



**MYCOCHEMICAL SCREENING AND DETERMINATION OF
NUTRITIVE POTENCY AND ANTIOXIDANT ACTIVITY OF EDIBLE
MACROFUNGI DACRYOPINAX SPATHULARIA (SCHWEIN) AND
SCHIZOPHYLLUM COMMUNE (FRIES)**

Amar Kumar^{1*}, Sarfaraz Ali², S. B. Lal³ and M. P. Sinha⁴

¹*Department of Zoology, K.S. College, Kolhan University, Jharkhand, India, 833219.

²P.G. Department of Biotechnology Magadh University Bodh Gaya.

³Department of Zoology, Kolhan University, Jharkhand, India, 833219.

⁴Department of Zoology, Ranchi University, Ranchi, Jharkhand, India, 834008.

Article Received on
14 July 2018,

Revised on 03 August 2018,
Accepted on 23 August 2018,

DOI: 10.20959/wjpr201816-13240

***Corresponding Author**

Amar Kumar

Department of Zoology,
K.S. College, Kolhan
University, Jharkhand,
India, 833219.

ABSTRACT

Present communication reveals that the two edible macrofungi, viz; *Schizophyllum commune* and *Dacryopinax spathularia* have been subjected for screened mycochemical constituents and the extracts have been further analysed for their antioxidant activity and nutritive potency. Both the macrofungi were found containing mycochemicals like **Tannins, Saponins, Flavonoids, Alkaloids, Phenolics etc.** The nutritive value of *D. spathularia* has been found to be 2.99 cal/gm which is more than the nutritive value of *S. commune* which is 2.75 cal/gm. Comparing the results to BHA (Butylated Hydroxy Anisole) standard (EC 50 = 5.0 µg/mL), the *S. commune* extract shows 19.65%

and the *D. spathularia* extract shows 14.75% free radical scavenging activity at 100 microgram/ml concentration. The superoxide anion scavenging activity of *D. spathularia* extract is found to be 6.45% and that of *S. commune* extract is found to be 4.84% at 100 microgram/ml concentration. The hydroxyl radical scavenging activity has been found to be 7.50% for *S. Commune* extract and 7.36% for *D. Spathularia* extract at 100 microgram/ml concentration. The total antioxidant capacity (TAC) of *Dacryopinax spathularia* extract has been found to be 7.30% and 12.20% at 50 microgram/ml and 100 microgram/ml concentration respectively, whereas the TAC of *Schizophyllum commune* extract has been found to be 6.15% and 12.15% at 50 and 100 microgram/ml concentration respectively. Thereby both the experimental macrofungal species are very much potential sources of

natural antioxidants, fibre and nutrients.

KEYWORDS: Macrofungi; Mycochemical; Antioxidant; Nutritive value; *D. spathularia*; *S. commune*; TAC; BHA; Nephroprotective; Hepatoprotective.

1. INTRODUCTION

The metabolic processes and biochemical reactions taking place in the living body continuously produces potentially damaging chemical species called Free Radicals. The free radicals contain one or more unpaired electrons due to which they are highly unstable and therefore they can react with biomolecules like protein, lipid, amino acids, DNA which may lead to cell injury or numerous disease (Kitaz et al, 2016). The ROS (Reactive Oxygen Species) and reactive nitrogen species (NOS) are among the most potent free radicals including Super oxide anion radical ($O_2^{\cdot-}$), Hydroxyl radical (OH.), Alkoxy radical (RO.), Peroxyl radical (ROO.), Hydrogen peroxide, Nitric oxide (NO.) etc., which are continuously generated within the body through various endogenous pathways like respiratory chain reaction, phagocytosis of infected cells, prostaglandin synthesis, degradation of fatty acids and natural toxins, as well as exogenous factors like exposure to electromagnetic radiations (Miller et al, 1997; Pacher et al, 2007). The destructive impact of these free radicals is neutralized by the compounds called Antioxidants like Vitamin E, Vitamin C, Beta Carotenoids, Glutathione etc., which are either synthesized within the body or supplied with the dietary sources (Krishnaiah et al 2007). When the equilibrium between the free radicals (Oxidants) and anti-Oxidants get disturbed by any means, then the body undergoes in a condition of Oxidative Stress (Sen et al, 2010), which may lead to a wide range of human diseases like cardiovascular disease (Cottone et al, 2008), pulmonary disease (Jelic et al, 2008), cancers (Reuter et al, 2010) etc. It is an established fact that the natural anti-oxidants mainly come from dietary sources in the form of Flavonoids, Alkaloids as well as Phenolic compounds etc. (Lei et al, 2012; Hammas et al, 2016).

