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EFFECT OF EXTRACTION AND SOLVENT ON MYCOCHEMICALS AND PROXIMATE COMPOSITION OF LIGNICOLOUS FUNGI *GANODERMA LUCIDUM* AND *G. APPLANATUM*: A COMPARATIVE STUDY

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Abstract

Objectives: The objective of the present study was a comparative evaluation, the effect of extraction methods and solvents on the mycochemical, proximate composition, Total phenolic content, Total flavonoids content, Phenotipical characters in lignicolous fungi sporophore of *Ganoderma lucidum* and *G. applanatum* collected from different localities of district Krishna, Andhra Pradesh, India.

Methods: in the present study, Sporophore extracts of water, Methanol, Ethanol, and Hydromethanol were prepared using 'Green extraction' methods such as Maceration Maceration water bath, and reflux assisted solvent extraction. The extracts and sporophore of lignicolous fungi was screened for Phenotypic characters by standard methods of

identification, Mycochemical test and Proximate evaluation by Indian Pharmacopoeia Commission, total phenolic content total flavonoid content was estimated by spectroscopic methods taking Gallic acid, Rutin as standards respectively.

Results: *Ganoderma lucidum* differs from *G. applanatum* in phenotypical characters. The solvent used for extraction and extraction methods played an important role on mycochemical, Proximate composition, Total phenolics and flavonoids content of *G. lucidum* and *G. applantum* powder extract. This proximate composition work aims at standardization of white rot fungi sporophore powder of *G. lucidum* and *G. applanatum* that will help to identify the genuine species and check for adulteration of sporophore powder available commercially. The Maceration water bath assisted extraction method is the best extraction method compared to maceration and Reflux extraction methods for all parameters studied in this work. The highest total phenol content was shown 111.18 mg GAE/g in *G. lucidum* 50% methanol extract prepared by water bath method. The *G. applanatum* showed better total flavonoid content when compared to *G. lucidum*. The highest total flavonoids content was shown 39.28 mg rutin/g in *G. applanatum* 50% methanol extract prepared by water bath method

Conclusion: Present study proves that the effect of extraction method and solvent influencing the mycochemical composition like total phenol content total flavonoid content in lignicolous fungi. The proximate composition evaluation is very much useful for standardisation of *G. lucidum* and *G. applanatum* in powder form.

Keywords: Lignicolous fungi, Extraction method, Mycochemicals, Proximate composition, Ganoderma

Introduction

Ayurvedic treatment is one of the most ancient treatment systems against various diseases in the Indian subcontinent. The medicine of ayurvedic treatment came from various green plants, nongreen plant, and their plant parts. Chemical compounds naturally produced or derived from the nongreen plant-like fungus is mycochemicals**.** The macrofungi like Mushrooms produce a variety of secondary metabolites like mycochemicals includes alkaloids, flavonoids, phenolic compounds, polyketides, terpenes, and steroids. These mycochemical compounds bear tremendous importance for mankind, displaying a broad range of useful antibacterial,

Anti-cancerous, Anti-inflammatory, antiviral and pharmaceutical activities, at the same time bear less toxic effects (Asatiani et al., 2010). The Macro fungus Ganoderma (Lingzhi, Reishi or Mannentake) is used in traditional Chinese herbal medicine, nearly 4,000 years ago (WachtelGalor et al 2004). It belongs to family Ganodermataceae have 8 genera and around 250 to 400 species. Ganoderma lucidum is one of the fungi from this family that has been extensively used as various therapeutic agents. It contains approximately 400 different Mycochemical bioactive compounds showing a number of pharmacological effects. But the mycochemical compounds of G. lucidium are changed from one habitat to another habitat. Some important pharmacological properties of Ganoderma are an ability to reduce the risk of heart disease, cancer, and stimulation of the immune system (Russell and Paterson 2006). Beneficial properties of the Ganoderma species are because of mycochemical bioactive components like polysaccharides, triterpenes, sterols, lectins and some protein etc. (Ferreira et al., 2010). In our country, it is rare to investigate the biological principles of Ganoderma species. So, it is important to evaluate the mycochemical composition in different extracts, the effect of extraction method on mycochemicals, proximate compounds of white rot fungi like Ganoderma species. In the present study two white rot fungi i.e. *G. lucidum* and *G. applanatum* are compared in terms of mycochemical extraction methods, different solvent extracts, Proximate compounds, total phenolic content, total flavonoid content.

