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**Research Article**


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## PRELIMINARY BIOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF *HYGROCYBE CANTHARELLUS* (SCHWEIN.) MURRILL

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### ABSTRACT

The present study was carried out to preliminary biochemical screening and antibacterial activities of *Hygrocybecantharellus* (Schwein.) Murrill on selected three plant and six human bacterial pathogens such as *Xanthomonascampestris*, *Pseudomonas syringae*, *Agrobacterium tumefaciens*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*. For antibacterial test, well diffusion technique was used and the zone of inhibition of microorganisms was measured in mm. The biochemical analysis of the mushroom extracts of various solvents contained alkaloids, saponins, glycosides, flavonoids and phenols but did not contain detectable levels of steroids, tannins, triterpenoids. The fruit body of *H. cantharellus* showed potential antibacterial activities against the selected strains and maximum inhibition zone 17 mm followed by 16 mm, 15 mm and 14 mm was recorded from 100 mg of methanol and petroleum ether extracts of *H. cantharellus* fruit body against *P. aeruginosa* followed by *S. typhi*, *A. tumefaciens*, *S. aureus* and *X. campestris* and minimum (8 mm and 6 mm) by the *S. paratyphi* and *K. pneumoniae* at 12.5 mg of extracts. However, the activity was less than the standard tetracycline and ciprofloxacin. The extract shows increasing inhibitory activity with increase in concentration (12.5 % - 100 %). The study concluded that the different solvent extracts of fruiting body of *H. cantharellus* contain potential compounds that inhibit growth of both plant and human pathogenic bacteria.

**Key words:** Fruit bodies, Antibacterial activity, Pathogenic bacteria, Solvent extracts, Agar well diffusion method.

### INTRODUCTION

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Since ancient times mankind has exploited nature for all kind of useful production and enjoyed the colors, flavors and fragrances of flowers, food etc. The Rigveda [1] which is the oldest book in the library of man supplies curious information on the subjects. Nature is an extremely rich source of highly diverse and innovative chemical structures [2].

The relationship existing between plants and humans is as old as mankind, dated back to the origin of human civilization [3]. Mushrooms belong to a special group of macroscopic fungi. Macromycetes arranged in the phylum Basidiomycota and some of them in the Ascomycota are known as the higher fungi [4, 5]. It is estimated the existence of about 1, 40, 000 different species of mushrooms in the planet, however, only about 10% is known. Half of them present nutritious properties. 2000 species of mushrooms are safe and approximately, 70 are known for presenting some pharmacological properties. Edible mushrooms are attractive because of their flavor, taste and delicacy [6]. Although many

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species of edible mushrooms exist in the nature, less than 20 species are used as food and only 8-10 species are regularly cultivated in significant extent.

Its diversity is unmatched due to the presence of 16 agroclimatic zones, 10 vegetative zones and 15 biotech provinces. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effect. Men and women, led by instinct, taste, experience, used plants for healing which were not a part of their normal diet; the physical evidence for herbalism goes some 60,000 years to a Neanderthal burial site uncovered in 1960 [7]. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. The country has 15,000-18,000 flowering plants, 23,000 fungi, 25,000 algae, 1,600 lichens, 1,800 bryophytes and 30 millions micro organisms. India also has equivalent to 3/4<sup>th</sup> of its land exclusive economic zone in the oceans harbouring a large variety of flora and fauna, many of them with therapeutic properties [8].

In the recent years, the human pathogenic microorganisms have developed multiple drug resistance owing mainly to the indiscriminate use of commercial antimicrobial drugs [9]. There is an urgent need for discovery of novel antimicrobial chemotherapeutic agents. Mushrooms have long been used as garnish for tonics in the folk medicine. *Lentinusedodes* is well-known for its antitumour activity; it has been demonstrated to increase the host resistance to bacterial and viral infections Jong and Birmingham [10]. Several compounds extracted from the mushrooms displayed antifungal and antibacterial activity [11, 12].

