KAVAKA 58 (2) : 11-16 (2022) DOI: 10.36460/Kavaka/58/2/2022/11-16

Characterization of chemical constituents of *Coprinopsis cinerea* (KX468975), a coprophilous mushroom

S. Mohankumar[1*] and J. Savitha[2]

[1]*St. Joseph's College (Autonomous), Langford Road, Shantinagar, Bengaluru- 560027. Karnataka, India*

[2]*Department of Microbiology, Biotechnology and Food Technology, Bangalore University, Jnanabharathi campus, Bangalore-560056, Karnataka, India.*

[*]*Corresponding author Email: drsvtj@yahoo.co.in*

(Submitted on April 28, 2022; Accepted on June 11, 2022)

ABSTRACT

Exploration of coprophilous mushrooms as a source of biologically active compounds is gaining importance in recent years. The present study was designed to determine the mycochemicals present in the methanolic extracts of fruit bodies and mycelia of coprophilous fungus *Coprinopsis cinerea* (KX468975) by qualitative methods and evaluation of their volatile components by gas chromatography coupled with mass spectroscopy. The qualitative mycochemical analyses of fruit bodies and mycelia have shown the presence of important chemical constituents such asalkaloids, terpenoids, flavonoids, cardioglycosides, quinones, phenols and tannins. Further, GC-MS analysis of methanolic extracts of fruit bodies and mycelia have led to the identification of 16 types of organic compounds belonging to n-alkanes, 1-alkenes, 1-alkanols, free fatty acids, alkyl esters, saturated and unsaturated fatty acids, sterols, triterpenes, mono and sesquiterpenes, 1-amines, aldehydes and amide groups. This provides baseline information about the bioactive constituents of *Coprinopsis cinerea* in providing valuable compounds of with significant medicinal values

Keywords: Corpophilous fungus, *Coprinopsis cinerea*, Methanolic extract, Bioactive compounds, Mycochemicals, GC-MS analysis

INTRODUCTION

Mushrooms are widely used as food not only for its delicacy but for its nutritional and medicinal values. They are low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals (Chen and Ho, 1986; Lindequist *et al.,* 2005; Barros *et al.,* 2007).

Bioactive molecules produced by mushrooms are mainly belonging to polysaccharides; glucans, terpenoids, phenolic compounds, lectins, statins, etc. having immune-modulating, antioxidant, genoprotective, antitumor, hypocholesterinemic, antidiabetic, hepatoprotective and other medicinal effects (Tiwari *et al.,* 2011; Alves *et al.,* 2012; Badalyan, 2014). Many pharmaceutical substances with potent and unique health enhancing properties have been isolated from mushrooms either from the mycelia or fruit bodies and are consumed in the form of capsules, tablets or extracts. Metabolite information on coprohilous mushrooms is essential to understand their pharmaceutical and nutraceutical importance.

In the last few years, gas-chromatography coupled mass-spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in living organisms (Petrova *et al.,* 2007). And also many reports are available till date on the identification of volatile constituents of wild and cultivated mushroom extracts using GC-MS (Cho *et al.,* 2008).

Coprinopsis cinerea, a coprophilous basidiomycete also called as inky cap mushroom belonging to the family *Psathyrellaceae,* is used as a model organism to study fruit body development in mushrooms (Kües and Liu, 2000). According to several reports, *C. cinerea* is extensively used in the production of pharmaceutically important antimicrobial peptides (Essig *et al.,* 2004), insecticidal peptides, cellulases, industrially important xylanases (Kaur *et al.,* 2011) and laccases (Wang *et al.,* 2014). The primordium of *C. cinerea* is chiefly used as a culinary food in Srilanka, Malaysia and Thailand countries. Although, several reports are available on the application of *Coprinopsis cinerea* on various fields, its chemical constituents and metabolite profile is not much reported. Hence, the present work was carried out to elucidate the mycochemical and volatile constituents of the methanolic extracts of *Coprinopsis cinerea* (KX468975) mycelia and fruit bodies using qualitative methods and GC-MS.

MATERIALS AND METHODS

Chemicals and reagents : All the chemicals and reagents used in the present study were of AR grade and HPLC grade purchased from Hi-Media and Sisco research laboratories (SRL), Mumbai, India.

