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COMPARATIVE ANTIBACTERIAL STUDY OF DIFFERENT EXTRACT OF *CLAVERIA ROSEA* FRIES AGAINST GRAM NEGATIVE AND GRAM POSITIVE PATHOGENS

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ABSTRACT:

Objective: To compare the antibacterial activity of different solvent extract of *Clavaria rosea*.

Method: Different solvent extract of *Claveria rosea* was prepared. Standard cultures of *Escherichia coli* (MTCC-1698), *Klebsiella pneumoniae* (MTCC-7028), *Pseudomonas aeuroginosa* (MTCC-1934), *Salmonella typhi* (MTCC-733), *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604) and *Agrobacterium tumefaciens* (MTCC-431), gram negative. *Staphylococcus aureus* (MTCC-902) and *Streptomyces pneumoniae* (MTCC-4734), gram positive were used for the study. The antibacterial tests used were the agar well plate method. Tetracycline and Ciprofloxacin was used as the positive control.

Results: The chloroform and methanol extract of *Claveria rosea* does not show any activity against *X. campestris, E. coli, S. typhi, P. aeuroginosa* and *P. syringae* at all concentration in gram negative pathogens. Whereas petroleum ether extract cannot show against *S. pneumoniae* at all concentration in gram positive pathogens. However, the activity was less than the standard Tetracycline and Ciprofloxacin. The extract shows increasing inhibitory activity with increase in concentration (50%-100%).

Conclusions: The use of natural products including medicinal mushrooms is increasing day by day and the growth of the medicinal mushroom for this reason our investigation, for screening different solvent extract of *Claveria rosea* the results obtained confirmed therapeutic potency of some mushroom used in traditional medicine. The mushroom could be potential source of new antimicrobial agent.

INTRODUCTION: Antimicrobial resistance has become a global problem. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Strategies to improve the current situation include research in finding new and innovative antimicrobials [1].

This has forced scientists to search for new antimicrobial substances from different sources especially medicinal plants [2]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics [3]. In 1997, the 30th World Health Assembly adopted a resolution urging interested governments to utilize their traditional systems of medicine with regulations suited to their national health care systems [4].

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Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria [5]. Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microorganisms and their control, incidence of the epidemics due to drug-resistant microorganisms and the emergence of unknown disease causing microorganisms, pose enormous public health concerns [6].

In the industrialized countries, consumers are seeking visible alternatives to modern medicine where there is already a problem of over-medication; accompanied by development of resistance to same [7].

There are over 2, 500 mushroom varieties are grown in the world today [8]. It is estimated that more than 10 million metric tons of edible and medicinal mushrooms were produced last year in various countries [9]. Therefore, mushroom family has received significant attention from medical and pharmacological researchers as sample source of biologically active compounds [10]. The use of herbal supplements in the Europe including United States is continuously growing and raises concerns about safety, efficacy, and how they affect safe patient care in most of the cases [11].

The search of natural bioactive compounds that can serve as antioxidant and antimicrobial agents has increased tremendously for the last three decades [12]. Because, living cells including those of man, animals and plants are continuously exposed to a variety of challenges, that exert oxidative stress, leads to the generation of reactive oxygen species [13-15]. Because, many mushroom reported to produce a wide range of secondary metabolites having high therapeutic values such as antioxidant, antitumor, antibacterial, antiviral, cholesterol lowering, hematological agents and immunomodulating properties [16, 17].

The climatic conditions and floral diversity of the Asian regions with a high diversity of wild edible mushrooms are most important, because of their high consumption by the rural population [18]. On this standpoint, the use of natural products including medicinal mushrooms is increasing day by day and the growth of the medicinal mushroom product industry have led to increase concern regarding their safety scale [19]. Therefore, the present study has been undertaken to investigate the antibacterial activity against Gram negative and Gram positive bacteria.

MATERIAL AND METHODS:

Mushroom: The *Claveria rosea* were collected from semi evergreen forest region (13˚51'56.30"N, 75˚03'12.50"E) which is located in Haniya, Hosanagar taluk, Shimoga district, Karnataka, India, during the month of June to August 2012. The *Claveria rosea* 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 like [20-22].

The voucher specimen (KUABARN-265) has been deposited at the herbarium of mycology laboratory, Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shimoga district, Karnataka, India.

Preparation of Mushroom extracts: The dried and powdered by grinder (Bajaj Electrical Limited-Twister Mixer) mushroom material (200 g) was extracted successively with 1500 ml pet ether following 1500 ml of chloroform and methanol with a Soxhlet extractor for 48 h at temperature not exceeding the boiling point of the solvent [23]. The extracts were concentrated in a vacuum at 40°C using a rotary evaporator.

For the entire analysis, compounds of extract were dissolved in dimethylsulfoxide (DMSO). The yield of extracts obtained from pet ether was 10.88 gm, followed by chloroform (11.21gm) and methanol (51.16gm). Each extract was transferred to glass vials and kept at 4°C before use.