Materials and methods

Collection of fungi

The sporophores of *Ganoderma lucidum* and *G. applanatum* were collected from Andhra Pradesh, India, during the rainy season (July–September) of the years 2014 to 2017. Field characters like habit, host, name of the locality, and other morphological features were recorded for sample specimens. Voucher specimens of *G. lucidum* (ALC 2) and *G. applanatum* (ALC 10) have been deposited at the herbarium of the Museum of Botany Department, Andhra Loyola College (ALC), Vijayawada, Andhra Pradesh, India.

Taxonomic studies

Macroscopic features like abhymenial, hymenial surfaces, context, and pore tubes of both species were examined. Microscopic features like hyphae, basidiospores, and pilear crust were observed by preparing crush mounts and free-hand sections in water, 5% KOH solution, and staining was done with cotton blue (1%, in lactophenol), and Melzer's reagent for

identification of specimen (Nagadesi et al., 2016). Basidiospore measurements like mean length, mean width, size range, and spore index were determined for 10 basidiospores (Parmasto et al., 1987).

Preparation of Extraction

The fruiting body of *G. lucidium G. applanatum* was cut into fine pieces, powdered by using mixer grinder and sieved by using sieve number 100 having 0.15mm diameter. For preparing the extracts water, methanol, and ethanol was used as solvents.

Maceration

Exactly 5 grams of sporophore powder is soaked in 100 ml of solvents comprises of water, methanol, ethanol, and hydro-methanol (50:50) separately. All the samples are left at room temperature for 24 hr in dark. Then the samples are filtered by using Whatman filter paper No.1 and the filtrates stored at 4°C for further use to perform mycochemical qualitative and quantitative tests.

Maceration Water Bath

Exactly 5 grams of Ganoderma species powder is soaked in 100 ml of solvents like water, methanol, ethanol, and hydro-methanol (50:50) separately. All the samples extract are prepared by heating in Maceration Water Bath at 80^0C for 1 hour. Then the extracts are filtered through Whatman filter paper No.1 and the filtrates stored at 4°C for further use to perform mycochemical qualitative and quantitative tests.

Reflux apparatus

For every 5 gram of Ganoderma powder, 100 ml of solvent is used and it is subjected to extraction by using a reflux apparatus. After the completion of extraction, it is centrifuged and the supernatant was filtered through Whatman No. 1 filter paper and the filtrates stored at 4°C for further use to perform various assays for determination of bioactivity mycochemicals.

Mycochemical tests

The screening of bioactive mycochemicals in fresh sporophores of *G. lucidum* and *G. applanautm* is tested by using standard methods of Indian Pharmacopoeia followed by Evans and Trease 1989, Gokhale 1993, Trease, and Evans 1996, Harborne 1973 and Shanmugam and Kumar, 2010.

Proximate evaluation

The pulverized sporophore of *G. lucidum* and *G. applanatum* was used for the standardization of mycochemical parameters in triplicate. Foreign matter, moisture content, extractive values, ash values (Gaithersburg 2000, Indian Pharmacopoeia Commission (2007).) dry matter (Kornerup and Waanscher 1978), absorption properties, foaming properties (Aremu et al., 2007), emulsion values (Yatsumatsu et al.,1972), dispersibility (Kulkarni 1991), flow characteristics, swelling index (Terangpi 2013) were determined.

Quantification of mycochemicals

Total phenolic content (TPC)

TPC in all extracts was determined by the Folin- Ciocalteu calorimeter method based on the procedure of Singleton and Rossi (1965). The extract (50mg) was mixed with folin-Ciocalteu reagent (0.5ml) and deionized water (7.5ml). The mixture was kept at room temperature for 5 minutes and then 10ml of 7% sodium carbonate was added to the mixture and then incubated for 90 minutes at room temperature. After incubation, the absorbance against the reagent blank was determined at 760nm. Total phenolics content was calculated from calibration curve equation of gallic acid ($y = 1.511x - 0.283$, $R^2 = 0.996$) and expressed as mg of gallic acid (GAE) equivalents per gram of sporophore powder (Yogita et al 2013). All samples were analyzed in triplicates for each method and solvent.