Many researchers have observed the antimicrobial activity of mushrooms [13-15]. An extensive examination of over 200 species of Basidiomycetes demonstrated that about 50% of them showed significant antibiotic activity against a range of test organisms [16]. An antibacterial protein from

*Cordyceps sinensis* showed effective antibacterial activity against human pathogens. The extracts from fruiting body, mycelium and culture filtrate have some biologically active compounds with a wide-range of antimicrobial activity [17]. The chloroform and ethyl acetate extracts of the dried mushroom *L. edodes* showed antibacterial activity against Gram-positive, Gram-negative human pathogenic bacteria and effectively inhibited the growth of *Candida albicans* [18-21].

The increasing uses of herbal products demand extra attention with particular focus on their safety, effectiveness and drug interactions. Over the last few decades, a substantial body of scientific evidence is available demonstrating wide range of pharmacological and nutraceutical activities of medicinal herbs [22].

So it was thought worthwhile to carry out antibacterial activity of wild mushrooms of *Hygrocybe cantharellus* on plant and human pathogenic bacteria.

## MATERIAL AND METHODS

### *Collection and identification of mushrooms material*

The *Hygrocybe cantharellus* (Fig-1) were collected from semi evergreen forest region (13°51'56.30"N, 75°03'12.50"E) which is located in Haniya, Hosanagartaluk, Shimoga district, Karnataka, India, during the month of June to August 2013. The *H. cantharellus* of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and air dried in an oven at 40°C for 48 h. dried mushroom samples were powdered mechanically for further use.

Identification was done by comparing their morphological, anatomical and physiological characteristics with the help of standard literatures [23-26]. The voucher specimen (KUABARN-67) has been deposited at the herbarium of mycology laboratory, Department of Applied Botany, Kuvempu University, JnanaSahyadri, Shimoga district, Karnataka, India.



Fig 1: Natural habitat of *Hygrocybecantharellus*(Schwein.) Murrill

### Chemicals

All the solvents viz., petroleum ether, chloroform and methanol used in this study were purchased from Hi-Media Laboratories, Pvt. Ltd, of analytical grade.

### Preparation of solvent extracts

The dried and powdered by grinder (Bajaj Electrical Limited-Twister Mixer) mushroom material (200 g) was extracted successively with 2000 ml petroleum ether following 2000 ml of chloroform and methanol with a Soxhlet extractor for 48 h at temperature not exceeding the boiling point of the solvent [27]. The extracts were concentrated in a vacuum at 40°C using a rotary evaporator. For the entire

analysis, compounds of extract were dissolved in dimethylsulphoxide (DMSO). Each extract was transferred to glass vials and kept at 4°C before use. All the solvent extracts were stored for further activity.

### Bacterial strains

The antibacterial activities of crude extracts were tested against three plant and six human pathogenic bacteria namely *Canthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604), *Agrobacterium tumefaciens* (MTCC-431), *Klebsiella pneumonia* (MTCC-7028), *Staphylococcus aureus* (MTCC-902), *Salmonella paratyphi* (MTCC-1088), *Salmonella typhi* (MTCC-968), *Pseudomonas aeruginosa* (MTCC-1934) and *Escherichia coli* (MTCC-1698). These organisms were received and authenticated from IMTECH, Chandigarh, India. The viability of the organisms was antibiotics until now [28], maintained by regular transfer into freshly prepared nutrient agar (Hi-Media) and stored at 4°C until used.

### Biochemical analysis

The methods described [29] were used to test for the presence of alkaloids, steroids, saponins, tannins, glycosides, triterpenoids, flavonoids and phenols in the test samples (Table-I).

