Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 10, pp.2731-2734, October, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *Scleroderma bermudense* CORKER. (SCLERODERMATACEAE)

***Ashok Chittaragi, Raja Naika, Roopashree, A. C. and Chandrashekar, M. B.**

Department of P. G. Studies and Research in Applied Botany, Mycology Lab, Jnana Sahyadri, Kuvempu University, Shankaraghatta-577451, Shimoga (District), Karnataka, India

ARTICLE INFO

Article History:
Received 14th July, 2013
Received in revised form
20th August, 2013
Accepted 19th September 2013
Published online 10th October, 2013

Key words:
Scleroderma bermudense extracts,
Antibacterial activity,
Pathogens, Wild mushrooms,
Western Ghats.

ABSTRACT

In this study, *in vitro* antibacterial properties of *Scleroderma bermudense* extracted using three different solvent systems (Petroleum ether, Chloroform and Methanol) were studied against plant and human pathogens viz., *Xanthomonas campestris*, *Pseudomonas syringae*, *Agrobacterium tumefaciens*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Salmonella parathyphi*, *Salmonella typhi*, *Pseudomonas aeroginosa* and *Escherichia coli* by agar well diffusion method. The three organic solvent extracts, showed more effective inhibitory activity against all the tested bacteria. Author conclude, the present results of the study evidence the utilize of the mushrooms in traditional biomedicine for the curing of ailments caused by the microorganisms.

INTRODUCTION

The higher Basidiomycetes include about 10,000 species from 550 genera and 80 families in the Basidiomycetes class with macroscopic fruiting bodies. Furthermore, approximately 700 species of higher Basidiomycetes have been found to possess significant pharmacological activities (Mizuno, 1995, Wasser, 1995, 2002). The Basidiomycota contains an abundance of species that produce large fruiting bodies including typical mushrooms, coral fungi, puffballs, bracket fungi, and so on. Some species are frequently used as a food source such as the common field mushroom, *Agaricus bisporus* (J. E. Lange) Imbach (Agaricaceae); others have been used chiefly in medicine (Boa, 2004; Molitoris, 1994), whereas some are known for their notoriously toxic properties such as Amanitas. Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from microorganisms, mainly based on their use in traditional medicine. In the past century, however, an increasing role has been played by microorganisms in the production of antibiotics and other drugs (Fenical, 1993). The medicinal use of mushrooms has a very long tradition in the Asia and Africa, whereas their use in the Western countries has been increasing only moderately in recent decades (Ding *et al*., 2008; Kirk *et al*., 2008; Kono *et al*., 2001). In recent years, a number of pharmacological actions have been intensively investigated, including cytotoxic/ antitumor, immune suppressive, antipruritic, antierythema, and antifungal, antioxidant and free radical scavenging activities. The recently-founded scientific publication International Journal of Medicinal Mushrooms (Begell House, Editor-in-Chief S. P. Wasser) and international conferences on this topic have confirmed this trend, as have several books and reviews concerning medicinal mushrooms and biologically active compounds from fungi (Buechi *et al*., 1965; Hutchinson 1999; Hyodo *et al*., 1994; Itabashi *et al*., 1993; Laakso *et al*., 2003). Fungi from the division Basidiomycota have been widely studied as an alternative source of metabolites with pharmacological properties, including anticancerigenous, antitumor, immune modulating, antibacterial and cytotoxic activities (Wasser, 2002; Daba and Ezeronye, 2003; Fan *et al*., 2006; Borchers *et al*., 2008). Antibiotic resistance of human pathogenic bacteria has become a major worldwide public health concern (Finch, 2002; Harbarth and Samore, 2005), this is why the search for new substances with antimicrobial activity is a priority (Livermore, 2005). Antimicrobial activity has already been documented in extracts from the mycelium (Suay *et al*., 2000) and fruiting bodies (Zjawioney, 2004) of different wild species from Basidiomycota. Extracts of various fungal fruiting bodies such as *Pleurotus ostreatus* (Jacq.) P. Kumm. (Iwalokun *et al*., 2007), *Pholiota adiposa* (Fr.) P. Kumm. (Strophariaceae) (Dulger, 2004), *Coprinus digitalis* (Batsch) Fr. (Agaricaceae) (Efremenkova *et al*., 2003), *Podaxis pistillaris* (L.) Fr. (Agaricaceae) (Al-Fatimi *et al*., 2006), *Lycoperdon pusillum* Batsch [now *Bovista pusilla* (Batsch) Pers.], and *Lycoperdon giganteum* Batsch [now *Calvatia gigantea* (Batsch) Lloyd] (Lycoperdaceae) (Jonathan & Fasidi, 2003) have shown activity against a range of different Gram+ve and Gram-ve bacteria and also fungi. Stamets (2006) mentioned that macrofungi produce numerous novel pharmaceuticals. Therefore, considering the previous reports on the antimicrobial potential of macrofungi and in view of the continuous need for the development of new antimicrobials, the present study aimed to evaluate a sample of *Scleroderma bermudense* for their antibacterial activity against gram positive and gram negative bacteria by agar well diffusion method.