Total flavonoid content (TFC)

The TFC was measured following a spectrophotometer method (Dewanto et al., 2002). 1ml (100ug/ml) of the extract was diluted with water (4ml) in a 10ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.3ml) was added to each volumetric flask. After 5 minutes, 10% $AlCl_3$ (0.3ml) was added and 2.4ml water was then 3 added to the reaction flask and mixed well. The absorbance of the reaction mixture was read at 510nm/425nm for Rutin. Total flavonoid content was calculated from calibration curve equation of rutin ($y = 2.342x - 0.07$, $R^2 = 0.997$). The total flavonoids content of the extracts was expressed as mg rutin equivalents per gram of sporophore powder (Abdurrahman et al., 2013). Three readings were taken for each sample and the method

Results

Taxonomic studies

A survey was conducted during 2014-2016 for the collection of wood decay fungi from living trees in Krishna district, Andhra Pradesh, India. The two species of Ganoderma was identified as *Ganoderma lucidum* (Curtis) P. Karsten, *Ganoderma applanatum* (Per.) Pat. and their macroscopic and microscopic characters are described in Table 1, 2. When you compare both species, *G. lucidum* have annual habit whereas *G. applanatum* has a perennial habit. *G. lucidum* have stalk whereas *G. applanatum* not having the stalk. The context was brown in lucidum whereas light brown in applanatum. The pores per mm were 5-6 in lucidum whereas 4-5 in applanatum. The hyphal system was trimitic in case of both Ganoderma species. Basidiospores were brown, ovoid, 9.33 – 14.25 x 5 - 7.25 µm in size in lucidum whereas brown, ellipsoidal 5.25- 8.33 X 4.1 – 6.8 µm in size in applanatum. The spore index was 1.45 in lucidum whereas 1.25 in case of applanatum.

Table 1. Comparison of taxonomic features of *G. lucidum* and *G. applanatum*.

Character	G. lucidum	G. applanatum
Habit	Annual	Perennial
Basidiocarp	Stipitate, corky becoming woody later, 12-15 x 8-12 x 1-3 cm.	sessile, applanate, single, corky soon becoming hard and woody in dry condition, 10 - 16 X 8 - 10 X 2 - 4 cm
Pilear crust	Hymenioderm	Hymenioderm
Context layer	brown, 2-8 mm thick,	context light brown, interspersed with white lint, material, fibrous, with silky shine, 2-3.5 cm thick 2 mm broad
Tube layer	Up to 6-7 mm long, unstratified	Up to 5 - 8 mm long, stratified
Pores	5–6 per mm, small, brown, 90-250 µ diameter,	4-5 per mm, pore surface white when fresh turning light brown on drying, pores round,
Hyphal system	Trimitic	Trimitic
Basidiospores	9.33 – 14.25 x 5 - 7.25 µm. brown, ovoid, thick-walled, minutely verrucose, truncate at base,	5.25- 8.33 (10) X 4.1 – 6.8 (8) µm. brown, broadly ellipsoid, thick-walled with outer wall smooth, inner wall echinulate, truncate

Table 2. Basidiospore biometrics of *G. lucidum* and *G. applanatum*

No.	Basidiospore character	*G. lucidum* (n = 10)	*G. applanatum* (n = 10)
1.	Mean length	11.50	10.0
2.	Mean width	7.90	8.0
3.	Spore index (SI)*	1.45	1.25

Spore index (SI)* = spore length / spore width.

Preparation of Extraction

Three extraction methods were employed in order to obtain the biologically active myco-chemical components in water, methanol, ethanol and 50% hydro-methanol as the solvent. The results are summarized in Table 3, 4, 5. The best extraction method was Maceration Water bath assisted extraction of bioactive mycochemicals when compared to maceration and reflux apparatus extraction method.

