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(RESEARCH ARTICLE)

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Antimicrobial and Antioxidant properties of medicinal mushroom *Ganoderma* P. Karst

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Abstract

Ganoderma species have been known all over the world as highly medicinal mushrooms. Antimicrobial activity of it is an attractive approach which raises the global interests from the scientific community. In this study, the antimicrobial assay of ethanol and methanol extracts of *Ganoderma* were prepared by using the dried mycelial powder obtained from five different liquid media; was performed against seven plant pathogenic fungi *viz., Alternaria macrospora, Aspergilus niger, A. flavus, Colletotrichum falcatum, Fusarium oxysporum, Pestalotiopsis mangiferae* and *Penicillium sp*. and two plant pathogenic bacteria *Xanthomonas oryzae* and *Ralstonia solanacearum*. The extracts of mycelia obtained from Yeast Wine Media exhibited the highest inhibition percentage as compared to rest. At concentration 1000ppm, there was complete inhibition of mycelial growth for *Alternaria macrospora, Aspergilus niger* and *A. flavus* while for *Fusarium oxysporum* and *Penicillum sp*. complete inhibition was observed at 500ppm but for *Colletotrichum falcatum* and *Pestalotiopsis mangiferae*, more than 80% mycelial inhibition at concentration 1000ppm in both ethanol and methanol extracts. In the case of *Xanthomonas oryzae* and *Ralstonia solanacearum*, at concentration 1000ppm, methanol extract showed the highest inhibition zone (3.50mm, 3.75mm). *Ganoderma* exhibited antagonistic effect against plant pathogenic fungi could add to the interest of developing *Ganoderma* as a successful bioagent in the near future. Antioxidant activities were evaluated by using DPPH radical scavenging assay and Metal chelating activity on ferrous ions. The DPPH radical scavenging effect was detected in methanol extract (Inhibition% = 27.312%) was higher than that of the ethanol extract (Inhibition% = 24.79%) and also Ferrous ion chelating ability of methanol extract (Inhibition% =22.27%) was higher than the ethanol extract (Inhibition% =12.55%). It is clearly indicated that both methanol and ethanol extract of the *Ganoderma* show antioxidant properties and *Ganoderma* extracts act as an effective antioxidant agents.

Keywords: Extract; Antifungal activity; Antibacterial activity; DPPH; Ferrous ion

1. Introduction

Ganoderma have attracted great attention all over the world because of their wide range of pharmacological values. It is an edible mushroom that has been used for centuries in Traditional Chinese Medicine for its health promoting properties. *Ganoderma* species are not listed among the group of edible mushrooms because the fruiting bodies are always thick, corky and tough and, do not have the fleshy texture characteristics of true edible mushroom [1], [2]. Although *Ganoderma* species could not be used for eating directly, they have been known all over the world as highly medicinal mushrooms [3], [4]. Its extracts are used worldwide as ingredients in health foods, herbal medicines and dietary supplements. Recent studies have proven that *Ganoderma* extract shows antimicrobial activity against various plant pathogens. It has inhibitory effect on growth and germination of *Alternaria alternata*; antimicrobial activities on *Botrytis cinera, Colletotrichum gloeosporioides*, and *C. miyabeanus*. Antimicrobial activity of it is an attractive approach which raises the global interests from the scientific community.

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Oxidation processes are essential to many living organisms for the production of energy to fuel biological processes. The uncontrolled production of reactive oxygens species (ROS) and the unbalanced mechanism of antioxidant protection result in the onset of many diseases and accelerate ageing. Oxidative damage plays a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage. Oxygen-centered free radicals and other reactive oxygen species that are continuously produced *in vivo*, result in cell death and tissue damage. The antioxidants in human diets are of great interest as possible protective agents to help human body reduce oxidative. *Ganoderma* act to prevent lipid oxidation in food and to decrease the adverse effects of reactive species (both ROS and reactive nitrogen species) on normal physiological functions in humans.

Ganoderma has been well known for its antimicrobial and antioxidant activities. In this study, the antimicrobial activity of ethanol and methanol extracts of *Ganoderma* against some plant pathogens was evaluated and the contents of scavenging effects on radicals and chelating effects on ferrous ion were determined.

