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IN VITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF WILD MUSHROOMS FROM SOUTHERN WESTERN GHATS, INDIA

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ABSTRACT

The present study is aims to investigate the antibacterial activities of crude methanol extracts of 24 southern Western Ghats wild mushrooms. Crude methanol extracts from 24 mushrooms from southern Western Ghats, India were evaluated for their antibacterial activity against *Eggerthella lenta*, *Vibrio parahaemolyticus* and *Enterococcus faecalis* by agar well diffusion method. The study revealed that about 67% of the mushrooms inhibited growth of all the test bacteria, 29% was active against any of the two test bacteria and 4% were completely inactive. Amoxicillin used as standard reference. 15 mushroom species showed strong antibacterial activity against *Eggerthella lenta* higher than the standard antibiotic (Amoxicillin). 11 mushrooms showed strong antibacterial activity against *Vibrio parahaemolyticus* higher than the standard antibiotic (Amoxicillin). The best *in vitro* antibacterial activity was by *Gymnopilus junonius* (26.0 mm against *Eggerthella lenta;* 25.0 mm against *Vibrio parahaemolyticus*) followed by *Tricholoma equestre* (21.0 mm against *Vibrio parahaemolyticus*). *Gymnopilus junonius, Tricholoma equestre* and *Trametes versicolor* have higher antibacterial activity than that of standard antibiotic.

Keywords: Antibacterial activity, Macrofungi, Western Ghats, *Gymnopilus junonius, Tricholoma equestre, Trametes versicolor*.

INTRODUCTION

Mushrooms are a promising source for a variety of potential antimicrobial compounds and are relatively less explored. They have been shown to be rich sources of natural antibiotics [1] and accumulate a variety of chemicals with strong anti-oxidant properties [2]. The search for new natural antimicrobial agents which have no or low impact on the environment as well as human health are highly needed in the present scenario of development of drug resistance in pathogenic bacteria, emergence of new microbial diseases and adverse side effects of synthetic drugs. Wild and cultivated mushrooms contain a huge diversity of biomolecules with nutritional [3] and/or medicinal properties [4-7]. Numerous mushroom extracts have been reported as having antimicrobial activity against bacteria.

As a matter of fact, macrofungi need secondary metabolites for their survival in various harsh environmental conditions. These chemical compounds are usually antibacterial and antifungal in nature. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for humans. Bioactive molecules have been isolated not only from edible, but also from inedible species [8-9]. The mushrooms and their medicinal properties, have long been recognised in China, Korea, and Japan, e.g., hypotensive and renal effects [10-11], immunomodulatory and antitumour activities of polysaccharide-protein complex (PSPC) from mycelial cultures, immunomodulatory and antitumour activities of lectins from edible mushrooms [12-18], isolation and characterization of a Type-I Ribosome-Inactivation protein from *Volvariella volvacea* [19], and medicinal effects of *Ganoderma lucidum* [20-21]. The responsible bioactive compounds belong to several chemical groups which are often polysaccharides or triterpenes [22-23]. One macrofungi species can have various bioactive compounds and pharmacological effects [5].

A significant amount of work has been carried out on the antimicrobial activities and chemical content of medicinal mushrooms including edible one but in Western Ghats of Tamil Nadu has not yet been explored for their antibacterial potential. Therefore, this study seeks to

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identify and survey the properties of the species of wild mushrooms growing in Western Ghats of Tamil Nadu state in India.

MATERIALS AND METHODS

Collection and preparation of mushroom species

Macrofungal species were collected from November 2013 to October 2014 from from southern Western Ghats in the Fingerpost Reserve Forest of North Zone of Nilgiri, Tamilnadu, India. In order to have wide range of species as possible, specimens were collected from forests, fields and woods. Identification of the mushrooms was done by comparing their morphological, anatomical and physiological traits with the standard description and the keys provided by the Directorate of Mushroom Research, Solan, India. The mushrooms were dried in the shade to prevent mushroom cells from sun light which destroy the cell and ground to powder using mortar and pestle.