****Corresponding author: Ashok Chittaragi,***
Department of P. G. Studies and Research in Applied Botany, Mycology Lab,Jnana Sahyadri, Kuvempu University, Shankaraghatta-577451, Shimoga (District), Karnataka, India

Table: 1. Antibacterial activities of *Scleroderma bermudense* extract against plant pathogenic bacteria by agar well diffusion method

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*	22	19	19	18	20	20	20	16	20	20	17	11	-	40
2	*P. syringae*	19	17	15	15	22	20	18	16	18	17	19	12	-	38
3	*A. tumefaciens*	22	20	18	17	23	20	18	17	20	19	15	18	-	30

'-' No activity.

Table: 2. Antibacterial activities of *Scleroderma bermudense* extract against human pathogenic bacteria by agar well diffusion method

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*	22	18	17	16	18	17	15	14	20	21	18	17	-	26
2	*S. typhi*	20	18	17	15	17	17	16	14	22	19	17	12	-	28
3	*P. aeroginosa*	20	18	17	16	16	15	14	13	19	21	18	16	-	32
4	*E. coli*	25	20	18	15	18	17	16	15	18	17	16	16	-	33
5	*K. pneumonia*	18	17	16	15	18	16	20	14	19	17	16	12	-	28
6	*S. aureus*	18	19	16	16	19	17	18	15	20	16	15	14	-	30

'-' No activity.

MATERIALS AND METHODS

The *Scleroderma bermudense* were collected from Haniya, Hosanagar taluk, Shimoga district, Karnataka during the month of September and October 2012. The S. *bermudense* 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 (Purkyastha and Aindrila, 1978) and also through the electronic data on identification keys of mushrooms (Kuo, 2004).The specimen was deposited at the herbarium of mycology laboratory, Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shimoga district, Karnataka, India.

Preparation of extracts

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.

Test microorganisms

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.

Antibacterial tests

Antibacterial activity of extracts was determined by agar well diffusion method (Bauer *et al*., 1966; Chew *et al*., 2011). The agar well diffusion method was employed for the determination of antibacterial property of the extracts. The pteriplates containing 20 ml of Mueller Hinton agar medium were seeded with 24 h culture of the microorganism. The wells (6 mm in diameter) were cut from the agar and the extract solution (5 mg/ml) was then added into it. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader) in millimeters for the organisms and comparing to the control (Anjum *et al*., 2013). DMSO served as control and 10 µg/ml of tetracycline and cifroflaxacin served as standard. Each experiment was performed in triplicates, repeated twice and were tabulated.