2. Methodology

This experiment was carried out in the laboratory of Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, and Gujarat. Survey for the collection of naturally growing *Ganoderma* was conducted at the different regions of Navsari *viz.,* Navsari Agricultural University campus, Dandi and Gandevi. The sites of collection were soil, open lands, farm lands, roadside, research field etc. The data was analysed using CRD and the difference among mean value was tested by using critical differences (CD) values at 5% level of probability.

3. Material and methods

3.1. *Ganoderma* and test Pathogens

The fruiting bodies of *Ganoderma* were collected from the different locations of Navsari, Gujarat during June 2018 to September 2018. They were found to be growing wildly on dead stump of unknown trees and stem of Pink shower, *Cassia grandis* [5]. The test pathogens used for these studies were seven plant pathogenic fungi *viz*., *Alternaria macrospora, Aspergilus niger, A. flavus, Colletotrichum falcatum, Fusarium oxysporum, Pestalotiopsis mangiferae* and *Penicillium sp.* and two plant pathogenic bacteria *Xanthomonas oryzae* and *Ralstonia solanacearum*.

3.2. Preparation of the extracts

The collected specimens were isolated using tissue culture technique on sterilized Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) media in the petri plates [6]. Mycelial biomass production of *Ganoderma sp.* was carried out using five different liquid media *viz*., Glucose Aspargine Media, Hwang Liquid Media, Potato Dextrose Broth, Yeast Wine Media and Glucose Peptone Liquid Media [7]. The mycelial mats obtained from different five media were ground and the air-dried powder was used for preparation of extracts. Ethanol extracts were prepared by adding 0.01g of dried mycelial powder in 10ml of methanol and wrapped with aluminium foil, leaving it for 6 hours. Then the extracts were centrifuged at 1000rpm for 15 minutes and the supernatant was used for further studies [8]. It was considered as pure 1000ppm solution and labelled as 'stock solution', from which 500ppm and 250ppm solutions were prepared by adding required amount of ethanol. Methanol extract was also prepared in the similar way but methanol was used instead of ethanol.

3.3. Antimicrobial activities

3.3.1. Antifungal activities

The antifungal activity of *Ganoderma* extracts was determined by poisoned food technique [9]. Three concentrations *viz*., 250ppm, 500ppm and 1000ppm of both methanol and ethanol extracts of all five dried mycelia powder obtained from five different liquid media were used against the test pathogens.

3.3.2. Antibacterial activities

The antibacerial activity of *Ganoderma* extracts was performed by Disc diffusion method using paper disc as reservoir against the test plant pathogens *viz*., *Xanthomonas oryzae* and *Ralstonia solanacearum*. Here, the filter paper discs of 6mm diameter were impregnated with 20µl of three concentrations viz., 250ppm, 500ppm and 1000ppm of both methanol and ethanol extracts of all five dried powder obtained from five different liquid media and placed on inoculated agar plates.

3.4. Antioxidant properties

3.4.1. DPPH radical scavenging assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) using the method of Blois (1958) [10]. Both methanol and ethanol extract solutions were added to a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and kept at room temperature for 30 minutes in the dark. Then the absorbance was measured at 517nm against a blank in spectrophotometer [11]. Butylated hydroxyanosole (BHA) and Butylated hydroxytoluene (BHT) were used as positive controls. The inhibition of DPPH free radical as a percentage (I%) was calculated according to the formula:

$$I\% = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100$$

Where, $A_{Control}$ is the absorbance of the blank at 517nm; A_{Sample} is the absorbance of the test compound at 517nm