Extracts preparation

To perform antimicrobial activity, fruiting bodies of the mushroom samples air-dried at 40ºC and300mg were ground using the pestle and mortar with methanol and then filtered using WhatmanNo.1 filter paper. After that, the extract was centrifuged at 5000rpm for 15min and the supernatant was stored at 4ºC for further experiment.

Antimicrobial activity

The antimicrobial activities of each of the methanolic extracts were tested against standard Gram positive bacteria (*Eggerthella lenta* (ATCC 43055), *Enterococcus faecalis* (ATCC 29212) and Gram negative bacterium (*Vibrio parahaemolyticus* (MTCC 451). Kirby-Bauer well diffusion assay technique [24-25] was used here. Muller-Hinton agar served as the basal medium to carry out the assay. Sterilized media plates were seeded with bacterial suspension using sterile swab. Wells (6mm) were loaded with fungal extracts at a desired concentration and were placed onto the bacteria seeded plates. Methanol was used as negative control and antibiotic as positive control. The plates were incubated at 37°C for 24 h. Two replicates were performed for each extract against each of the tested organisms. Simultaneously, controls involving the addition of amoxicillin and methanol instead of the extracts were included. Upon the completion of incubation the diameter of the resultant inhibition zones were measured and tabulated as means.

RESULTS

In the present experiment, 24 species of wild mushroom collected from the Western Ghats of Tamil Nadu, India were evaluated for their antibacterial potential. All the mushrooms used in this study were found to exhibit various degrees of antimicrobial effects against the tested microorganisms (Plate 1, 2 & 3). The zone of inhibition exhibited more than the positive control *i.e.*, Amoxicillin was considered as highly active for extracts. The study revealed that about 67% of the mushrooms inhibited growth of all the test bacteria, 29% was active against any of the two test bacteria and 4% were completely inactive (Table 1). The best *in vitro* antibacterial activity was by *Gymnopilus junonius* (26.0 mm against *Eggerthella lenta;* 25.0 mm against *Vibrio parahaemolyticus*) followed by *Tricholoma equestre* (21.0 mm against *Vibrio parahaemolyticus*).

15 mushroom species showed strong antibacterial activity against *Eggerthella lenta* higher than the standard antibiotic (Amoxicillin). 11 mushrooms showed strong antibacterial activity against *Vibrio parahaemolyticus* higher than the standard antibiotic (Amoxicillin). Whereas, only 4 mushroom species have showed strong antibacterial activity against *Enterococcus faecalis* higher than the standard antibiotic (Amoxicillin).

Figure 1 summarizes the antibacterial activity of methanolic extract of various wild mushrooms of Western Ghats with Positive control in relation to their susceptibility to the tested microorganisms. These results reveal that *Gymnopilus junonius*, *Tricholoma equestre* and *Trametes versicolor* had comparatively similar concentrations of standard antibiotics, which confirms the presence of bioactive components in these wild mushrooms.

Fig 1. Antibacterial activity of wild mushrooms from Western Ghats, India.