RESULTS

The antibacterial activities of petroleum ether, chloroform and methanol extract of mushroom *S. bermudense* was analyzed in vitro by agar well diffusion method. The growth inhibitory effect of crude extracts of *S. bermudense* 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 *S. bermudense* against plant pathogenic bacteria were presented in Table-1. The three organic solvent extracts, showed more effective inhibitory activity against all the tested bacteria. The maximum antibacterial activity of chloroform extracts of *S. bermudense* was found against *A. tumefaciens* (23mm) at 100% concentration, followed by *P. syringae* (22mm) and *X. campestris* (20mm) and moderate against *P. syringae* (18mm) and *A. tumefaciens* at 50% concentration, followed by *A. tumefaciens* (18mm) and *P. syringae* (16mm) at lower concentration. The petroleum ether extract showed maximum activity against *X. campestris* (22mm) and *A. tumefaciens* (22mm) the minimum inhibition zone (15mm) was recorded against *P. syringae.*

The methanol extract showed moderate degree of inhibition zone against *X. campestris* (20mm) and *A. tumefaciens* (20mm) at 100% concentration and mild activity against *P. syringae* (12mm) and X. *campestris* (11mm) at 12.5% concentration. The antibacterial effects of different solvent extracts of *S. bermudense* were tested against six human pathogenic bacteria and results were tabulated in Table 2. Among the three organic solvent extracts, showed more effective inhibitory activity against all the tested bacteria. The petroleum ether extracts were showed more active antibacterial proficiency against *E. coli* (25mm) and *S. parathyphi* (22mm) at 100% concentration, moderate effect against *S. typhi* (20mm) followed by *P. aeroginosa* (20mm), *K. pneumonia* (18mm) and *S. aureus* (18mm). The methanol and chloroform extract were highly active against *S. typhi* (22mm) at 100% concentration, followed by *S. parathyphi* (21mm) and *P. aeroginosa* (21mm) at 50% concentration.

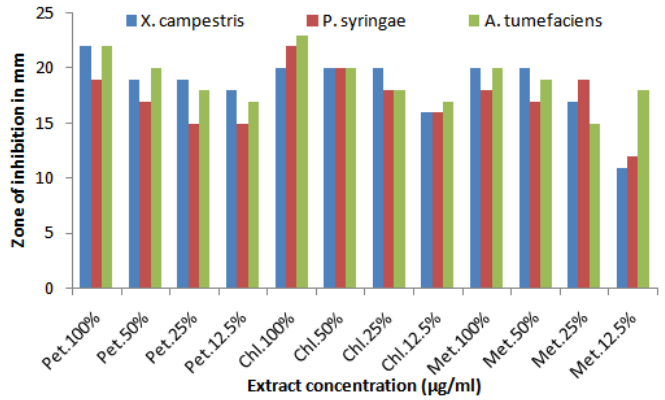

Fig. 1. Graphical representation of antibacterial activity of *Scleroderma bermudense* against plant pathogens

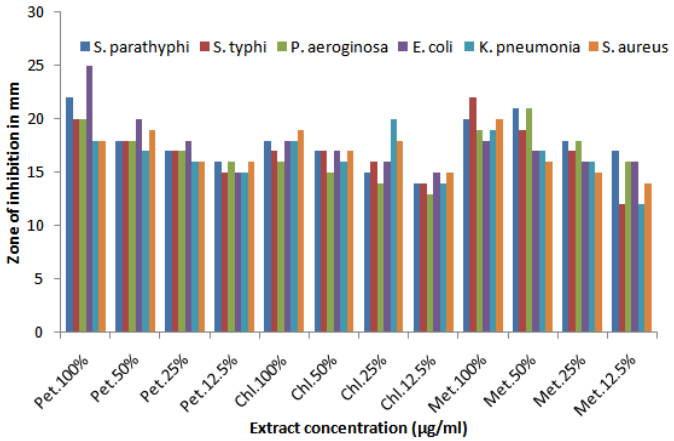

Fig. 2. Graphical representation of antibacterial activity of *Scleroderma bermudense* against human pathogens

DISCUSSION

The results of the present study revealed that antibacterial potentialities of petroleum ether, chloroform and methanol extracts varied in usefulness which may be attributed, the author also notable the antibacterial activity, which agrees with the findings of Divya *et al*., (2011). 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 (Iwalokun *et al*., 2007), 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 (Gao *et al*., 2005). However, basidiomycetes may be a source of new and useful bioactive compounds. Further studies on isolation and characterization of the active compounds may provide a better source for developing new therapeutic and pharmacological agents.