Table I: Qualitative biochemical analysis

Test	Observation	Inference
500mg potassium iodide + 136mg mercuric chloride mixed in 10ml d/w + extract	Turbid pink precipitate	Presence of alkaloids
0.5g of the extract was dissolved in 2 ml of chloroform H <sub>2</sub> SO <sub>4</sub> was added carefully from the side of the tube to form a lower layer	No reddish brown colour	Absence of steroid/sterols
2 gm of extract + 20ml d/w boiled in water bath and filter + 10 ml filtrate + 5ml d/w and shaken vigorously for stable persistent froth. The froth is mixed with 3 drops olive oil and shaken vigorously	Formation of emulsion	Presence of saponins
0.5 gm of extract + 20 ml boiled water in test tube + filtered + few drops of 0.1 ml ferric chloride added	No brownish green or blue black colouration	Absence of tannins
5 ml of extracts + 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution + 1ml of conc. sulphuric acid	Development of violet indicates	Presence of glycosides
5 ml of each extract + 2 ml of chloroform + conc. sulphuric acid carefully added to form a layer along sides	No reddish brown colouration of the interface	Absence of triterpenoids
Extract + 10 ml ethyl acetate over a steam bath for 3 min and filtered. 4 ml of filtrate shaken with 1ml of dil. ammonia solution	Yellow colouration	Presence of flavonoids
Test solution + few drops of 5 % glacial acetic acid and 5 % sodium nitrate	Muddy yellow, olive, niger brown or deep chocolate coloured precipitate	Presence of phenols

### Antibacterial activity by 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 [30] 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 [31].

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. Antibacterial activity was determined by measuring the radius of the clear inhibition zone around each well [32]. Tetracycline and Ciproflaxacin was used as Standard antibiotic for comparison. The results are tabulated (Table-III and IV).

**Table III: Analysis of Antibacterial activity of *Hygrocybecantharellus* against plant pathogenic bacteria**

Pathogens	Zone of inhibition(mm)													
	P E Extract				CH Extract				ME Extract				Standard antibiotic	Control
	Concentration (mg/ml)													
	12.5 %	25 %	50 %	100 %	12.5 %	25 %	50 %	100 %	12.5 %	25 %	50 %	100 %	Tetracycline	DMSO
XC	6	8	10	14	NS**	6	7	9	NS**	6	8	12	30	-
PS	NS**	7	9	12	6	7	9	10	NS**	6	7	9	36	-
AT	8	10	13	15	NS**	6	8	10	NZ*	NS**	7	9	32	-

Note: NZ\*---- No Zone; NS\*\*----No Significant Zone; "--"---No Activity

**Table IV: Analysis of Antibacterial activity of *Hygrocybecantharellus* against human pathogenic bacteria**

Pathogens	Zone of inhibition(mm)													
	P E Extract				CH Extract				ME Extract				Standard antibiotic	Control
	Concentration (mg/ml)													
	12.5 %	25 %	50 %	100 %	12.5 %	25 %	50 %	100 %	12.5 %	25 %	50 %	100 %	Ciprofloxacin 11 I	-
~	NS**	6	9	11	NS**	6	8	9	NS**	6	7	8	28	-
ST	8	10	12	15	6	7	8	10	NS**	6	8	12	26	-
PA	10	13	14	16	6	8	9	11	10	12	14	17	30	-
EC	7	8	10	14	NS**	NS**	9	10	NS**	8	9	10	32	-
KP	6	8	9	10	NS**	6	9	11	6	7	9	10	28	-
SA	6	8	12	14	NS**	8	9	12	8	12	14	17	30	-

## RESULTS AND DISCUSSION

### Biochemical analysis

Qualitative biochemical analysis yielded positive results for most of the solvents extracts of *Hygrocybecantharellus*. The biochemical analysis of the mushroom extracts of various solvents contained alkaloids, saponins, glycosides, flavonoids and phenols

but did not contain detectable levels of steroids, tannins, triterpenoids based on the procedure used.

These biochemical findings and patterns from the present study are well in line with findings from several other studies in China, Poland

and South Africa [33-35]. The presence of these bioactive compounds also account for

the antibacterial activity of the mushroom species (Table-II).