Edible macrofungi or mushrooms belong to group Basidiomycota which includes nearly 10000 species out of which approximately 700 species have been reported for their pharmacological properties (Karaman et al, 2012; Dandapat et al, 2015). *Dacryopinax spathularia* (Schewin) and *Schizophyllum commune* (Fries) are the two edible macrofungi belonging to group Basidiomycota and has been used traditionally for the treatment of various diseases and disorders such as antiviral, antitumour, antibacterial, and immunomodulating, anti-inflammatory, anti-diabetic, nephroprotective, hepatoprotective

activities (Mitko et al, 2008; Adebayo et al, 2012). But there is paucity of scientific authentication of pharmacological and medicinal property and efficacy of these species. Therefore, the present study has been undertaken to analyse the mycochemical composition, nutritive potentiality and the antioxidant potency of the two edible macrofungi *D. spathularia* and *S. Commune*, which are easily available in India.

2. MATERIAL AND METHODS

2.1 Extraction

Following the standard Soxhlet method of extraction from the fresh fungi has been washed, disinfected by treating with HgCl₂ and then subjected to repeated washing. The fungi had been dried in shade under room temperature for six to seven days. After proper dehydration the fungi samples have been grinded to produce into powdered form. 50g of the fine powder has been subjected to extraction by soxhlet using distilled water for aqueous extract or other organic solvent like acetone, ethanol. The extract obtained has been filtered, concentrated and dried in rotary flash evaporator maintained at 45°C for proper dehydration. Percentage yield of each extract has been calculated and the dried extract has been stored in air tight containers at room temperature for further use.

2.2 Mycochemical analysis

Estimation of moisture content, ash content, and nutritional potentiality, pH of fruiting body, flavonoids, tannins, saponins and alkaloids content of fungal extracts was done following the method of Sofowara (2008). Total phenol was determined by Folin Ciocalteu reagent, following Ramamoorthy and Bono (2007). The amount of crude fibre was determined using the method described by Watanables and Olsen (1965). The moisture content was determined in terms of the loss in weight of the plant material on overnight heating at 150°C Sadasivam and Manickam (1996). Micro Kjeldahl method was used for the determination of protein. Crude fat, carbohydrate and nutritive value were calculated, following Nile and Khobragade (2009).

2.3 Antioxidant activity analysis

The free radical scavenging activity was assayed using a stable free radical, 1, 1- diphenyl-2-picryl hydrazyl (DPPH) and using BHA (Butylated Hydroxyl Anisole) as reference standard, following Moon and Terao (1998). The superoxide anion scavenging activity was assayed following Gulcin et al (2005) and the Hydroxyl radical scavenging activity was assayed following Klein et al (1991). Determination of total antioxidant capacity has been done by the

spectrophotometric quantification of phosphomolybdate complex using Ascorbic acid as reference standard (Preito et al, 1999).

3. RESULTS AND DISCUSSION

3.1 Mycochemical properties and Nutritive value

The present work reveals that both the macrofungi contain Phenolics, Alkaloids, Flavonoids, Tannins, Saponins and other mycochemical components. The results of mycochemical screening of both experimental macrofungi have been shown in Table 1 and 2. The *D. spathularia* contains comparatively more amount of Tannins, Alkaloid, and Saponins than those in *S. commune*. On the other hand the *S. commune* contains comparatively more amount of Phenolics and Flavonoids than those in *D. spathularia* (Table 1). Udu-Ibiam et al (2014) has studied the mycochemical composition of two edible macrofungi *Tricholoma nudum* and *Psalliota campestris* and found that *T. nudum* contains 64.12 ± 1.2 mg/g phenols, 0.016 ± 0.001 mg/g flavonoids, 0.28 ± 0.04 mg/g saponins, $0.1 \pm 0.04\%$ alkaloids, $0.014 \pm 0.003\%$ tannins, whereas *P. campestris* contains 6.012 ± 0.91 mg/g phenols, 0.031 ± 0.02 mg/g flavonoids, 0.27 ± 0.008 mg/g saponins, $2.0 \pm 0.01\%$ alkaloids and $0.014 \pm 0.001\%$ tannins. The present study reveals that the two experimental edible macrofungi contains comparatively more amount of the mycochemical constituent compounds except the phenolic content which is more in *T. nudum*. Regarding the nutritional aspects, the *D. spathularia* contains comparatively more amount of crude carbohydrate and crude fat than those in *S. commune*, whereas the *S. commune* contains comparatively more amount of crude protein and crude fibre than *D. spathularia* (Table 2). The Nutritive value of *D. spathularia* is found to be 2.99 cal/gm which is more than the nutritive value of *S. commune* that is 2.75 cal/gm The above results shows that the two edible macrofungi studied have significant nutritive potency and they are good source of carbohydrate, protein, fats and fibres.