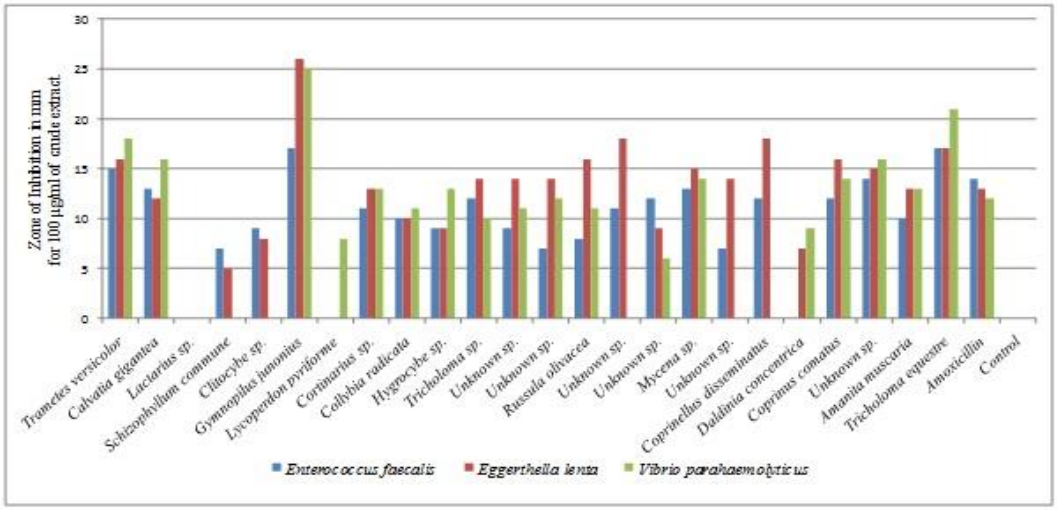

Plate 1. Antibacterial activity of Methanolic extract of Wild Mushroom species against *Enterococcus faecalis* (ATCC-29212)