**Table II: Biochemical analysis of *Hygrocybecantharellus* (Schwein.) Murrill**

Secondary metabolites	Petroleum ether extract	Chloroform extract	Methanol extract
Alkaloids	-	-	+
Steroids / Sterols	-	-	-
Saponins	+	+	+
Tannins	-	-	-
Glycosides	+	+	+
Triterpenoides	-	-	-
Flavonoides	-	-	+
Phenols	-	+	+

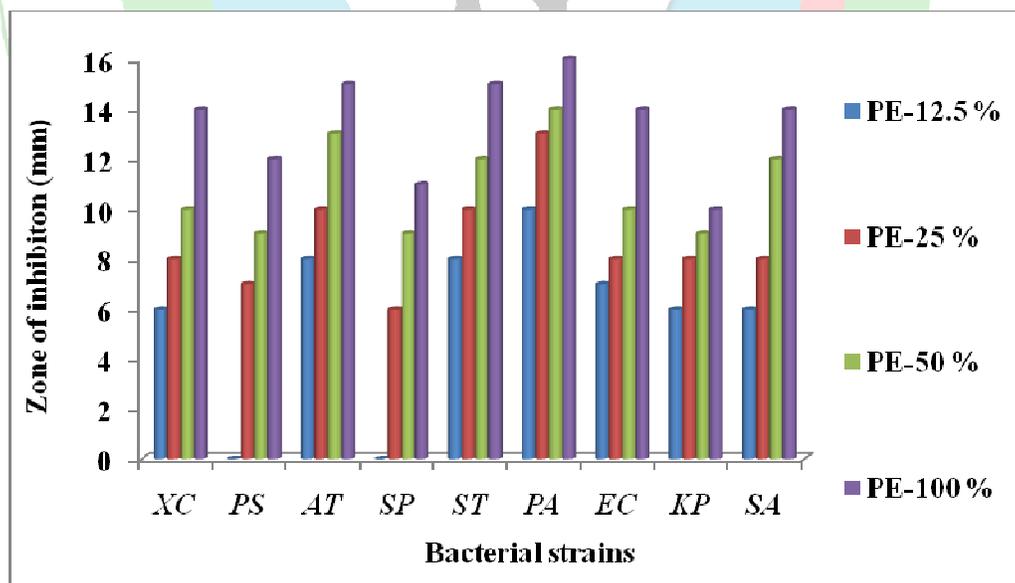
Note: '+' is Present, '-' is Absent

#### Antibacterial Activity

The various solvent extract obtained were screened for antibacterial activity against plant and human pathogenic bacterial strains. Analysis of antibacterial activity is reported in the form of zone of inhibition (Table-III and IV).

Among all the solvent extracts petroleum ether extract was found to be most effective. It significantly inhibited the growth of bacteria. The chloroform and methanol extracts also

show moderate activity against bacteria. These findings indicated the presence of antibacterial compounds in the mushroom species. Among the three various solvent extracts of *Hygrocybecantharellus*, the antibacterial activity of petroleum ether extracts were compared and it was found that more effective than chloroform and methanol shows moderate activities against bacteria (Fig-II, III and IV).