Fungal culture : The pure culture of *C. cinerea* (KX468975) isolated from horse dung was used for the present study and it was maintained in 2% wheat flour agar (WFA) medium at 4°C until use (Mohankumar and Savitha, 2017).

Submerged fermentation : Submerged fermentation was carried out to cultivate *C. cinerea* (KX468975) in 2% wheat flour broth (WFB) medium incubated for 7 days at 30°C, pH 6 under dark. After the fermentation the mycelia was separated from culture filtrate and used for the extraction of metabolites (Mohankumar and Savitha, 2017).

Extraction of metabolites

The fresh fruit bodies and mycelia of *C. cinerea* (KX468975) were dried in an oven at 50ºC until constant weight was obtained. Ten grams of dried material was extracted with 100 ml of analytical methanol (Himedia, India) for 72 hrs, the process was continued three times. The combined extracts were filtered through Whatman filter paper no. 1 and concentrated to dryness using rotary vacuum evaporator. The concentrated extract was dissolved in methanol (10mg/ml) and used for qualitative myco-chemicals and GC-MS analysis (Roy and Krishnappa, 2018).

Qualiatative mycochemical analysis of methanolic extracts of *C. cinerea* (KX468975) : The methanolic extract

of *C. cinerea* (KX468975) fruit bodies and myceliaprepared in the previous step was analysed for the presence of mycochemicals including carbohydrates, cardioglycosides, terpenoids, tannins, phenolic compounds, proteins and quinones according to the methods described by Gull *et al.,* (2013).

Test for carbohydrates : One ml of each extract was taken in test tubes separately and treated with 5 ml of Fehling's solution (Solution A: 34.6 g of copper (II) sulfate pentahydrate dissolved in 500 ml distilled water, Solution B: 125 g of potassium hydroxide and 173 g of potassium sodium tartrate tetrahydrate dissolved in 500 ml of distilled water, combine solution A and solution B (1:1) just before use). The test tubes were placed in boiling water bath for 5 minutes and observed for the appearance of yellow or red colour precipitate, which indicates the presence of reducing sugars.

Test for cardioglycosides : Five ml of each extracts was taken in test tubes separately and treated with 2 ml of glacial acetic acid having a drop of ferric chloride solution. One ml of the concentrated sulphuric acid was added to each test tube. The tubes were observed for the appearance of brown colored ring at the interface indicating the presence of cardio-glycosides.

Test for terpenoids : Five ml of each extract was taken in test tubes separately and mixed with 2 ml of chloroform. Further, concentrated sulphuric acid was added along the tubes to form a layer and observed for the appearance of reddish brown colour at the interface.

Test for tannins : Two ml of each extract was mixed with few drops of 0.1% ferric chloride solution in test tubes and observed for the appearance of brownish green colour.

Test for phenolic compounds : One ml of each extract was mixed with 4 drops of ethanol and 3 drops of 0.1% ferric chloride solution in test tubes separately and observed for the appearance of red colour.

Test for proteins : One ml of each extract was taken in test tubes separately and two drops of freshly prepared 0.2% ninhydrin reagent (2.5 g of ninhydrin dissolved in 50 ml n-butyl alcohol on mild heating and stirring and diluted 500 ml with n-butyl alcohol) was added. Then the test tubes were heated for few minutes and were observed for the appearance of blue colour.

Test for quinones : Few drops of 1N sodium hydroxide solution were mixed with 1 ml of each extract in test tubes separately. Test tubes were observed for the appearance of red colour indicating the presence of quinones.

GC-MS analysis : GC-MS analysis of volatile constituents of *C. cinerea* (KX468975) fruit bodies and mycelia was carried out on gas chromatograph (Hewlett Packard 5890) linked to a mass spectrometer (Hewlett Packard 5972) with a capillary column SPB-50 (30 m × 0.32 mm, 0.25 µm film thickness). A helium carrier gas was used with a temperature programme set at 270°C - 290°C at a rate of 4°C min^{-1} with a 20 min hold. The ion source was set at 250°C with the ionization voltage at 70 eV3. The MS transfer line was maintained at a temperature of 250°C and TSQ 8000 Triple Quadrupole MS detector was used for the analysis. The obtained MS data was evaluated for identification and quantification of compounds by total ion count (TIC) (Roy and Krishnappa, 2018).