Effects of extraction method on Mycochemical composition in G. lucidum

Mycochemical compounds screening of water, methanol, ethanol and 50% Hydro-methanol extracts of *G. lucidum, G. applanatum* is prepared by using Maceration, Maceration Water bath, Reflux apparatus methods and the results of the effect of extraction methods on myco chemical compounds are presented in Tables 3, 4, 5, respectively. Screening of different solvent extracts of *G. lucidum* indicating the presence of alkaloids, carbohydrates, proteins, phenolic compounds, flavonoids, tannins, terpenoids, diterpenoids, and Anthocyanin. The methanol extract prepared by maceration method showed an excellent concentration of Tannins and Flavonoids whereas Ethanol extract showed an excellent concentration of Tannins, Flavonoids, Terpenoids, and DiTerpenoids. The methanol extract prepared by Maceration water bath method showed an excellent concentration of Alkaloid, Tannins, Flavonoids, and Phenols, Ethanol extract showed an excellent concentration of Tannins, Flavonoids, Terpenoids, and DiTerpenoids, and 50% methanol showed an excellent concentration of phenols. The Ethanol extract prepared by Reflux apparatus showed an excellent concentration of Terpenoids, and DiTerpenoids, whereas 50% methanol showed an excellent concentration of Tannins, Flavonoids, Phenols, and Terpenoids.

Table 3: Mycochemicals screening of different extracts of *G. lucidum*

Extraction by maceration	Alkaloid	Carbohydrates	Proteins	Amino Acids	Tannins	Flavonoids	Phenols	Terpenoids	Di Terpenoids	Anthocyanins
Water	+	++	+	-	++	+	++	+	+	+
Methanol	+++	++	+	-	++++	++++	+++	+++	+++	++
Ethanol	++	++	-	-	++++	++++	++	+++	+++	++
50% methanol	+++	+	+	-	+++	+++	+++	+++	++	++
Extraction by Water Bath	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**Di Terpenoids**	**Anthocyanins**
Water	++	+++	++	-	+++	++	++	++	++	++
Methanol	++++	++	+	-	++++	+++++	+++	+++	+++	++
Ethanol	+++	+++	-	-	++++	+++++	+++	+++	+++	+++
50% methanol	+++	++	++	-	+++	+++	+++	+++	++	+++
Extraction by reflux apparatus	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**Di Terpenoids**	**Anthocyanins**
Water	+	+	+	+	++	+	++	+	++	++
Methanol	++	++	+	-	+++	+++	++	++	++	+
Ethanol	+	++	-	-	+++	+++	+++	+++	+++	++
50% methanol	++	+	+	-	++++	++++	+++	+++	++	+++

+= present, ++ (or) +++= moderately present, ++++ (or) +++++= Excellent

Effects of extraction method on the Mycochemical compound in G. applanatum

Mycochemical analysis of sporophore extract of G. applanatum proves the presence of alkaloids, carbohydrates, proteins, amino acids, phenolic compounds, flavonoids, tannins, terpenoids, diterpenoids, and Anthocyanin (Table 4, 5). The methanol extract prepared by maceration method showed an excellent concentration of Tannins and Flavonoids whereas ethanol extract showed an excellent concentration of Tannins Flavonoids Terpenoids and DiTerpenoids. The methanol extraction prepared by water bath assisted method showed an excellent concentration of Alkaloid, Tannins, and Flavonoids, the ethanol extraction showed an excellent concentration of Tannins, Flavonoids, Terpenoids, Phenols, and DiTerpenoids. The methanol extract prepared by Reflux apparatus showed an excellent concentration of Flavonoids whereas 50% methanol extract showed an excellent concentration of flavonoids and phenols

Table 4: Mycochemicals screening of different extracts of *G. aplanatum*

Extraction by maceration	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**DiTerpenoids**	**Anthocyanins**
Water	+	++	+	+	+	+	++	++	++	+
Methanol	++ +	+	+	-	++++ +	++++	++	+++	++	+++
Ethanol	++	-	+	-	++++	++++	+++	+++ ++	+++ ++	+++
50% methanol	++	+	++	-	+++	+++	++	+++	++	++
Extraction by Water Bath	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**DiTerpenoids**	**Anthocyanins**
Water	++	++	+	+	++	++	+++	++	++	++
Methanol	++++	+	+	-	++++	++++	++	+++	++	+++
Ethanol	+++	+	+	-	++++	++++	+++ +	+++ ++	+++ ++	+++ +
50% methanol	+++	+	+++	-	+++	+++	+++	+++	++	+++