3.4.2. Metal chelating activity on ferrous ions (Fe^{2+})

Metal chelating activity was determined according to the method proposed by Decker and Welch (1990) [12]. In brief, 1ml of the *Ganoderma* extract was mixed with 0.05ml of 2mM $FeCl_2$ and 0.1ml of 5mM ferrozine. Then, the mixture was shaken vigorously and kept at room temperature for 10 minutes. Then, its absorbance was measured spectrophotometrically at 562nm. Ethylene diamine tetraacetic acid (EDTA) was used as positive controls. The percentage of inhibition of ferrozine-ferrous ion complex formation was calculated using the formula given below:

$$I\% = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100$$

Where, $A_{Control}$ is the absorbance of the blank at 562nm

A_{Sample} is the absorbance of the test compound at 562nm

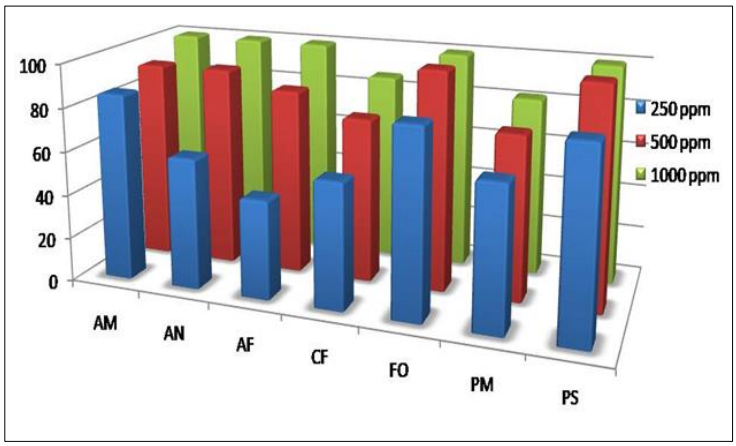
3.5. Statistical analysis

The data was analyzed using CRD and the difference among mean value was tested by using critical differences (CD) values at 5% level of probability.

4. Results

4.1. Antimicrobial activity

4.1.1. Antifungal activities



AM - *Alternaria macrospora*, **AN -** *Aspergilus niger*, **AF -** *A. flavus*, **CF -** *Colletotrichum falcatum*, **FO -** *Fusarium oxysporum*, **PM -** *Pestalotiopsis mangiferae*, **PS -** *Penicillium sp*

Figure 1 Effect of methanolic extract of the dried mycelia powder of *Ganoderma* obtained from the Yeast Wine Media on mycelial growth of different fungi

It was observed that *Ganoderma* extract demonstrated various degrees of antifungal activities against the seven test fungi (Fig. 1). For both ethanol and methanol extracts, the highest antifungal activity was demonstrated by extract of the dried mycelia powder obtained from the Yeast Wine Media. There was complete inhibition of mycelial growth for *Alternaria macrospora, Aspergilus niger* and *A. flavus* at concentration 1000ppm while for *Fusarium oxysporum* and *Penicillum sp.* complete inhibition was observed at 500ppm but minimum inhibition observed at 250ppm; whereas at concentration 1000ppm more than 80% inhibition of mycelia of *Colletotrichum falcatum* and *Pestalotiopsis mangiferae* was observed in both ethanol and methanol extracts. In control sets, however no fungal or mycelia inhibition was observed.

4.1.2. Antibacterial activities

In the case of *Xanthomonas oryzae*, at concentration 1000ppm, methanol extract of mycelia obtained from Yeast Wine Media showed the highest inhibition zone (3.50mm) than the ethanol extract (3.25mm). Whereas in *Ralstonia solanacearum*, methanol extract of mycelia obtained from Yeast Wine Media showed the highest inhibition zone (3.75mm) and no inhibition was recorded in ethanol extract (Table 1 and 2).

Table 1 Inhibitory effect of *Ganoderma* extract against *Xanthomonas oryzae* by disc diffusion method

Sr. No.	Synthetic liquid Media	Zone of Inhibition (mm)	
		Ethanol Extract	Methanol Extract
		1000 ppm	1000 ppm
1.	Glucose Aspargine liquid Media	0.71 (0.00)	1.40 (1.50)
2.	Hwang liquid Media	0.71 (0.00)	1.49 (1.75)
3.	Potato Dextrose Broth	0.71 (0.00)	0.71 (0.00)
4.	Yeast Wine Medium	1.93 (3.25)	2.00 (3.50)
5.	Glucose Peptone Liquid Media	0.71 (0.00)	0.71 (0.00)
	S. Em±	0.03	0.07
	CD @ 0.05	0.08	0.21