Identification of compounds : Identification of compounds was carried out by searching mass spectra of the components with the data available in National Institute of Standards and Technology (NIST) library.

RESULTS

Mycochemical constituents of methanolic extracts of *C. cinerea* (KX468975) fruit bodies and mycelia : The results of qualitative mycochemical screening of methanolic extract of *C. cinerea* (KX468975) fruit bodies and mycelia revealed the presence of carbohydrates, proteins, cardioglycosides, terpenes, quinones, tannins, alkaloids, flavonoids and phenolics in varying proportions (**Table 1**). Tannins were absent in all the extracts.

Table 1: Mycochemical constituents of methanolic extracts of *C. cinerea* (KX468975) fruit bodies and mycelia

Mycochemical constituents	Methanolic extract	
	Fruit bodies	Mycelia
Carbohydrates	+	+
Terpenoids	-	+
Cardioglycosides	+	+
Proteins	+	+
Phenols	+	+
Quinones	+	+
Tannins	-	-
Alkaloids	+	+
Flavonoids	+	+

('+' Indicates present, '-'Indicates absent)

GC-MS analysis : In this study, the GC-MS analysis of methanolic extracts of *C. cinerea* (KX468975) fruit bodies and mycelialed to the identification of 16 volatiles in varying proportions (**Fig. 1 & 2**). All the 16 mycochemicals with their retention time, molecular formula, molecular weight and concentration (peak area %) are presented in the **table 2 & 3**. The mass spectrometer analyzes the nature and structure of the compounds eluted at different times. The most prevailing compounds were p-anisaldehyde (13.96%), hexadecanoic acid (4.55%), stearolic acid (34.52%), cis-vaccenic acid (14.12%) and resorcinol pentadecyl (16.63%) in the methanolic extract of *C. cinerea* (KX468975) fruit bodies (**Table 2**) and succinic acid dimethyl ester (33.67%), palmitic acid methyl ester (11.80), 9,12-octadecadienoic acid methyl ester (14.40%) and methyl lignocerate (8.62%) in the methanolic

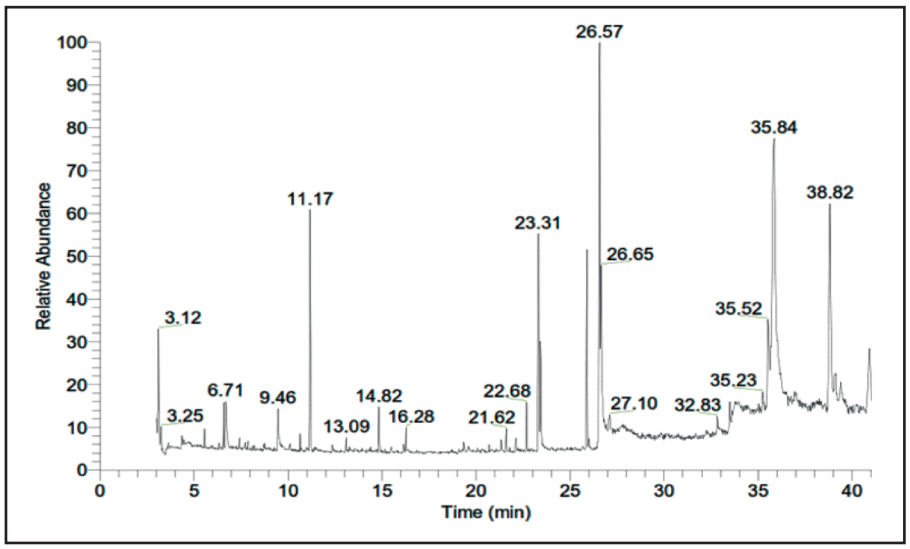

Fig. 1: GC-MS chromatogram of methanolic extract of *C. Cinerea* (Kx468975) fruit bodies.