Extraction by Reflux apparatus	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**Di Terpenoids**	**Anthocyanins**
Water	++	++	+	+	+	+	++	+	++	+
Methanol	++	+	+	-	+++	++++	++	+++	++	+++
Ethanol	++	+	+	-	+++	+++	+++	+++	++	++
50% methanol	+++	+	++	+	+++	++++	+++ +	++	++	++

+= present, ++ (or) +++= moderately present, ++++ (or) +++++= Excellent

Table 5. Comparison of different mycochemicals in Ganoderma lucidum and G. applanatum.

Solvent	**Extraction method**	**G. lucidum.**	**G. applanatum**
Water	**Maceration**	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Amino Acids Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Methanol		Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Ethanol		Alkaloid Carbohydrates Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
50% methanol		Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Water	**Water bath**	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Amino Acids Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins

Methanol		Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Ethanol		Alkaloid Carbohydrates Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
50% methanol		Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Water	**Reflux apparatus**	Alkaloid Carbohydrates Proteins Amino Acids Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Amino Acids Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Methanol		Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
Ethanol		Alkaloid Carbohydrates Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins
50% methanol		Alkaloid Carbohydrates Proteins Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins	Alkaloid Carbohydrates Proteins Amino Acids Tannins Flavonoids Phenols Terpenoids Di Terpenoids Anthocyanins

Proximate evaluation

The proximate composition of both G. lucidum and G. applanatum was recorded in Table 6. The percentage of foreign matter, dry matter, water-soluble extractives, total ash, water soluble ash, oil absorption capacity, emulsifying capacity, foaming capacity, foaming stability, swelling index was high in G. lucidum compared to G. applanatum. The moisture content,

ethanol soluble extractives, acid soluble ash, water absorption capacity, emulsion stability, dispersibility, bulk density was high in G. applanatum compared to G. lucidum.

Table 6. Physicochemical evaluation sporophores of *G.lucidum G. aplanatum*

Parameters		*G. lucidum*	G. aplanatum
	Foreign matter (%)	0.06	0.04
	Moisture content (%)	2.48	3.54
	Dry matter (%)	98	89
Extractive values (%)	**Ethanol soluble extractives**	4.65	5.23
	Water soluble extractives	5.86	2.43
Ash content (%)	**Total ash**	7.78	5.54
	Acid insoluble ash	2.66	3.5
	Water soluble ash	5.5	2.5
Absorption properties (ml/g)	**Oil absorption capacity**	10.45	8.78
	Water absorption capacity	62.58	74.46
Emulsion properties (%)	**Emulsifying capacity**	35.65	21.34
	Emulsion stability	11.35	18.62
	Dispersability (%)	30	35
Flow properties	**Bulk density (g/ml)**	1.3	1.5
	Tapped density (g/ml)	1.8	1.8
Foaming properties (%)	**Foaming capacity**	48.80	40.30
	Foaming stability	37.63	35.54
	Swelling Index (%)	75	50

Quantification of mycochemicals

Effects of extraction method on total phenolics content

In case of G. lucidum and G. applanatum, total phenolic contents of water, methanol, ethanol and 50% methanol extracts prepared from Maceration, Water bath assisted and Reflux apparatus methods are shown in Table 7. The water bath extraction method was the best method of quantification for total phenols in both G. lucidum and G. applanatum when