Selection of the Microorganisms: Nine different bacterial strains were used: *Escherichia coli* (MTCC-1698), *Klebsiella pneumoniae* (MTCC-7028), *Pseudomonas aeuroginosa* (MTCC-1934), *Salmonella typhi* (MTCC-733), *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae*

(MTCC-1604) and *Agrobacterium tumefaciens* (MTCC-431), gram negative. *Staphylococcus aureus* (MTCC-902) and *Streptomyces pneumoniae* (MTCC-4734), gram positive. They were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The viability of the organisms were maintained by regular transfer into freshly prepared nutrient agar (Himedia) and stored at 4°C until used.

Antibacterial activity by Agar Well Diffusion Method: The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37°C and maintained on nutrient agar slants at 4°C and stored at -20°C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 37°C for overnight. The overnight broth cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The agar plates were prepared by pour plate method using 20ml M-H medium. The sterile M-H agar medium is cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 10^8 cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test extracts were added.

The agar plates were incubated at 37°C for 24hrs. The diameter of zones of inhibition was measured in mm using HiMedia zone reader [24].

RESULTS AND DISCUSSION: The antibacterial activities of petroleum ether, chloroform and methanol extract of mushroom *Claveria rosea* was analyzed in vitro by agar well diffusion method. The growth inhibitory effect of crude extracts of *Claveria rosea* were tested against gram positive and gram negative bacteria *viz., X. campestris, P. syringae, A. tumefaciens, K. pneumoniae, S. aureus, S. pneumoniae, S. typhi, P. aeuroginosa* 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 *Claveria rosea* against gram negative pathogenic bacteria were presented in **Table 1**. The maximum antibacterial activity of petroleum ether extracts of *Clavaria rosea* was found against *P. aeuroginosa* (14mm) at 100% concentration, followed by *P. syringae* (13mm), *K. pneumoniae* (13mm), *S. typhi* (13mm) and *X. campestris* (12mm) and does not show any activity against *A. tumefaciens* at lower concentration.

TABLE 1: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *CLAVERIA ROSEA* AGAINST GRAM NEGATIVE PATHOGENS

Name of the bacteria	Crude extract	Diameter of Zone of Inhibition(in mm)					
		Extract concentrations (µg/ml)				Standard	Control
		12.5%	25%	50%	100%	Tetracycline (30µg/ml)	DMSO (100%)
Xanthomonas campestris	Petroleum ether	8	11	-	12	40	-
	Chloroform	-	-	-	-		
	Methanol	-	-	-	-		
Pseudomonas syringae	Petroleum ether	9	-	-	13	38	-
	Chloroform	-	-	6	7		
	Methanol	-	-	-	-		
Agrobacterium tumefaciens	Petroleum ether	-	-	-	11	30	-
	Chloroform	-	-	-	10		
	Methanol	-	-	-	8		
Escherichia coli	Petroleum ether	8	-	9	11	33	-
	Chloroform	-	-	-	-		
	Methanol	-	-	-	-		
Klebsiella pneumoniae	Petroleum ether	-	11	-	13	28	-
	Chloroform	7	-	-	11		
	Methanol	-	-	-	-		
Pseudomonas aeuroginosa	Petroleum ether	11	12	13	14	32	-
	Chloroform	-	-	-	10		
	Methanol	-	-	-	-		
Salmonella typhi	Petroleum ether	-	7	9	13	28	-
	Chloroform	-	-	-	-		
	Methanol	-	7	10	13		

'-'no activity

The antibacterial activities of *Claveria rosea* against gram positive pathogenic bacteria were presented in **Table 2**. Among the three organic solvent extracts, showed more effective inhibitory activity against *S. aureus* (9mm-12mm) at 100% concentration followed by (7mm-10mm) at lower concentration. *S. pneumoniae* does not show inhibition zone at all concentration in petroleum ether extract also there is no activity against *S. pneumoniae* in chloroform and methanol extracts at lower concentration.

TABLE 2: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *CLAVERIA ROSEA* AGAINST GRAM POSITIVE PATHOGENS

Name of the bacteria	Crude extract	Diameter of Zone of Inhibition(in mm)					
		Extract concentrations (µg/ml)				Standard	Control
						Ciprofloxacin (30µg/ml)	DMSO (100%)
		12.5%	25%	50%	100%		
Staphylococcus aureus	Petroleum ether	-	8	10	12	30	-
	Chloroform	-	7	-	9		
	Methanol	-	10	-	12		
Streptomyces pneumoniae	Petroleum ether	-	-	-	-	26	-
	Chloroform	-	-	-	9		
	Methanol	-	-	-	9		

'-'no activity

However, the activity was less than the standard Tetracycline and Ciprofloxacin. The extract shows increasing inhibitory activity with increase in concentration (12.5% -100%). 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 [25]. In this context, this novel medicinal mushroom *Claveria rosea* needs further elaborative study, pharmacological investigations, clinical trials and public awareness.