3.2 Anti-oxidant potential

It has been a well-established fact that Flavonoids, Tannins and Alkaloids possess anti-oxidising effects (Tiane et al, 2014), and both the macrofungi studied have been found to contain these anti-oxidant components in significant quantities, therefore The antioxidant potential of the two edible macrofungi studied underlines their use as antioxidant supplement. Table 3 shows that the Hydroxyl radical scavenging activity of *D. spathularia* extract ($7.36 \pm 0.10\%$) is more or less similar to that of *S. commune* extract ($7.50 \pm 0.08\%$) at 100 microgram concentration. Table 4 Shows the DPPH radical scavenging activity of both fungal extracts.

Comparing the results to BHA standard (EC 50 = 5.0 µg/mL), the *S. commune* extract shows comparatively more free radical scavenging activity ($19.65 \pm 0.27\%$) than the *D.spathularia* extract ($14.75 \pm 0.16\%$). Table 5 and 6 explains that the super oxide anion scavenging activity of *D. spathularia* extract ($6.45 \pm 0.12\%$) is more than that of *S. commune* extract ($4.84 \pm 0.08\%$). Total antioxidant capacity (TAC) means the capacity of free radical scavenging by the bioactive constituents present in the test sample (Niki, 2010). The total antioxidant analysis reveals more or less similar antioxidant activities in *D. spathularia* extract ($12.20 \pm 0.14\%$) and *S. commune* extract ($12.15 \pm 0.08\%$).

Mshvildadze et al. (2004) reported that antioxidant activities are directly related to the saponin content. Whereas Rodrigues et al (2005) reported that the beneficial effects of saponin on serum lipids were related to a direct antioxidant activity of saponins. Elekofehinti et al (2012) concluded that saponin content of *Solanum anguivis* is capable of improving the antioxidant defense in rats. Satayanshu et al (2013) reported phenolic content in *Vitex trifolia* (74.5 GAE g⁻¹), *T. chebula* (531.5 74.5 GAE g⁻¹), *T. bellerica* (362.5 74.5 GAE g⁻¹), *E. officinalis* (221.6 74.5 GAE g⁻¹), *A. racemosus*(10.0 74.5 GAE g⁻¹) and found a linear relation between antioxidant activity and phenolic contents of plants. Melo et al (2010) screened some plants for their antioxidant activity and Tannin content. They reported highest tannin content in *Pyramidalis queiroz* (8.17 ± 0.64 µg/g) and lowest in *Cyperus distans* (1.22 ± 0.02 µg/g), they attributed the antioxidant activity of studied plants to their tannin content. Van et al (1996) concluded that flavonoids can be used as cardioprotective agents in doxorubicin-induced cardiotoxicity, which is caused by the formation of free oxygen radicals. Benabdesselam et al (2007) concluded that antioxidant activity of *Fumariacar peolata* and *Fumaria bastardii* are due to their alkaloid content. on the basis of the results obtained, it can be concluded that both the edible macrofungi or mushrooms studied have been found to contain the mycochemical constituents like tannins, saponins, alkaloids, flavonoids etc. which have significant antioxidant properties.