compared to maceration, reflux apparatus. The highest total phenol content was shown 111.18 mg GAE/g in G. lucidum 50% methanol extract prepared by water bath method whereas lowest total phenol content was observed 37.06 mg GAE/g in case of G. lucidum water extract prepared by Reflux apparatus. For G. lucidum the water extract prepared by using Water bath assisted method showed the higher phenolic content of 60.88 mg GAE/g sporophore powder as compared to Maceration (45.00 mg GAE/g) and Reflux apparatus (37.06 mg GAE/g) methods. The methanol extract prepared by using Water bath assisted method showed the higher phenolic content of 84.71 mg GAE/g sporophore powder as compared to Reflux apparatus (71.47 mg GAE/g) and Maceration (66.18 mg GAE/g) methods. The Ethanol extract prepared by using Water bath assisted method showed the higher phenolic content of 100.59 mg GAE/g sporophore powder as compared to Reflux apparatus (84.71 mg GAE/g) and Maceration (79.41 mg GAE/g) methods. The 50% methanol extract prepared by using Water bath assisted method showed the higher phenolic content of 111.18 mg GAE/g sporophore powder as compared to Reflux apparatus (97.94 mg GAE/g) and Maceration (92.65 mg GAE/g) methods. In case of G. applanatum the water, methanol, ethanol, 50% methanol extract prepared by Water bath method show highest total phenolic content like 52.94 mg GAE/g, 79.41 mg GAE/g, 92.65 mg GAE/g, 105.89 mg GAE/g sporophore powder respectively when compared to remaining method of extraction.

Table 7. Total phenolic compounds in different solvents

	G. lucidum		***G. apllanatum***	
Solvent Maceration	**OD**	**Total Phenols**	**OD**	**Total Phenols**
water	0.17	45.00	0.15	39.70
Methanol	0.25	66.18	0.22	58.23
Ethanol	0.30	79.41	0.32	84.71
50% methanol	0.35	92.65	0.30	79.41
Solvent Maceration water bath	**OD**	**Total Phenols**	**OD**	**Total Phenols**
water	0.23	60.88	0.20	52.94
Methanol	0.32	84.71	0.30	79.41

Ethanol	0.38	100.59	0.35	92.65
50% methanol	0.42	111.18	0.40	105.89
Solvent Reflux apparatus	**OD**	**Total Phenols**	**OD**	**Total Phenols**
water	0.14	37.06	0.17	45.00
Methanol	0.27	71.47	0.25	66.18
Ethanol	0.32	84.71	0.34	90.00
50% methanol	0.37	97.94	0.36	95.30

Effects of extraction method on total flavonoids content

In case of G. lucidum and G. applanatum, total flavonoids contents of water, methanol, ethanol and 50% methanol extracts prepared by Maceration, Water bath apparatus and Reflux apparatus methods are shown in Table 8. The G. applanatum showed better total flavonoid content when compared to G. lucidum. The water bath method was the best method of quantification for total flavonoids in G. applanatum whereas reflux apparatus was the best method of quantification of flavonoids in G. lucidum when compared to maceration, reflux apparatus. The highest total flavonoids content was shown 39.28 mg rutin/g in G. applanatum 50% methanol extract prepared by water bath method whereas lowest total flavonoids content was observed 10.24 mg rutin/g in case of G. lucidum water extract prepared by Maceration method. In case of maceration method the water extract of applanatum shown 16.22 mg rutin/g flavonoids compared to lucidum, methanol extract of applanatum shown 23.05 mg rutin/g flavonoids compared to lucidum, ethanol extract of applanatum shown 29.88 mg rutin/g flavonoids compared to lucidum and 50% methanol extract of applanatum shown 35.86 mg rutin/g flavonoids compared to lucidum In case of water bath method, water extract of applanatum shown 17.93 mg rutin/g flavonoids compared to lucidum, methanol extract of applantum shown 25.61 mg rutin/g flavonoids compared to lucidum, ethanol extract of applantum shown 32.45 mg rutin/g flavonoids compared to lucidum, and 50% methanol extract of applantum shown 39.28 mg rutin/g flavonoids compared to lucidum In case of reflux method water extract of applanatum shown 17.07 mg rutin/g flavonoids compared to lucidum, methanol extract of lucidum shown 25.61 mg rutin/g flavonoids compared to applanatum, ethanol extract of applantum shown 31.59 mg rutin/g flavonoids compared to lucidum, and 50% methanol extract of applantum shown 37.57 mg rutin/g flavonoids compared to lucidum.