*Figures are Square Root (X + 0.5) transformed values; Figures in parenthesis are original value

Table 2 Inhibitory effect of *Ganoderma* extract against *Ralstonia solanacearum* by disc diffusion method

Sr. No.	Liquid Media	Zone of Inhibition
		Methanol Extract
		1000 ppm
1.	Glucose Aspargine Media	1.58 (2.00)
2.	Hwang liquid Media	1.65 (2.25)
3.	Potato Dextrose Broth	1.58 (2.00)
4.	Yeast Wine Medium	2.06 (3.75)
5.	Glucose Peptone Liquid Media	1.58 (2.00)
	S. Em±	0.04
	CD @ 0.05	0.13

*Figures are Square Root (X + 0.5) transformed values. Figures in parenthesis are original values

4.2. Antioxidant Properties

4.2.1. DPPH radical scavenging assay

The Table 3 showed that the percentage of inhibition of ethanol and methanol extracts of mycelia obtained from Hwang Liquid Media was significantly more (24.79% and 27.31%) as compared to the rest media and the DPPH radical scavenging effect in methanol extract (Inhibition% = 27.31%) was higher than the ethanol extract (Inhibition% = 24.79%). The Fig 2 showed that the scavenging abilities of *Ganoderma* extracts were in a concentration-dependent fashion. It increased with increasing of concentration.

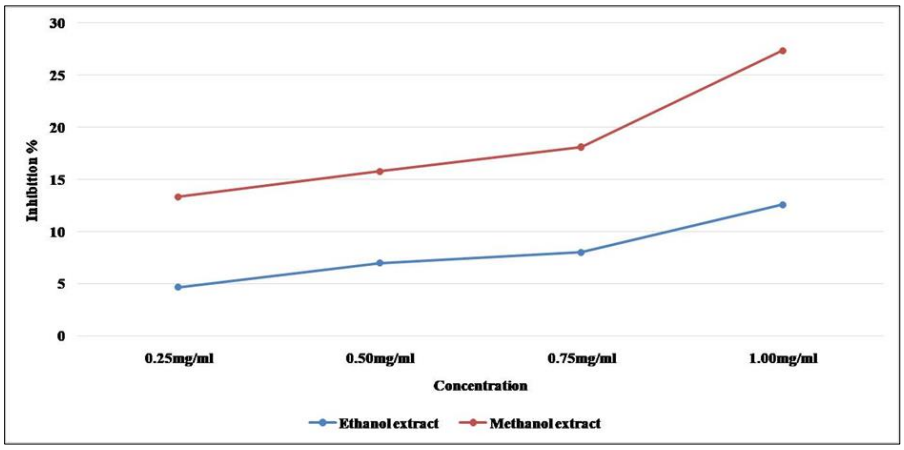


Figure 2 DPPH radical scavenging ability of *Ganoderma* extract obtained from Hwang liquid media at different concentrations

Table 3 DPPH radical scavenging ability of *Ganoderma* extract obtained from five different media

Sr. No.	Liquid media	Methanol extract (mg/ml)		Ethanol extract (mg/ml)	
		Absorbance	Inhibition %	Absorbance	Inhibition %
1	Glucose Aspargine liquid Media	1.78	25.12	1.93	19.02
2	Hwang liquid Media	1.73	27.31	1.79	24.79
3	Potato Dextrose Broth	1.83	22.90	1.94	18.88
4	Yeast Wine Medium	1.77	25.53	1.92	19.22
5	Glucose Peptone Liquid Media	1.85	22.10	1.931	18.91
6	Control	2.38	-	2.38	-
	S. Em±	0.02	-	0.03	-
	CD @ 0.05	0.06	-	0.10	-
	C. V. %	3.56	-	2.67	-