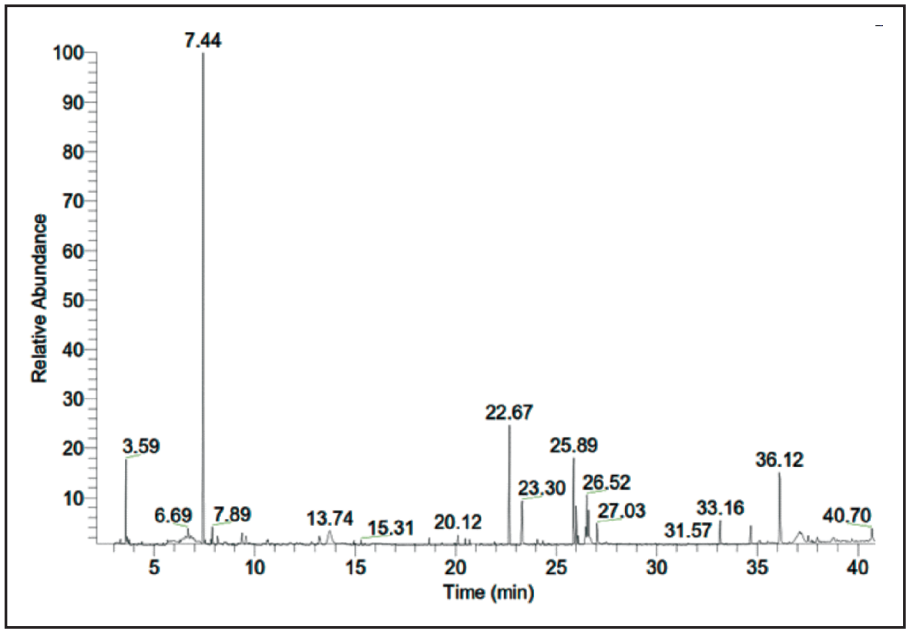

Fig. 2: GC-MS chromatogram of methanolic extract of *C. cinerea* (KX468975) mycelia.

extract of *C. cinerea* (KX468975) mycelia (**Table 3**).

DISCUSSION

The exploration for novel drugs from coprophilous fungi had advanced from past one decade due to the exclusive diversity of bioactive compounds reported (Bills *et al,* 2013). Gas chromatography coupled with mass spectroscopy (GC-MC) is proved to be suitable for the interpretation of the volatile constituents of the mushrooms (Zhang *et al.,* 2008; Cho *et al.,* 2008). Extraction is the main step for the recovery and isolation of bioactive mycochemicals from mushrooms, before component analysis (Karimi and Jaafar, 2010). Successful determination of biologically active compounds from mushrooms is largely dependent on the type of solvent used in the extraction procedure and are chosen based on the polarity of the solute of interest. It has been reported that extraction of biomolecules using methanol as a solvent offers successful recovery of polar and non polar constituents of great interest (Gómez-Romero *et al.,* 2010). Therefore, in our study we used methanol as a solvent for the extraction of mycochemicals and volatile constituents form fruit bodies and mycelia of *C. cinerea* (KX468975). The myco-chemical analysis showed positive results for alkaloids, terpenoids, flavonoids, cardioglycosides, steroids, phenols and carbohydrates. Proteins and tannins were found to be absent in all the extracts. These mycochemicals alone or in combination may have tremendous therapeutic potential in curing various ailments (Roy *et al.,* 2018: Sharma *et al.,* 2018). Our GC-MS results led to the identification of 16 types of organic compounds belonging to n-alkanes, 1-alkenes, 1-alkanols, free fatty acids, alkyl esters, saturated and unsaturated fatty acids, sterols, triterpenes, mono and sesquiterpenes, 1-amines, aldehydes and amide groups. Among the identified compounds hexadecanoic acid present in both methanolic extracts of fruit bodies and mycelia have the properties of antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, hemolytic, antimicrobial (Gomathi *et al.,* 2015) and larvicidal activities (Rahuman, 2000). Methyl lignocerate present in methanolic extract of mycelia is used in the production of biodiesel (Pratas, 2011). Octadeca-dienoic acid (Z, Z) detected in the fruit bodies extract have the property of anti-inflammatory, hypo-cholesterolemic and antiarthritic activities (Gomathi *et al.,* 2015). Stearolic acid is a major components present in the methanolic extract of fruit bodies and mycelia of *C. cinerea* has the property of DNA binding and antifungal activities against *Candida* and *Trichophyton* spp. (Li *et al.,* 2008). Further, study is aimed at establishing its pharmaceutical and nutraceutical importance.