Table 8. Total flavonoid compounds in different solvents

	G. lucidum		*G. apllanatum*	
Solvent Maceration	**OD**	**Total flavonoids**	**OD**	**Total flavonoids**
water	0.12	10.24	0.19	16.22
Methanol	0.20	17.07	0.27	23.05
Ethanol	0.33	28,18	0.35	29.88
50% methanol	0.25	21.34	0.42	35.86
Solvent Maceration Water bath	**OD**	**Total flavonoids**	**OD**	**Total flavonoids**
water	0.13	11.10	0.21	17.93
Methanol	0.23	19.64	0.30	25.61
Ethanol	0.30	25.61	0.38	32.45
50% methanol	0.23	19.64	0.46	39.28
Solvent Reflux Apparatus	**OD**	**Total flavonoids**	**OD**	**Total flavonoids**
water	0.15	12.80	0.20	17.07
Methanol	0.30	25.61	0.28	23.91
Ethanol	0.22	18.78	0.37	31.59
50% methanol	0.28	23.91	0.44	37.57

Discussion

Effects of extraction method on Mycochemical composition in G. lucidum

Qualitative chemical screening of various extracts revealed the presence of carbohydrates, proteins, amino acids, lipids, steroids, terpenoids, glycosides, phenolic compounds, alkaloids, and saponins, while tannins and mucilage were not detected in G. lucidum (Singh et al., 2014). In the present paper, tannins were observed in all extracts of G. lucidum prepared by different extraction methods. Preliminary phytochemical screening of *G.lucidum* unprocessed powder ethanol extract prepared by maceration, reveals the presence of alkaloids, cardiac glycosides,

tannins, terpenoids shows positive results and saponin shows negative results (Nithya et al., 2014). In the present paper, extracts prepared by maceration showed excellent mycochemicals in G. lucidum. Preliminary phytochemical analysis of the extract prepared by using a Soxhlet apparatus showed that the methanol extracts of G. lucidum contained Polyphenols, flavonoids, and terpenes (Sheena et al., 2003). In the present study, the methanol extract of G, lucidum prepared by reflux apparatus contained alkaloids, carbohydrates, proteins, phenolic compounds, flavonoids, tannins, terpenoids, diterpenoids, and Anthocyanin. Phytochemical analysis of aqueous extract of G. lucidium prepared by Sonication shows the carbohydrates, saponins, glycosides were present at high level, proteins, tannins, terpenoids, phlobatannins, phenolic compounds were present at moderate level and fats, alkaloids were present at a low level but phytosterol and flavonoids were not detected (Islam et al., 2018). In the present paper, the extracts prepared by maceration water bath showed the highest content of flavonoids. They have not investigated the efficient method for extraction of mycochemicals from G. lucidum (Sheena et al., 2003). In the present study, the mycochemical composition of Maceration, Maceration water bath, Reflux apparatus extracts of G. lucidum were compared and the results were given in Table 3. Higher levels of alkaloids, carbohydrates, proteins, phenolic compounds, flavonoids, tannins, terpenoids, diterpenoids, and Anthocyanin were obtained in Maceration Water bath extracts (Table 3). The present study results clearly showed that water bath assisted extraction was an efficient method than maceration method for extraction of mycochemicals from G. lucidum.

Effects of extraction method on the Mycochemical compound in G. applanatum

The wild macrofungi G.applanatum is a rich source of phytoconstituents containing phenols, terpenoids, flavonoids, saponins, steroids, alkaloids and glycosides (Nagaraj et al 2013). In the present paper, The different solvent extracts prepared by all three methods shown rich contents of mycochemicals. Phytochemical screening of G. applanatum showed that aqueous extracts of the mushroom prepared by decoction contain saponins, flavonoids, cardiac glycosides and steroids but did not contain detectable levels of alkaloids, tannins, and anthraquinone (Manasseh et al 2012). In the present paper also the extracts prepared by maceration water bath showed an excellent concentration of Alkaloid, Tannins, and Flavonoids in methanol whereas the ethanol extract showed an excellent concentration of Tannins, Flavonoids, Terpenoids, Phenols, and DiTerpenoids. Chemical screening of various prepared extracts revealed the presence of carbohydrates, proteins, amino acids, lipids, steroids, terpenoids, glycosides, phenolic compounds, alkaloids, and saponins, while tannins