4.2.2. Metal chelating activity on ferrous ion

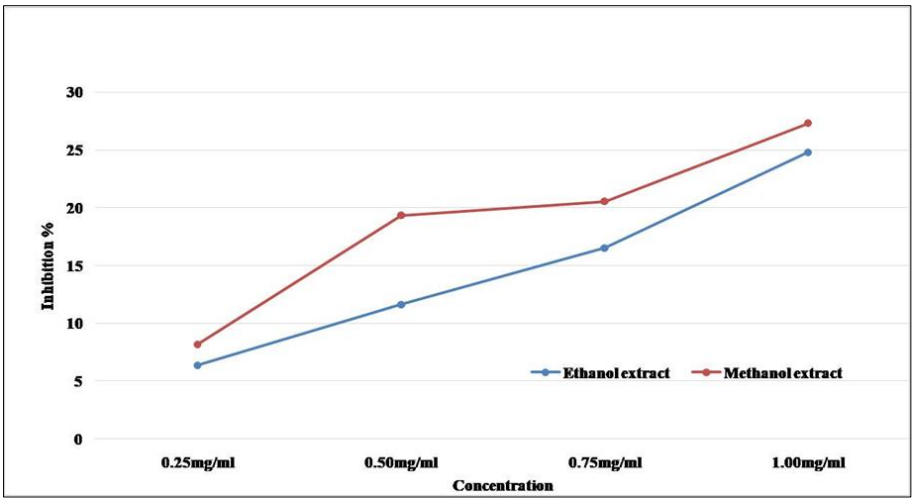


Figure 3 Metal chelating activity of *Ganoderma* extract obtained from Hwang liquid media at different conentrations on ferrous ion

The highest percentage of inhibition of ferrozine- Fe^{2+} complex formation was noted in ethanol and methanol extracts of mycelia obtained from Hwang Liquid Media (Table 4) and Ferrous ion chelating ability of methanol extract (Inhibition% =22.27%) was higher as compared to the ethanol extract (Inhibition% =12.55%). The Fig 3 showed that the chelating ability of the *Ganoderma* extracts also were in a concentration-dependent fashion. It increased with increasing of concentration.

Table 4 Metal chelating activity of *Ganoderma* extract obtained from five different media on ferrous ion

Sr. No.	Liquid media	Methanol extract (1mg/ml)		Ethanol extract (1mg/ml)	
		Absorbance	Inhibition %	Absorbance	Inhibition %
1	Glucose Aspargine Media	2.02	18.20	2.18	11.80
2	Hwang liquid Media	1.92	22.27	2.16	12.55
3	Potato Dextrose Broth	2.09	15.06	2.26	8.69
4	Yeast Wine Medium	2.04	17.33	2.19	11.19
5	Glucose Peptone Liquid Media	2.08	15.67	2.25	8.75
6	Control	2.47	-	2.47	-
	S. Em±	0.01	-	0.02	-
	CD @ 0.05	0.03	-	0.05	-
	C. V. %	3.87	-	2.49	-

5. Discussion

The results of antimicrobial activities of *Ganoderma* extract were supported by the Singh *et al.* (2014) [13] who reported that the all extracts of *Ganoderma* possessed strong antifungal activity against five fungal strains which were tested by using poisoned food technique. Moreover, the antifungal activities of almost all the extracts were found to be concentration dependent. Fidler *et al.* (2015) [14] reported the antimicrobial activity of alcoholic extracts of mycelial biomass of *Ganoderma* obtained in medium prepared with yeast wine was higher as compared to mycelial biomass obtained in other medium. Prakash and Sharma (2016) [8] observed the maximum antimicrobial activity of both aqueous and ethanolic extract of *G. lucidum* at a concentration of 1000ppm.

The findings of DPPH radical scavenging assay of *Ganoderma* extract are in close enough with the Celik *et al*. (2014) [11] who reported that DPPH radical scavenging effect was higher in the methanol extract as compared to the ethanol extracts. Fan *et al.,* (2012) [15] reported that the scavenging abilities of the polysaccharides of *Ganoderma* were in a concentration-dependent fashion. The results of metal chealting activity on Ferrous ion of *Ganodema* extract were in agreement with Celik *et al.* (2014) [11] who reported that methanol extract ferrous iron chelating ability was higher than the ethanol extract. In addition, they reported that the chelating effects of methanolic extracts from *G. lucidum* on ferrous ion increased with increasing concentrations. Mau *et al*. (2002) [16] found that the methanolic extracts of *G. lucidum* chelated ferrous ions.