Tables and Figures

***Table 1: Quantitative analysis of phytochemicals of *D. Spathularia* and *S. commune* (M ± SD; n=6).**

Sl. No.	Attributes	<i>D. spathularia</i>	<i>S. commune</i>
1.	Total Ash (gm%)	2.52 ± 0.24	2.12 ± 0.22
2.	Moisture content (gm%)	4.73 ± 0.32	3.54 ± 0.26
3.	pH	6.4 ± 0.46	6.3 ± 0.40

4.	Phenolics (mg/gm)	4.82 ± 0.34	10.80 ± 0.76
5.	Flavonoids (mg/gm)	2.89 ± 0.21	4.67 ± 0.23
6.	Alkaloids (mg/gm)	11.64 ± 0.52	4.26 ± 0.54
7.	Tannins (mg/gm)	5.81 ± 0.37	1.24 ± 0.16
8.	Saponins (mg/gm)	28.56 ± 1.14	23.83 ± 0.84

*(Data is author's own work, submitted for publication in Bioscan)

***Table 2: Nutritive value of *D. Spathularia* and *S. Commune* (M ± SD; n= 6).**

Sl. No.	Attributes	<i>D. spathularia</i>	<i>S. commune</i>
1.	Crude Protein (gm%)	14.25 ± 1.05	16.25 ± 0.85
2.	Crude carbohydrate (gm%)	55.24 ± 2.15	48.34 ± 2.28
3.	Crude Fat (gm%)	2.40 ± 0.25	1.82 ± 0.16
4.	Crude Fibre (gm%)	5.0 ± 0.45	15.0 ± 0.52
4.	Nutritive Value (Cal/gm)	2.99 ± 0.31	2.75 ± 0.33

*(Data is author's own work submitted for publication in Bioscan)

Table 3: Percentage Hydroxyl radical scavenging activity.

Concentration (microgm)	<i>D. spathularia</i>	<i>S. commune</i>	Ascorbic acid
10	1.81%	0.14%	14.31%
50	5.56%	4.44%	36.25%
100	7.36%	7.50%	65.56%

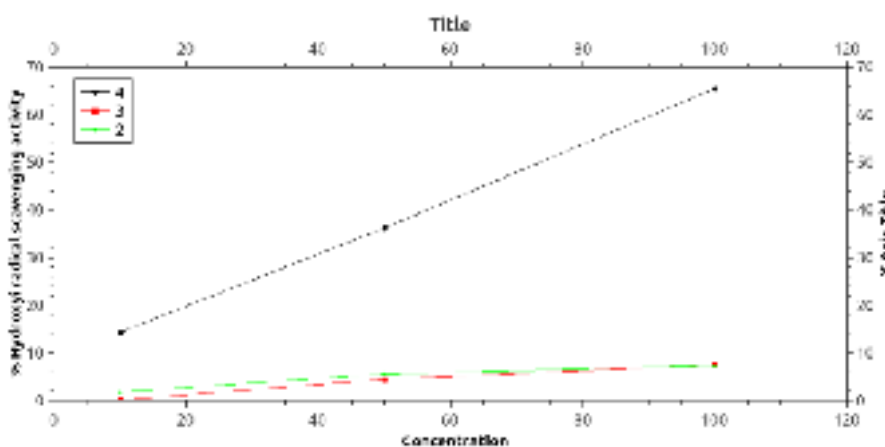


Fig. 1: Graph showing % Hydroxyl radical scavenging activity of 2 (*D. spathularia*), 3 (*S. commune*) and 4 (Ascorbic acid); (M ± SD; n= 6).

Table 4: Free radical scavenging activity.

Concentration (microgm)	<i>D. spathularia</i>	<i>S. commune</i>	BHA
10	1.02	2.00	44.84
50	8.57	9.84	78.08
100	14.75	19.65	102.00

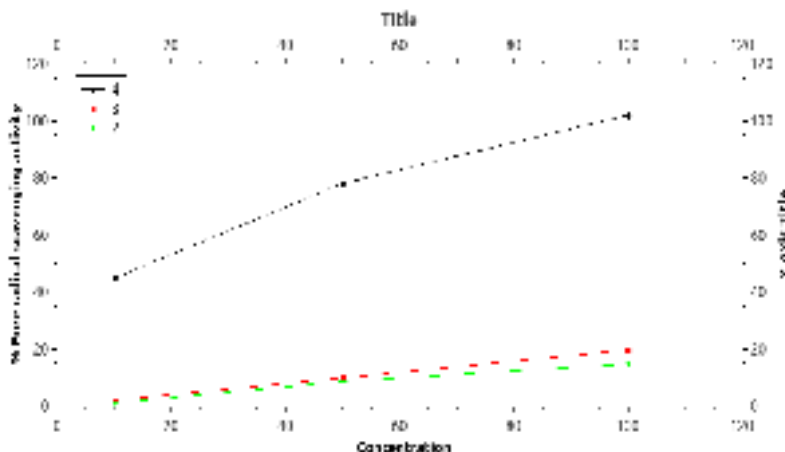


Fig. 2: Graph showing % Free radical scavenging activity of 2 (*D. spathularia*), 3 (*S. Commune*) and 4 (BHA); (M ± SD; n= 6).