**Fig II: Comparative Antibacterial Studies of Petroleum ether Extracts**

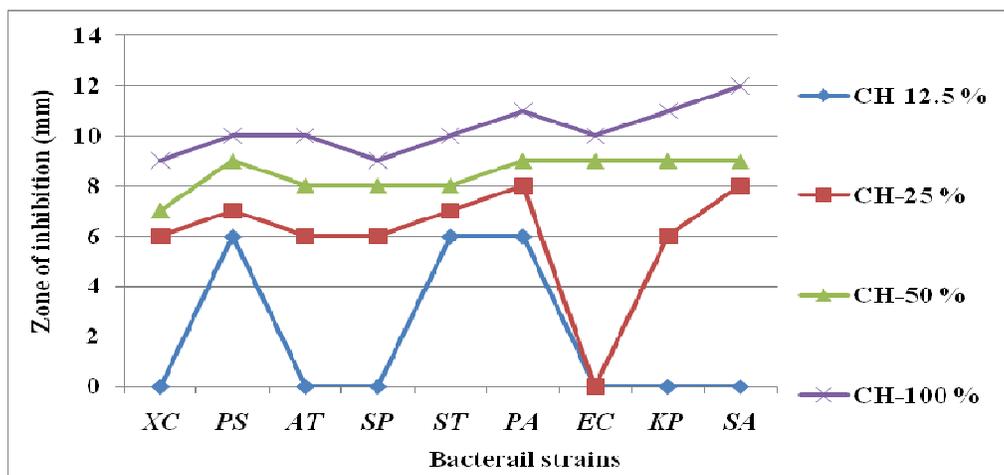


Fig III: Comparative Antibacterial Studies of Chloroform Extracts

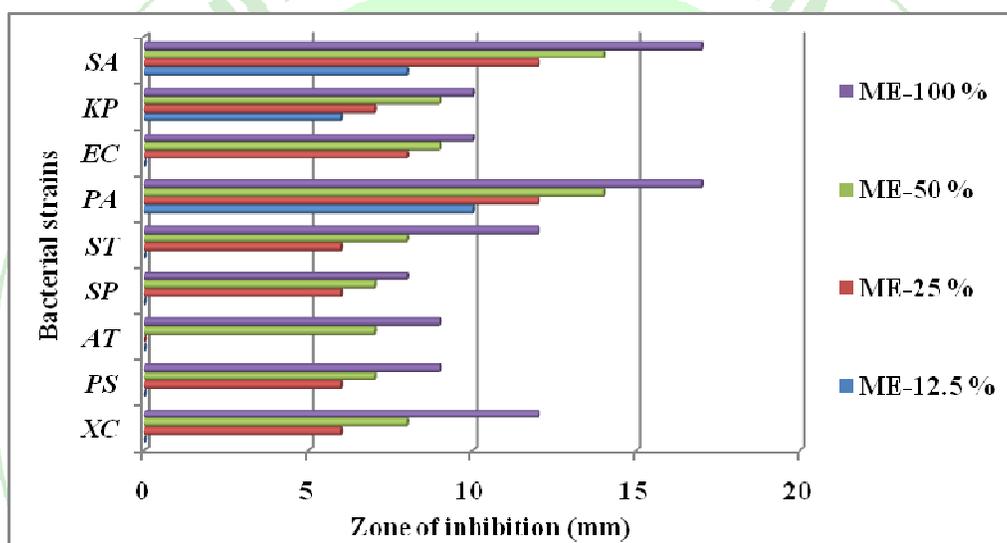


Fig IV: Comparative Antibacterial Studies of Methanol Extracts

Mushrooms have been appreciated as sources of food nutrients for centuries and especially, used for medicinal purposes in the orient for centuries [36]. The hot water extract (WE) from *Dictyophora indusiata* was able to inhibit the growth of both bacteria and fungi used as indicator organisms. The results displaying a wide spectrum antimicrobial activity at concentration of 200 mg/ml whilst [12] reported antimicrobial effect of phenolics extracts of Portuguese wild mushrooms to be between 10 to 300 mg/ml. In present study the significant antibacterial and antifungal activity of both methanol and aqueous extract of 200 mg level.

Hygrocybe samples were highly active against the plant as well as human pathogenic bacteria and hence are a broad spectrum antibiotic,

while these also showed strong activities against *Candida albicans* [37].

In this study also exhibited highly active against the plant as well as human pathogenic bacteria and hence are a broad spectrum antibiotic, while these also showed strong activities against *Xanthomonas campestris*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. In this event the local species of *Hygrocybe cantharellus* appears as a good source of antibacterial agent possessing various other pharmacological properties.

## CONCLUSION

The present study has revealed that, the antimicrobial activity of the wild mushroom

under study and suggest that the bioactive contents of the mushroom is the promising natural antibacterial agents that can be harnessed as potential antimicrobial agents. Petroleum ether 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, extensive studies are recommended for the mushroom to actually identify the bioactive components responsible for their antimicrobial activities. The effect of antibacterial potential was examined against plant and human pathogenic bacteria; petroleum ether extract of the *Hygrocybe cantharellus* has showed consistently significant inhibitory activity. Further study on this aspect has the potential to lead towards a few more astonishing facts towards the antibiotic property of its extracts. Further qualitative assessment of the separated compounds in column chromatography can be done by HPLC-MS, NMR etc.

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