6. Conclusion

It can be concluded that *Ganoderma* shows considerable antimicrobial potential. So, it may be an alternate ecofriendly option for plant disease management without having toxic residues in raw and processed food and can reduce the negative effect of chemical pesticides. Also some of the reports show that it possesses some specific characteristics like biodegradability and target specificity. In this study, it is clearly indicated that both methanol and ethanol extract of the *Ganoderma* show antioxidant properties and *Ganoderma e*xtracts act as an effective antioxidant agent. So, further work is needed towards the field evaluation of their antimicrobial potential, antioxidant property, identification of bioactive principles and elucidation of their mechanism of action.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

[1] Jong SC, Birmingham JM, Medicinal benefit of the mushroom *Ganoderma*, Advances in Applied Microbiology. 1991; 37: 104-132.

[2] Jonathan SG, Kigigha LT, Ohimain E, Evaluaton of the Inhibitory Potentials of Eight higher Nigerian Fungi against Pathogenic microorganisms, African Journal of Biomedical Reaserch. 2008; 11: 197-202.

[3] Yoon SY, Eo SK, Kim YS, Lee CK, et al., Antimicrobial activity of *Ganoderma* extract alone and in combination with some antibiotics, Archives of Pharmacol. Reseach. 1994; 17(6): 438-442.

[4] Wasser SP and Weis AL, Medicinal properties of substances occurring in higher basidiomycetes mushrooms current perspectives (Review), International Journal of Medicinal Mushroom. 1999; 1: 31-62.

[5] Suansia A & John P, Morphological and cultural Characteristics of Different Collections of Medicinal White-Rot Bracket Fungi *Ganoderma* P. Karst., International Journal of Current Microbiology and Applied Science. 2020; 9(1): 2636-2644.

[6] Kapoor P & Sharma BM, Studies on different growth parameters of *Ganoderma lucidum*. International Journal of Science, Environment and Technology. 2014; 3: 1515-1524.

[7] Suansia A, John P, Mycelial biomass production of medicinal mushroom *Ganoderma* P. Karst. International Journal of Agricultural Sciences. 2021; 17 (1): 15-19.

[8] Parkash V & Sharma A, In vitro efficacy of bracket fungi for their potential antimicrobial activity. Journal of microbiology, biotechnology and food sciences. 2016; 6: 818-822.

[9] Prakash V, Aggarwal A, Sharma S, Gupta S, et al., Antifungal activity of Withania somnifera L. Dunal on some phytopathogenic fungi. Bulletin of the National Institute of Ecology, 2005; 15: 15-218.

[10] Blois MS, Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181: 1199-1200.

[11] Celik GY, Onbasil D, Altinsoy B & Alli, H, In vitro antimicrobial and antioxidant properties of *Ganoderma lucidum* extracts grown in Turkey. European Journal of Medicinal Plants. 2014; 4: 709-722.

[12] Decker EA, Welch B, Role of ferritin as a lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry. 1990; 38: 674-677.

[13] Singh J, Gupta S, Malviya S, Ahrwar B, In-vitro evaluation of antimicrobial activity of *Ganoderma lucidum*. International Journal of Advanced Research. 2014; 2: 460-466.

[14] Fidler G, Butu A, Rodino S, Butu M, et al., Antioxidant activity, bioactive compounds and antimicrobial effect of mushrooms extracts. Animal Science and Biotechnologies. 2015; 72: 32-35.

[15] Fan L, Li J, Deng K & Ai L, Effects of drying methods on the antioxidant activities of polysaccharides extracted from *Ganoderma lucidum*. Carbohydrate Polymers. 2012; 87: 1849-1854.

[16] Mau JL, Tsai SY, Sung YH & Huang SJ, Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill, Lebensmittel-Wissenschaft and Technologie. 2002; 38: 589-597.