Table 5: Super oxide anion scavenging activity.

Concentration (microgm)	<i>D. spathularia</i>	<i>S. commune</i>	Ascorbic acid
10	0.81	0.76	37.10
50	2.42	2.32	50.00
100	6.45	4.84	58.87

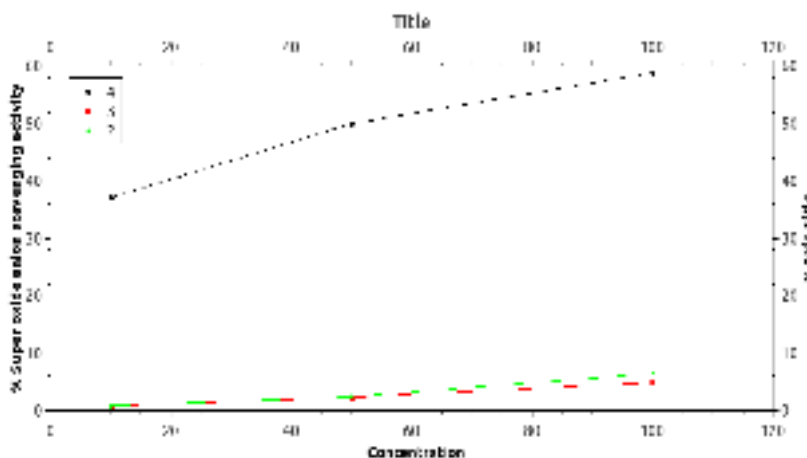


Fig. 3: Graph showing % Super oxide anion scavenging activity of 2 (*D. spathularia*), 3 (*S. commune*) and 4 (Ascorbic acid); (M ± SD; n= 6).

Table 6: Total anti-oxidant property.

Concentration (microgm)	<i>D. spathularia</i>	<i>S. commune</i>	Ascorbic acid
10	2.50	4.75	5.00
50	7.30	6.15	14.50
100	12.20	12.15	28.00

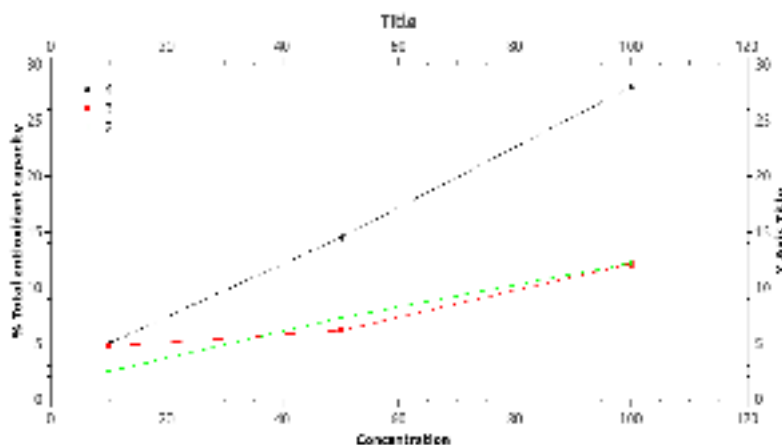


Fig. 4: Graph showing % Total antioxidant capacity of 2 (*D. spathularia*), 3 (*S. commune*) and 4 (Ascorbic acid); ($M \pm SD$; $n= 6$).

CONCLUSION

Present communication shows on the basis of the results obtained *Schizophyllum commune* and *Dacryopinax spathularia* (edible mushrooms) have been found to contain the mycochemical constituents like tannins, saponins, alkaloids, and flavonoids etc. which have significant antioxidant properties. Therefore it can be a good or healthy resource of antioxidant compounds and further work may lead to the use of these edible macrofungi as the source drug development against several diseases related to oxidative stress in the body.