CONCLUSION

In recent years, consumption of synthetic drugs has resulted in some side effects and has posed severe health risks. Thus, mushroom based compounds are preferred over the synthetic

Table 2: List of volatile constituents identified in methanolic extract of *C. cinerea* (KX468975) fruit bodies by GC-MS

Compound Name	Molecular formula	Molecular weight	Retention time	Peak area (%)
Toluene	C_7H_8	92	3.12	1.47
p −Anisaldehyde	$C_8H_8O_2$	136	11.17	13.96
Hexadecanoic acid	$C_{12}H_{32}O_2$	256	23.31	4.55
Stearolic acid	$C_{18}H_{32}O_2$	280	26.57	34.52
cis−Vaccenic acid	$C_{18}H_{34}O_2$	282	26.65	14.12
9,12−Octadecadienoic acid (Z, Z) − , 2,3 − dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	35.52	1.66
Resorcinol, pentadecyl	$C_{21}H_{36}O_2$	320	35.84	16.63
β − sitosterol	$C_{29}H_{50}O$	414	38.82	13.05

Table 3: List of volatile constituents identified in methanolic extract of *C. cinerea* (KX468975) mycelia by GC-MS.

Compound Name	Molecular formula	Molecular weight	Retention time	Peak area (%)
2,3−Butanediol	$C_4H_{10}O_2$	90	3.59	8.44
Succinic acid, dimethyl ester	$C_6H_{10}O_4$	146	7.44	33.67
Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	22.67	11.80
Hexadecanoic acid	$C_{16}H_{32}O_2$	256	23.30	6.01
9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	25.89	14.40
Stearolic acid	$C_{18}H_{32}O_2$	280	26.52	13.60
Behenic acid, methyl ester	$C_{23}H_{46}O_2$	354	33.16	3.42
Methyl lignocerate	$C_{25}H_{50}O_2$	382	36.12	8.62

one which causes minimal side effects. The volatiles represent a new frontier in bioprospecting, and study of these compounds promises the discovery of new products for human exploitation and will provide basis for future research work. In the present study, 7 mycochemicals and 16 bioactive constituents have been identified from methanolic extracts of fruit bodies and mycelia of *C. cinerea* (KX468975) by qualitative and GC-MS analysis. As far as we know, this work is the first approach to report the volatile constituents of *C. cinerea* (KX468975) by using GC-MS. The presence of these medically important mycocompounds justifies the nutritional and medicinal values of *C. cinerea* and can be exploited commercially. Further, studies on isolation and identification of bioactive compounds have to be performed to determine the bioactivity of each compound.

ACKNOWLDEGEMENTS

The authors would like to thank the Department of Microbiology and Biotechnology, Bangalore University, Bangalore for providing the facility to carry out this research work and Department of backward classes, government of Karnataka for financial support in the form of OBC PhD fellowship.

REFERENCES

Alves, M. J., Ferreira and I. C., Dias J. *et al.* 2012. A review on antimicrobial activity of mushroom (*Basidiomycetes*) extracts and isolated compounds. *Planta medica* **78 (16):** 1707-18.

Badalyan, S.M. 2014. Potential of Mushroom Bioactive Molecules to Develop Healthcare Biotech Products. *In Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8)*. New Delhi: Yugantar Prakashan Pvt. Ltd., pp. 373-378.

Barros, L., Baptista, P., Estevinho, L.M. and Ferreira, I.C. 2007. Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *Lactarius* sp. mushrooms. *Journal of Agricultural and Food Chemistry* **55 (21) :** 8766-71.

Bills, G.F., Gloer, J.B. and An, Z. 2013. Coprophilous fungi: antibiotic discovery and functions in an underexplored arena of microbial defensive mutualism. *Current Opinion in Microbiology* **16 (5):** 549-65.