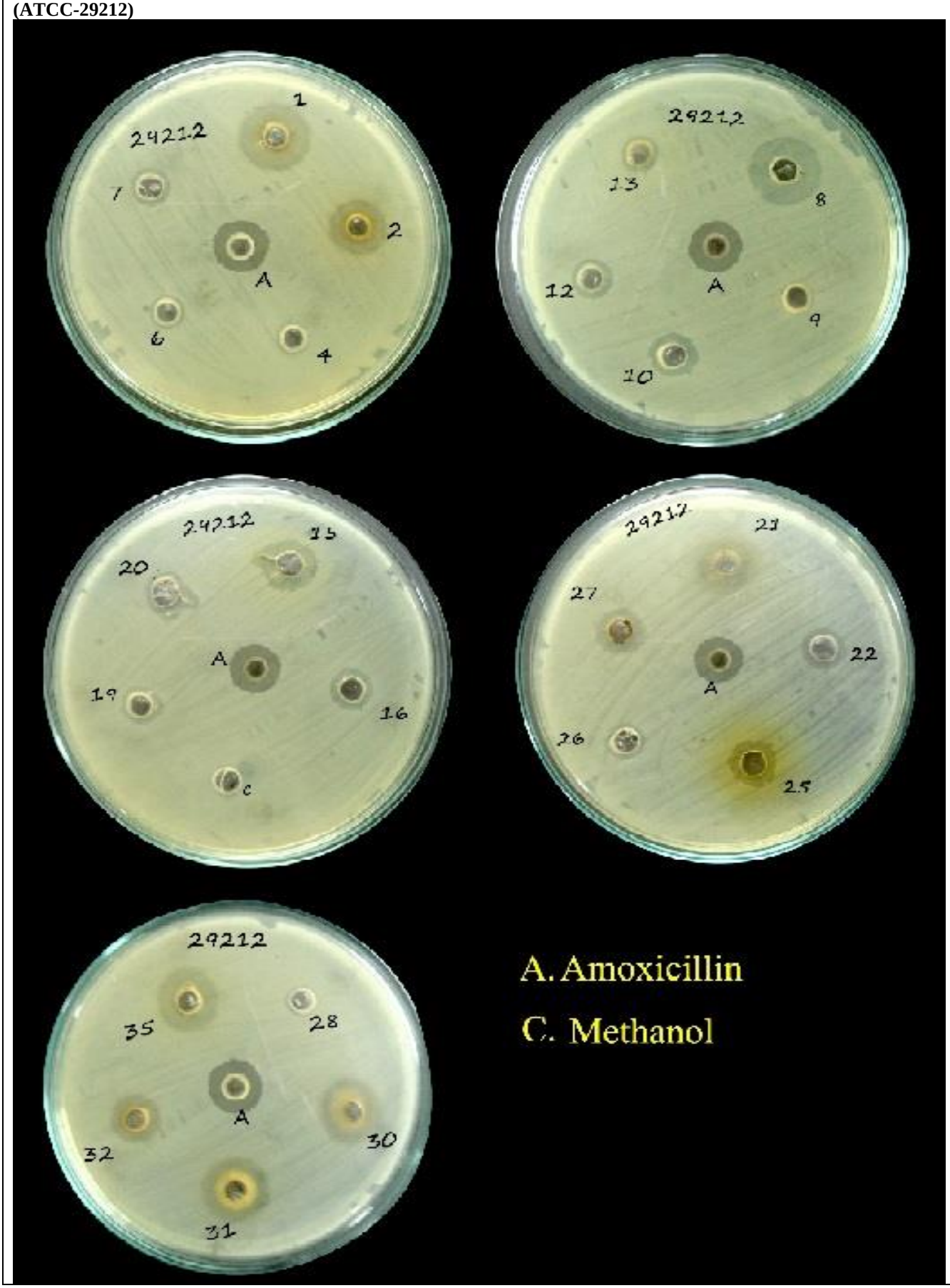

Plate 2. Antibacterial activity of Methanolic extract of Wild Mushroom species against *Eggerthella lenta* (ATCC-43055)