CONCLUSION: Petroleum ether extract was found to be effective against tested gram negative pathogenic bacteria compared to chloroform and methanol, whereas, all extract, was found to be moderate against tested gram positive pathogenic bacteria. The effect of antibacterial potential was examined against gram positive bacteria and gram negative bacteria; petroleum ether extract of the *Claveria rosea* has showed consistently significant inhibitory activity on different bacterial species tested except *S. pneumoniae.*

The use of natural products including medicinal mushrooms is increasing day by day and the growth of the medicinal mushroom for this reason our investigation, for screening different solvent extract of *Claveria rosea* the results obtained confirmed therapeutic potency of some mushroom used in traditional medicine. The mushroom could be potential source of new antimicrobial agent.

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REFERENCES:

1. Freeman CD: Antimicrobial resistance; implications for the clinician. Journal of Critical Care Nursing 1997; 20:21-35.
2. Thatoi HN, Panda SK and Dutta SK: Phytochemical and antimicrobial evaluation of three medicinal plants from Similipal: A note. Plant Science Research 2008; 30:48-51.
3. Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, Bories C, et al: Antiparasitic activities of medicinal plants used in Ivory Coast. Journal of Ethnopharmacology 2004; 90:91-97.
4. Panda SK and Dutta SK: Antibacterial activity from bark extracts of *Pterospermum acerifolium* (L.) Willd. International Journal of Pharmaceutical Sciences and Research 2011; Vol. 2(3): 584-595.
5. Kone WM, Kamanzi-Atindehou K, Terreaux C, Hostettmann K, Traore D and Dosso M: Traditional medicine in North Cote-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. Journal of Ethnopharmacology 2004; 93:43-49.
6. Iwu MM, Duncan AR and Okunji CO: New antimicrobials of plant origin. In: Janick J. (edition), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. 1999:457-462.
7. Akerele O: The conservation of medicinal plants. Proceedings of an International Consultation organised by the WHO, IUCN and the WWF. Cambridge University Press. Cambridge, UK, 1988: 266.

8. The Mushroom Council Report, February 2006 (http://www.mushroomcouncil.com/varieties/varieties.html).
9. Royse DJ: Forward to the fifth international conference on mushroom biology and mushroom products. Acta Edulis Fungi (Suppl.) 2005; 12:1-2.
10. Wasser SP: Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 2002; 60:258-274.
11. Lee KH: Research and future trends in the pharmaceutical development of medicinal herbs from Chinese medicine. Public Health Nutr 2000; 3(4A):515-22.
12. Demain AL: Pharmaceutically active secondary metabolites of microorganisms. Applied Microbiology and Biotechnology 1999; 52(4):455–463.
13. Ames BM, Shigenaga MK and Hagen TM: Oxidants, antioxidants and the degenerative diseases of aging. Proceedings of the National Academy Sciences 1993; 90:7915–7922.
14. Cheuion S, Roberts MA and Cheuion M: The use of cyclic voltammetry for the evaluation of antioxidant capacity. Free Radical Biology and Medicine 2000; 28:860–870.
15. Halliwell B and Gutteridge JMC: Free Radicals in Biology and Medicine. Oxford University Press, Oxford, UK, 2003.
16. Wasser SP and Weis AL: Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (Review). International Journal of Medicinal Mushrooms 1999; 1:31–62.
17. Yang J-H, Lin HC and Mau J-L: Antioxidant properties of several commercial mushrooms. Food chemistry 2002; 77:229–235.
18. Loganathan JK, Gunasundari D, Hemalatha M, Shenbhagaraman R and Kaviyarasan V: Antioxidant and phytochemical potential of wild edible mushroom *Termitomyces reticulatus*: Individual cap and stipe collected from South Eastern part of India International Journal of Pharmaceutical Sciences and Research 2010; 1 (7): 62-72.
19. Gogtay NJ, Bhatt HA, Dalvi SS and Kshirsagar NA: The use and safety of non-allopathic Indian medicines. Drug Saf 2002; 25(14):1005-19.
20. Purkayastha RP and Chandra A: Manual of Indian Edible Mushrooms, Today & Tomorrow's Printers and Publishers, New Delhi, India 1985.
21. Singer R: The Agaricales in Modern Taxonomy, Bishen Sing Mahendra Sing Publishers, Dehradun, India 1986.
22. Roy A and De AB: Polyporaceae of India, International Book Distributor, Dehradun, India 1996; 309.
23. Lin J, Opak W and Geheeb-Keller M: Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. Journal of Ethnopharmacology, 1995, 68: 267–274.
24. Kaladhar DSVGK and Siva Kishore N: Antimicrobial studies, biochemical and image analysis in *Mirabilis jalapa*. International Journal of Pharmacy &Technology 2010; 2(3): 683-693.
25. Manjunathan J, Kaviyarasan V. Solvent Based Effectiveness of Antibacterial Activity of Edible Mushroom *Lentinu stuberregium* (Fr.). Int. J. Pharm Tech Res. 2010, 2: 3, 1910-1912.

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