Conclusion

The presentation of the antibacterial potential of the mushroom extract of the *Scleroderma bermudense* against plant and human pathogenic microorganisms is confirmation that the extracts are potential source of antibiotics with a wide spectrum of properties. Results of this research confirm the utilize of the mushrooms in traditional biomedicine to cure ailments caused by the pathogenic bacteria. Further study will be required to bioassay indicated isolation to isolate, identify and characterization the structure of the biologically active compound accountable for pharmacological properties.

Acknowledgement

We are thankful to The Chairman, Department of Applied Botany, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shimoga (D), Karnataka, India, for providing laboratory facilities and the University Grant Commission (UGC), Government of India, for providing a research grant to carry out this study.

REFERENCES

Al-Fatimi, M.A., Jülich, W.D., Jansen, R. and Lindequist, U. (2006). Bioactive components of the traditionally used mushroom *Podaxis pistillaris*. Evid Based Complement Alternat Med 3:87–92.

Anjum, F.I., Shahid, M., Bukhari, S.A., Anwar, S. and Latif, S. (2013). Study of quality characteristics and efficacy of extraction solvent/technique on the antioxidant activity of bitter guard seed. J. Food Process Technol. 4:205.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol. 45:493-496.

Boa, E. (2004). Wild edible fungi a global overview of their use and importance to people. Non-Wood Forest Products 17. Rome, Italy: FAO.

Borchers, A.T., Krishnamurthy, A., Keen, C.L., Meyers, F.J. and Gershwin, M.E. (2008). The immunobiology of mushrooms. Exp. Biol. Med., 233(3): 259-276.

Buechi, G., White, J.D. and Wogan, G.N. (1965). The structures of mitorubrin and mitorubrinol. J Am Chem Soc 87:3484-3489.

Chew, Y.L., Chan, E.W.L., Tan, P.L., Lim, Y.Y., Stanslas, J. and Goh, J.K. (2011). Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC Complementary and Alternative Medicine. 11:12.

Daba, A.S. and Ezeronye, O.U. (2003). Anti-cancer effect of polysaccharides isolated from higher basidiomycetes mushrooms. Afr. J Biotechnol, 2 (12): 672-678.

Ding, G., Liu, S., Guo, L., Zhou, Y. and Che, Y. (2008). Antifungal Metabolites from the Plant Endophytic Fungus *Pestalotiopsis foedan*. J Nat Prod 71:615-618.

Divya, N., Mythili, S. and Sathiavelu, A. (2011). Phytochemical analysis and in vitro antimicrobial activity of *Andrographis paniculata* (Acanthaceae). Journal of Pharmacy Research. 4(7):2140-2142.

Dulger, B. (2004). Antimicrobial activity of the macrofungus *Pholiota adiposa*. Fitoterapia 75:395–397.

Efremenkova, O.V., Ershova, E.Y., Tolstych, I.V., Zenkova, V.A. and Dudnik, Y.V. (2003). Antimicrobial activity of medicinal mushrooms from the *genus Coprinus* (Fr.) S. F. Gray (Agaricomycetedeae). Int J Med Mush 5:37–41.

Fenical, W. (1993). Chemical studies of marine bacteria: developing a new resource. Chem Rev. 1673-1683.

Finch, R. (2002). Bacterial resistance- the clinical challenge. Clin. Microbiol. Infect. 8 (Suppl 3): 21-32.

Gao, Y., Tang, W., Gao, H., Chan, E., Lan, J., Li, X. and Zhou, S. (2005). Antibacterial activity of medicinal mushroom *Ganoderma*. Food Rev Int. 21: 211-229.