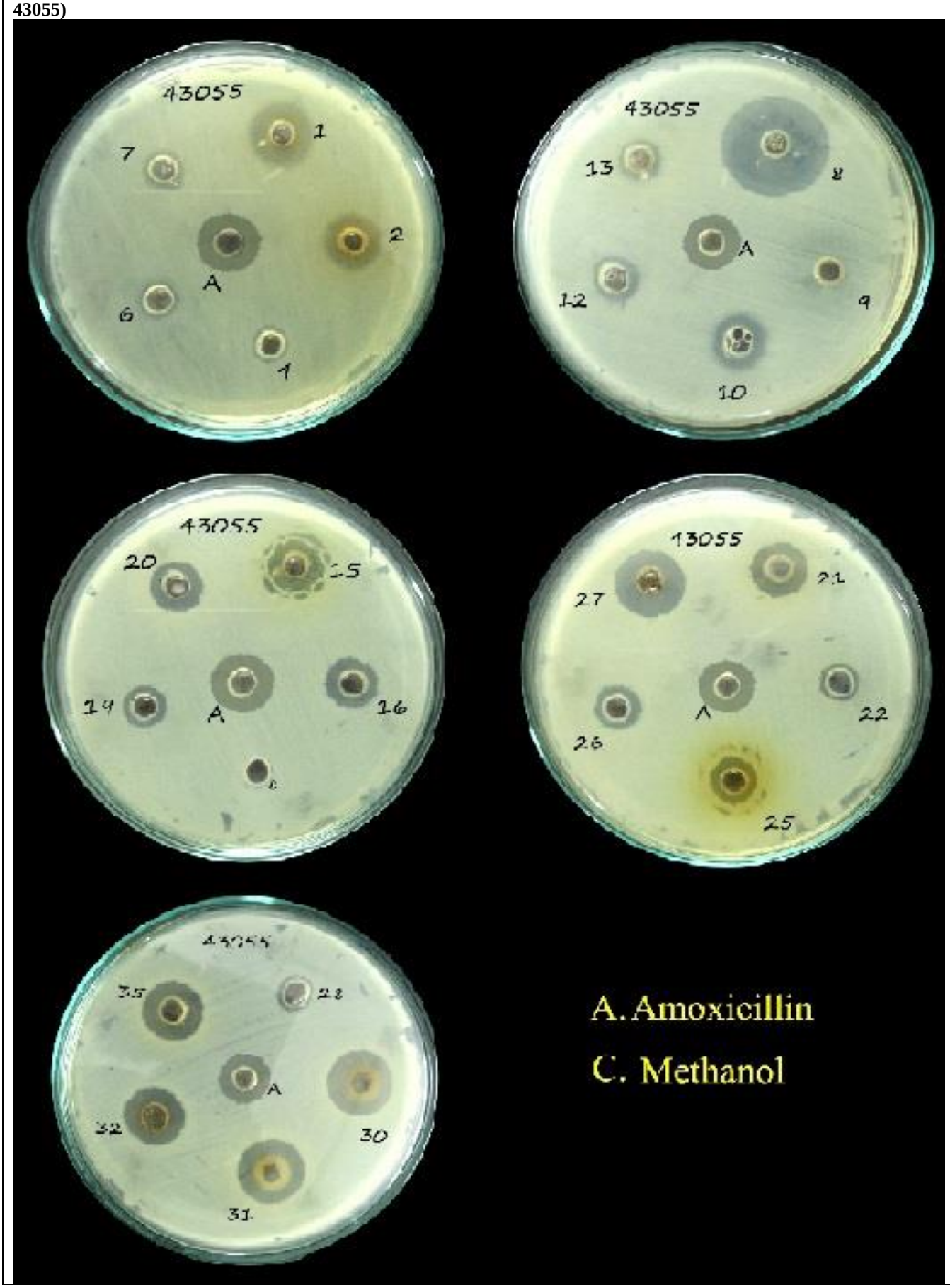

Plate 3. Antibacterial activity of Methanolic extract of Wild Mushroom species against *Vibrio parahaemolyticus* (ATCC-451)

