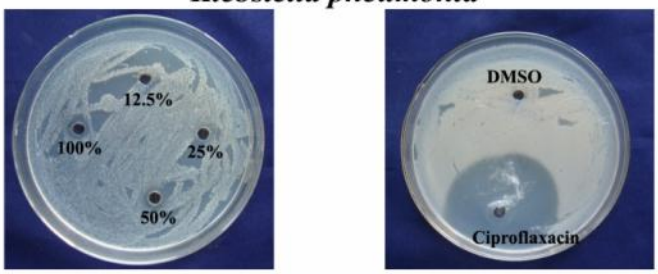
Plate: 2. Antibacterial activities of Petroleum ether extract of *Geastrum triplex* against human pathogens



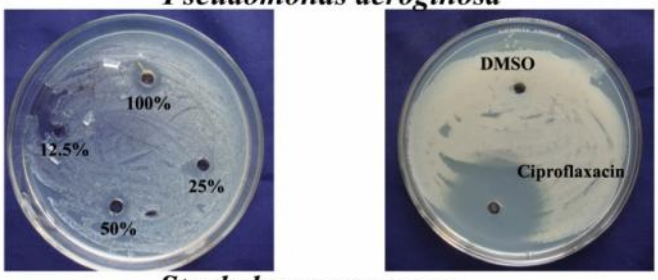
Escherichia coli



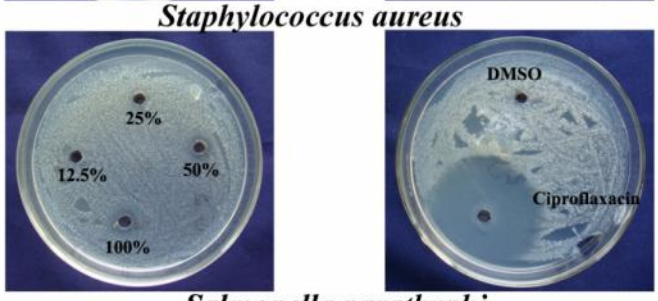
Klebsiella pneumonia



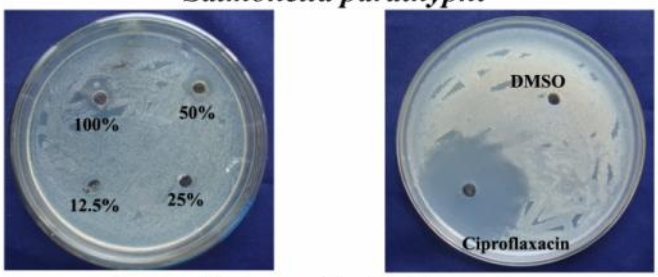
Pseudomonas aeroginosa



Staphylococcus aureus



Salmonella parathyphi



Salmonella typhi

Conclusion

Chloroform extract was found to be effective against tested plant pathogenic bacteria compared to methanol and petroleum ether, whereas, Petroleum ether extract, was found to be effective against tested human pathogenic bacteria compared to chloroform and methanol. Whole world is frantically in search of new antibiotics because of an alarmingly increase in bacterial resistance to existing antibiotics due to their inappropriate and indiscriminate use. Further studies onisolation and characterization of the active compounds from basidiomycetes may provide a better source for developing newtherapeutic and pharmacological agents.

Acknowledgements

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