Chen, C.C. and Ho. C.T. 1986. Identification of sulfurous compounds of shiitake mushroom (*Lentinus edodes* Sing.). *Journal of Agricultural and Food Chemistry* **34 (5):** 830-833.

Cho, I. H., Namgung, H.J., Choi, H.K. and Kim, Y.S. 2008. Volatiles and key odorants in the pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.). *Food Chemistry* **106 (1):** 71-6.

Essig, A., Hofmann, D., Münch, D. *Et al.* 2014. Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis. *Journal of Biological Chemistry* **289 (50):** 34953-64.

Gomathi, D., Kalaiselvi M. and Ravikumar G. *et al.* 2015. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. *Journal of Food Science and Technology* **52 (2):** 1212-1217.

Gómez-Romero, M, Segura-Carretero, A. and Fernández-Gutiérrez, A. 2010 Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry* **71 (16):** 1848-64.

Gull, I., Sohail., M, Aslam, M.S. and Athar, M.A. 2013. Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. *Annals of Clinical Microbiology and Antimicrobials* **12 (1):** 36.

Kalac, P. 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry* **113 (1):** 9-16.

Karimi, E. and Jaafar, H.Z. 2011. HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of *Labisia pumila* Benth. *Molecules* **16 (8):** 6791-805.

Kaur, H., Dutt, D. and Tyagi, C.H. 2011. Production of novel alkali-thermo-tolerant cellulase-poor xylanases from *Coprinopsis cinerea* HK-1 NFCCI-2032. *Bio Resources* **6 (2):** 1376-1391.

Kües, U. and Liu, Y. 2000. Fruiting body production in *Basidiomycetes*. *Applied Microbiology and Biotechnology* **54 (2):** 141-52.

Li, X.C., Jacob, M.R. and Khan, S.I., *et al.* 2008. Potent *in-vitro* antifungal activities of naturally occurring acetylenic acids. *Antimicrobial Agents and Chemotherapy* **52 (7):** 2442-8.

Lindequist, U, Niedermeyer, T.H. and Jülich, W.D. 2005. The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine* **2 (3):** 285-99.

Mohankumar, S. and Savitha, J. 2017. Wheat flour, an inexpensive medium for *in-vitro* cultivation of coprophilous fungus *Coprinopsis cinerea*. *Current Research in Environmental and Applied Mycology* **7 (3):** 144-54.

Petrova, A., Alipieva, K. and Kostadinova, E. *et al.* 2007. GC-MS studies of the chemical composition of two inedible mushrooms of the genus *Agaricus*. *Chemistry Central Journal* **1 (1):** 33.

Pratas, M.J, Freitas, S.V. and Oliveira M.B. *et al.* 2011. Biodiesel density: experimental measurements and prediction models. *Energy & Fuels* **25 (5):** 2333-2340.

Rahuman, A.A., Gopalakrishnan, G. and Ghouse, B.S. *et al.*

2000. Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia* **71 (5):** 553-555.

Roy, D.R. and Krishnappa, M. 2018. Mycochemical profiling of *Lentinus squarrosulus* Mont., A wild edible macrofungi using GC-MS. *International Journal of Pharmaceutical Sciences and Research* **9:** 349-354.

Sharma, D., Singh, V.P. and Singh, N.K. 2018. A review on phytochemistry and pharmacology of medicinal as well as poisonous mushrooms. *Mini Reviews in Medicinal Chemistry* **18 (13):** 1095-1109.

Tiwari, P., Kumar, B. and Kaur, M. *et al.* 2011. Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia* **1(1):** 98-106.

Wang, B., Wang, L. and Lin, Y., *et al.* 2014. Purification and characterization of a laccase from *Coprinopsis cinerea* in *Pichia pastoris*. *World Journal of Microbiology and Biotechnology* **(4):**1199-1206.

Zhang, Z.M., Wu, W.W. and Li, G.K. 2008. A GC-MS study of the volatile organic composition of straw and oyster mushrooms during maturity and its relation to antioxidant activity. *Journal of Chromatographic Science* **46 (8):** 690-696.