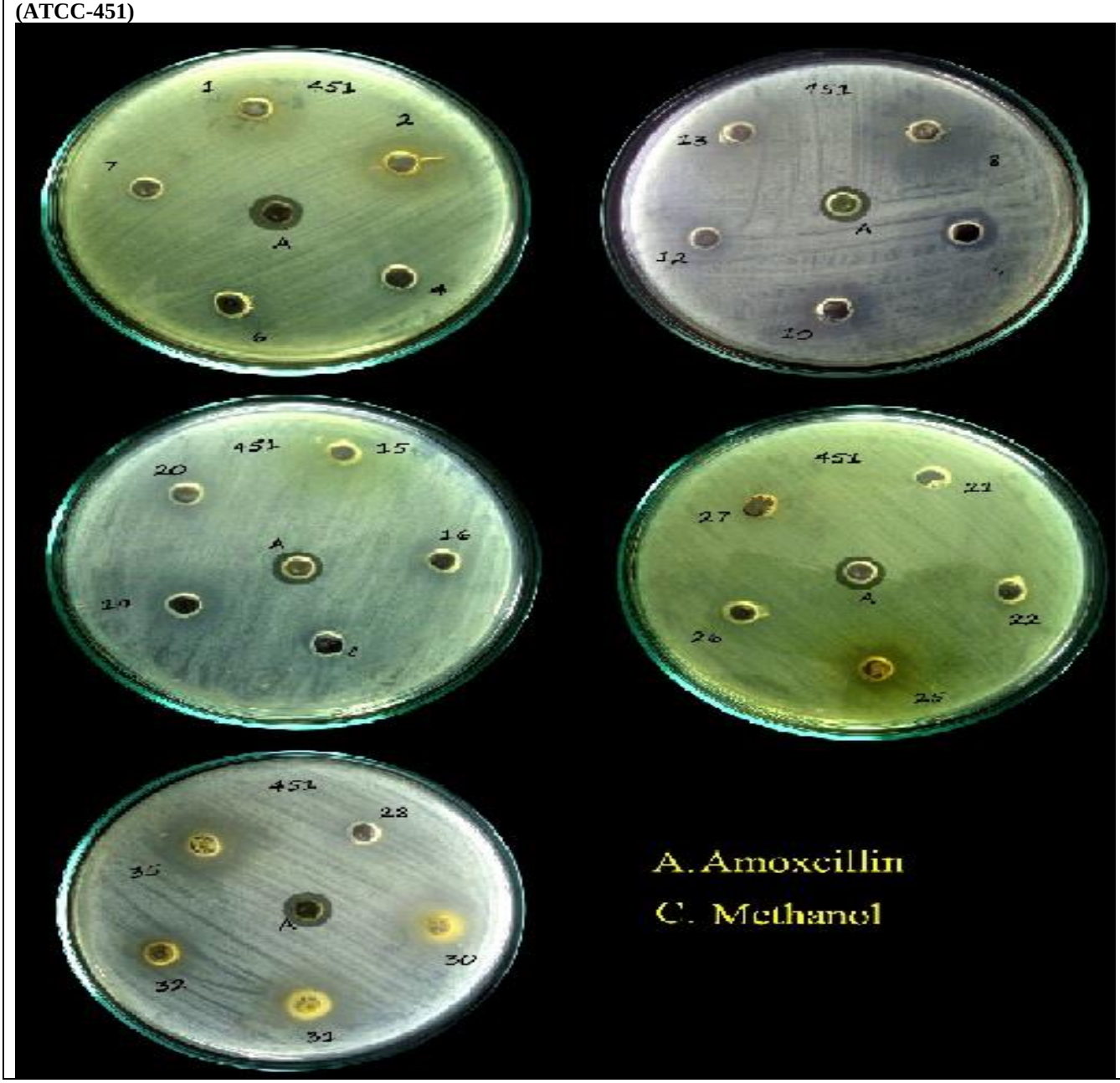

Table 1. Antibacterial activity of wild mushrooms from south Western Ghats

Sample No	Mushroom Species	Zone of Inhibition in mm for 100 µg/mL of crude extract		
		Gram Positive		Gram Negative
		Enterococcus faecalis	*Eggerthella lenta*	*Vibrio parahaemolyticus*
1	*Trametes versicolor*	15	16	18
2	*Calvatia gigantea*	13	12	16
4	*Lactarius* sp.	-	-	-
6	*Schizophyllum commune*	7	5	-
7	*Clitocybe* sp.	9	8	-
8	*Gymnopilus junonius*	17	26	25
9	*Lycoperdon pyriforme*	-	-	8
10	*Cortinarius* sp.	11	13	13
12	*Collybia radicata*	10	10	11
13	*Hygrocybe* sp.	9	9	13
15	*Tricholoma* sp.	12	14	10
16	*Unknown* sp.	9	14	11

19	*Unknown* sp.	7	14	12
20	*Russula olivacea*	8	16	11
21	*Unknown* sp.	11	18	-
22	*Unknown* sp.	12	9	6
25	*Mycena* sp.	13	15	14
26	*Unknown* sp.	7	14	-
27	*Coprinellus disseminatus*	12	18	-
28	*Daldinia concentrica*	-	7	9
30	*Coprinus comatus*	12	16	14
31	*Unknown* sp.	14	15	16
32	*Amanita muscaria*	10	13	13
35	*Tricholoma equestre*	17	17	21
	Amoxicillin	14	13	12
	Control	-	-	-

(-) = NZI – No Zone of Inhibition/Absence of susceptibility

DISCUSSION

Methanolic extract of *Agaricus bisporus*, the most cultivated mushroom in the world, showed activity against *Bacillus cereus, Micrococcus luteus, Micrococcus flavus, Staphylococcus aureus*, and *Staphylococcus epidermidis* [26-28]. Several other *Agaricus* species have also demonstrated antimicrobial activity. Methanolic extracts *Agaricus bitorquis* and *Agaricus essettei* showed an inhibitory effect upon all the tested gram-positive bacteria [28]. Methanolic extract of *Agaricus silvicola, Cantharellus cibarius, Lentinus edodes, Boletus edulis* and different *Cortinarius* sp. also revealed antimicrobial properties against *Staphylococcus aureus* and in some cases *Bacillus cereus* and *Bacillus subtilis* [29-34]. The mycelium of *Agaricus* cf. *nigrecentulus* and *Tyromyces duracinus* (ethyl acetate extracts) showed activity only against *Staphylococcus saprophyticus* [35].