REFERENCES

1. Adebayo E.A.1, Oloke J. K.1, Majolagbe O. N.1, Ajani R. A.2 and Bora T. C. Antimicrobial and anti-inflammatory potential of polysaccharide from *Pleurotus pulmonarius* LAU 09. African Journal of Microbiology Research, 2012; 6(13): 3315-3323.
2. Benabdesselam, F. M., Khentache, S., Bougoffa, K., Chibane, M., Chapeleur, Y., Max, H., Adach, S. and Mattar, D. L. Antioxidant activities of alkaloid extracts of two Algerian species of *Fumaria*: *Fumaria carpeolatai* and *Fumaria bastardii*. Rec. Nat. Prod., 2007; 1(2-3): 28- 35.
3. Cottone, S., M.C. Lorito, R. Riccobene, E. Nardi, G. Mule, S. Buscemi, C. Geraci, M. Guarneri, R. Rseno and G. Cerasola, Oxidative stress, inflammation and cardiovascular disease in chronic renal failure, J. Nephrol., 2008; 21(2): 175-179.
4. Dandapat S and Sinha M.P., Antioxidant and anti-inflammatory activity of *Pleurotus tuber-rigium* (Rumph ex Fr.) Singer. Advances in biological research, 2015; 9(3): 140-145.
5. Dandapat S, Kumar M, Kumar A, Sinha MP. Antipathogenic efficacy of methanolic leaf

- extract of *Cinnamomum tamala* (Buch.-Ham) and *Aegle marmelos* (L.) with their nutritional potentiality, *The Bioscan*, 2013; 8(2): 635-641.
6. Dandapat, S., M. Kumar, A. Kumar and M.P. Sinha, Therapeutic efficacy and nutritional potentiality of *Cinnamomum tamala* (Buch.-Ham), *Int. J. Pharm.* 2013; 3(4): 779-785.
 7. Elekofehinti, O. O., Adanlawo, I. G., Komolafe, K. and Ejelonu, O. C. Saponins from *Solanum anguivi* fruits exhibit antioxidant potential in Wistar rats. *Annals of Biol. Res.* 2012; 3(7): 3212-3217.
 8. Gulcin, I., Alici, H.A. and Cesur M. Determination of *in vitro* antioxidant and radical scavenging activities of Propofol. *Chem. Pharm. Bull.* 2005; 53(3): 281-285.
 9. Hammas D. Kitaz A., Sabbagh G. Total phenolic content, flavonoid concentration and antioxidant activity of leaves and bark extracts of *Celtis australis* L., *Int. J. of Pharm. Sc. And Nanotech. (IJPSN)*, 2016; 9(2): 3188-3192.
 10. Jelic, S. and J.T.H. Le, Inflammation, oxidative stress and the vascular endothelium in obstructive sleep apnea, *Trends Cardiovas. Med.*, 2008; 18(7): 253-260.
 11. Karaman, M., m. Vesic, M. Stahl, M. Novakovic, L. Janjic and M. Matavuly, Bioactive properties of wild growing mushrooms species *Ganoderma applanatum* (Pers.) Pat. From Fruska Gora forest (Serbia). *Ethnomedicine and Therapeutic validation.*, 2012; 32: 361-377.
 12. Kitaz A, Al-Kayali R, Sabbagh G Phytochemical screening and antioxidant activity of selected wild plants in Liliaceae Family growing in Syria, *Int J Pharmacog Phyt Res.* 2016; 8(12): 2025 – 2032.
 13. Krishnaiah D, Sarbatly R and Bono A. Phytochemical antioxidants for health and medicine – A move towards nature. *Biotech. And Mol. Bio. Review*, 2007; 1(4): 97-104.
 14. Klein, S.M., Cohen, G., Cederbaum, A.I. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical scavenging system. *Biochemistry*, 1991; 20: 6006-6012.
 15. Lei J., Yanlong Z., Linmao Y., Yulong G. and Lixin N. Phenolic compounds and antioxidant activity of bulb extracts of six liliaceae species native to China. *Molecules*, 2012; 18: 690-700.
 16. Melo, J. G. et al. Antiproliferative Activity, Antioxidant Capacity and Tannin Content in Plants of Semi-Arid North eastern Brazil. *Molecules*, 2010; 15: 8534-8542.
 17. Miller, DM, Buettner GR and Aust SD Transition metals as catalysts of autoxidation reactions, *free Radical Biol. Med.*, 1990; 8: 95-108.
 18. Mitko Karadelev¹, Katerina Rusevska¹ & Sofija Stojanovska. *Ecology And Distribution*