Harbarth, S. and Samore, M.H. (2005). Antimicrobial resistance determinants and future control. Emerg. Infect.Dis.,11(6):794-801.

Hutchinson, C.R. (1999). Microbial polyketide synthases: More and more prolific. Proc Natl Acad Sci USA 96:3336–3338.

Hyodo, S., Fujita, K., Kasuya, O., Takahashi, I., Uzawa, J. and Koshino, H. (1994). Structures of phospholipase A2 inhibitors, ergophilone A and B. Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 36:760-767.

Itabashi, T., Nozawa, K., Nakajima, S. and Kawai, K.I. (1993). A new azaphilone, falconensin H, from *Emericella falconensis*. Chem Pharm Bull 41:2040-2041.

Iwalokun, B.A., Usen, U.A. Otunba, A.A. and Olukoya, D.K. (2007). Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. African J Biotech. 6: 1732-1739.

Iwalokun, B.A., Usen, U.A., Otunba, A.A. and Olukoya, D.K. (2007). Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. Afr J Biotech 6:1732–1739.

Jonathan, S.G. and Fasidi, I.O. (2003). Antimicrobial activities of two Nigerian edible macrofungi *Lycoperdon pusilum* (Bat. Ex) and *Lycoperdon giganteum* (Pers.). Afr J Biomed Res 6:85–90.

Kirk, P.M., Cannon, P.E., Minter, D.W. and Stalpers, J.A. (2008). Dictionary of fungi, 10th edition, CABI Europe – UK.

Kono, K., Tanaka, M., Ono, Y., Hosoya, T., Ogita, T. and Kohama, T. (2001). S-15183a and b, new sphingosine kinase inhibitors, produced by a fungus. J Antibiot 54:415-420.

Kuo, M. (2004). Contributors Retrieved from the MushroomExpert.Com. 2004. Available from: http://www. mushroomexpert.com/contributors.

L., Pan, H., Soccol, A.T., Pandey. A. and Soccol, C.R. (2006). Advances in mushrooms research in the last decade. Food Technol. Biotechnol, 44(3): 303-311.44(3): 303-311.

Laakso, J.A., Raulli, R., McElhaney-Feser, G.E., Actor, P., Underiner, T.L., Hotovec, B.J., Mocek, U., Cihlar, R.L. and Broedel, SEJr. (2003). CT2108 A and B: new fatty acid synthase inhibitors as antifungal agents. J Nat Prod 66:1041-1046.

Livermore, D. (2005). Minimising antibiotic resistance. Lancet Infect. Dis., 5(7): 450-459.

Mizuno, T. (1995). Bioactive biomolecules of mushrooms: Food function and medicinal effect of mushroom fungi. Food Rev. Int. 11, 7-21.

Molitoris, H.P. (1994). Mushrooms in medicine. Folia Microbiol (Praha) 39:91–98.

Purkyastha, R.P. and Aindrila, C. (1978). Manual of Indian Edible Mushrooms. New Delhi: Today's and Tomorrow's Printers and Publication; 346.

Stamets, P. (2006). Can mushrooms help save the world? Explore 2:152–161.

Suay, I,, Arenal, F., Asensio, F.J., Basilio, A., Cabello, M.A., Díez, M.T., García, J.B., del, Val. A. G., Gorrochategui, J., Hernández, P., Peláez, F. and Vicente, M.F. (2000). Screening of basidiomycetes for antimicrobial activities. Antonie Van Leeuwenhoek, 78(2): 129-139.

Wasser, S.P. (1995). New and noteworthy species of the genus Agaricus L.: Fr. emend. Karst. from Israel. Documents Mycologiques. 25, 469-478.

Wasser, S.P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol, 60(3):258-274.

Zjawiony, K.J. (2004). Biologically active compounds from Aphyllophorales (Polypore) Fungi. J. Nat. Prod., 67(2): 300-310.

****