Eggerthella lenta is an emerging pathogen which is susceptible to amoxicillin-clavulanate, cefoxitin, metronidazole, ertapenem, piperacillin-tazobactam, and meropenem; resistant to penicillin and piperacillin-tazobactam [36]. The present study is, the first of its kind, finding the antibacterial activity by crude extract of wild mushrooms against *Eggerthella lenta*.

Lactarius sp. showed no activity against all the tested bacteria. However, Aqueous and organic (hexane, ethyl acetate and methanol) basidiocarp extracts of the edible mushroom L*actarius indigo* were showed varied activity against various diarrheagenic *Escherichia coli* strains, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Salmonella enterica* [37].

The extracts obtained from six *Lactarius* species such as *L. deterrimus, L. sanguifluus, L. semisanguifluus, L. piperatus, L. deliciosus* and *L. salmonicolor* showed varied inhibiting activity against *Escherichia coli, Micrococcus luteus, Stapylococcus aureus, Salmonella thyphi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Corynebacterium xerosis, Bacillus cereus, Bacillus megaterium, Mycobacterium smegmatis, Candida albicans* but no antagonistic effect on *Saccharomyces cerevisiae* [38].

Lactarius controversus showed no activity against *Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica* and showed activity against *Shigella flexneri* as well as at higher concentration (30 □L) showed inhibiting activity against *Pseudomonas aeruginosa* whereas *Lactarius deliciosus* showed no activity against *Candida albicans, Escherichia coli, Salmonella enterica* and showed inhibiting activity against *Pseudomonas aeruginosa* and *Shigella flexneri* [39].

The antimicrobial activity of aqueous, methanol, hexane, and ethyl acetate extracts from edible wild and cultivated mushrooms such as *Agaricus silvicola*, *Clitocybe nebularis* and *Tricholoma equestre* against *Vibrio parahaemolyticus* and *Staphylococcus aureus*, foodborne pathogenic bacterial strains was screened and antimicrobial activity of gram-positive bacteria were more sensitive than gram-negative bacteria to fungal extracts [40].

Gymnopilus junonius a poisonous mushroom, formerly known as *Gymnopilus spectabilis*, belonging to the family Cortinariaceae, is found growing in dense clusters on stumps and logs of hardwoods and conifers. Two polyacetylenes were isolated from *Gymnopilus spectabilis*, namely hepta-4,6-diyn-3-ol and 7-chloro-hepta-4,6-diyn-3-ol which showed biological activity against *Bacillus brevis, B. subtilis, Streptococcus pyogenes* and *Staphylococcus aureus* [41]. Extracts of *Gymnopilus spectabilis* showed positive antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and resistance to *Escherichia coli* [42]. A lectin was isolated from fruiting bodies of the mushroom *Gymnopilus spectabilis* inhibited the growth of *Staphylococcus aureus* and *Aspergillus niger* [43].

Polysaccharides, sesquiterpenes, lectins, phenolic compounds, α-d-glucan and other biomolecules with different therapeutic effects (antibacterial, antifungal, cytotoxic, antitumor, anti-inflammatory, insecticidal, nematocidal, antioxidant and others) were detected in several medicinal ectomycorrhyzae forming basidiomycetous mushrooms esp. various species of *Tricholoma* such as *T. lobayense, T. giganteum, T. matsutake, T. mongolicum* and *T. portentosum* [44-46].

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Nil

CONFLICT OF INTEREST

No interest

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