- Of Genus *Phellinus* (Hymenochaetaceae) In The Republic Of Macedonia. Proceedings of the III Congress of Ecologists of Macedonia, 2008; 197-207.
19. Moon, J.H. and Terao, J., Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low density protein. *J. Agri. And Food Chem.*, 1998; 46: 5062-65.
 20. Mshvildadze, V., Gülçin, I. G. A. and Elias, R. Antioxidant activity of saponins isolated from ivy; alpha-hederin, hederasaponin- c, hederacolchiside-E and hederacolchiside-F. *Planta Med.*, 2004; 70(6): 561- 563.
 21. Nile and Khobragade Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. *J Med. Plants*, 2009; 8(5): 79-88.
 22. Niki, E., Assessment of antioxidant capacity in vitro and in vivo. *Free radical Bio. Med.*, 2010; 49: 503-515.
 23. Pacher P, Beckman JS and Liaudet L Nitric oxide and peroxynitrite in health and disease, *Physiological Reviews*, 2007; 87: 315 – 324.
 24. Prieto P, Pineda M and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation and phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 1999; 269: 337-341.
 25. Reuter, S., Gupta, S.C., Chaturvedi, M.M. and Aggrawal, B.B. Oxidative stress, inflammation, and cancer: how they are linked? *Free radical Biol. MED.*, 2010; 49(11): 1603-1616.
 26. Ramamoorthy, P.K. and Bono, A. Antioxidant activity, total phenolics and flavonoids content of *Morindacitrifolia* fruit extracts from various extraction processes. *J. Engg. Sci. and Tech.*, 2007; 2(1): 70-80.
 27. Rodrigues, H. G., Diniz, Y. S., Faine, L. A., Galhardi, C. M., Burneiko, R. C., Almeida, J. A., Ribas, B. O. and Novelli, E. L. Antioxidant effect of saponin: potential action of a soybean flavonoid on glucose tolerance and risk factors for atherosclerosis. *Int J. Food Sci. Nutr.*, 2005; 56(2): 79-85.
 28. Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y.S.R. and De, B. Free radicals, antioxidants, disease and phytomedicines: current status and future prospect. *Int. J. Pharmaceutical Sc.*, 2010; 3(1): 91-100.
 29. Sofowara A. Screening plants for bioactive agents, in *Medicinal plants and traditional medicines in Africa*, 3rd ed. Spectrum Books Ltd., Sunshine House, Ibadal, Nigeria, 2008; 134-156.
 30. Sadasivam S, Manickam A. *Biochemical methods*, 2nd ed. New Age International Limited Publishers, New Delhi, India, 1996; 159-160.

31. Satayanshu, K., Sonal, K. and Tushar, K. Comparative evaluation of antioxidant potential of extract of *Vitex negundo*, *Vitex trifolia*, *Terminalia bellerica*, *Terminalia chebula*, *Embelica officinalis* and *Asparagus racemosus*. IPP., 2013; 1(1): 44-53.
32. Tiane CF, Aldo JPD, Joao APH, Mariana RE. A review on general nutritional compounds and pharmacological properties of *Lentinula edodes* Mushroom. Food and Nutrition Sciences, 2014; 5(12): Article ID 47339.
33. Udu-Ibiam, O.E., O. Ogbu, U.A. Ibiam, A.U. Nnachi, M.V. Agah, C.O. Ukaegbu, O.S. Chukwu, N.B. Agumah and K.I. Ogbu, 2014. Phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi state, Nigeria, Pharm. Bio. Sci., 2014; 9(3): 86-91.
34. Van, A. S. A., Van, D. B. D. J., Tromp, M. N., Griffioen, D. H., Van, B. W. P., Van, D. V. W. J. and Bast, A. Structural aspects of antioxidant activity of flavonoids. Free Radic. Biol. Med., 1996; 20(3): 331- 42.
35. Watanble, FS and Olsen SR. Test for ascorbic acid method for determining phosphorus in water and sodium bicarbonate extract of soil. Proc. Soil Sci Soc Am., 1965; 29: 677 – 678.