and mucilages were not detected in either of the species studied (Singh et al 2014b). In the present study, the four extracts of G. applanatum contain all tested mycochemicals except amino acids Chemical analysis of different extracts revealed some differences in the constituents of the G. applanatum studied. Petroleum ether extract of both the species showed the presence of lipids, steroids, and terpenoids. Chemical examination of the chloroform extract of G. applanatum revealed the presence of lipids and steroids Carbohydrates, proteins, amino acids, alkaloids, phenolic compounds, flavonoids, and glycosides were detected in methanolic extract of both the species. Aqueous extract of G. applanatum showed the presence of carbohydrates, reducing sugars, phenolic compounds, flavonoids, saponins and glycosides (Singh et al 2014b). In the present studies based on the solvent used for extraction contains various mycochemicals except for proteins and amino acids. The presence of these primary and secondary metabolites attributes to the high nutritional and medicinal values of G. applanatum (Singh et al 2014b). In the present studies the white rot fungi G. applanatum is able to produce mycochemicals in all solvents.

Proximate evaluation

Standardization of natural products is essential to ensure their identity, quality, and purity. Macroscopic and microscopic are the simplest methods to establish the correct identity of source materials (Jafari et al., 2013 Singh et al., 2014). In the present study, also different macroscopic and microscopic characters were used to identify the sporophore powder. Proximate parameters like foreign matter, moisture content, ash content, and extractive values are used to determine quality and purity (WHO, 1998). A drug containing appreciable quantities of foreign matter may produce a critical impact on health. Therefore, the parameter must not be neglected. The permissible limits for foreign matter as per standards should not be more than 2% (Soni et al., 2011 Singh et al., 2014a). In the present study, both G. lucidum and G. applanatum showed 0.1% foreign matter. High moisture content may lead to the activation of enzymes and promotes susceptibility to microbial growth, which accelerates spoilage (Usman et al., 2012). The percentage moisture content for G. lucidum was 10.54%, w/w (Singh et al., 2014a). In the present study, the moisture content for G. lucidum was 2.48% for G. lucidum and 3.54% for G. applanatum respectively

High ash content of a drug gives an idea about the earthy matter or inorganic composition and other impurities present along with the drug. The results of the present studies show that the ash content values are comparatively higher than those reported for G. lucidum (5.93%, w/w) previously (Usman et al., 2012 Singh et al., 2014a). In the present paper, the ash content

values are 7.78% w/w for G. lucidum and 5.54% w/w for G. applanatum, Extractive values give an indication about the nature of the chemical constituents present in the drug. Water soluble extractives were higher as compared to alcohol soluble extractives, which showed that G. lucidum and G. applanatum had more water-soluble polar constituents (Singh et al., 2014a, b). In the present study, the water-soluble extractives were higher compared to ethanol extractives in G. lucidum whereas in G. applanatum it was reverse. The absorption properties, emulsion properties, and foaming properties were also favorable, for making Ganoderma powder available to use in many drug formulations where foaming, emulsification, reconstitutability, and retention of flavor are required (Singh et al., 2014a). In the present study also for making Ganoderma powder to used in many drug formulations the physicochemical characters like absorption properties, emulsion properties, and foaming properties were studied. Absorption properties describe the ability of association of powder and water or oil, which is a useful indication of whether powder or isolates can be incorporated into aqueous or oily food and drug formulations (Udensi and Okoronkwo, 2006 Singh et al., 2014a). In the present study, the oil absorption capacity was high for lucidum whereas water absorption capacity was high for applantum.

Emulsion properties determine the ability of the powder to emulsify the oil. Emulsions play an important role in pharmaceutical preparations such as cosmetics, pastes, or cod liver oil. Emulsions have also been used for treating skin diseases and lacerations and for drug delivery, etc. (Khan et al., 2011 Singh et al., 2014a). In the present study the emulsifying capacity was high for G. lucidum compared to G. applanatum, so lucidum was useful in the preparation of emulsions for treating various skin diseases. Foaming properties determine the ability of a powder to form a foam. The foaming ability is related to the amount of solubilized protein (Odoemelam, 2005). Saponins are also involved in the process of foam formation. Foaming properties are important in the preparation of shampoos, liquid detergents, kinds of toothpaste, and beverages (Chen et al., 2010 Singh et al., 2014a). In the present study, both Ganoderma species show foaming capacity so they were used in the preparation of beverages, kinds of toothpaste. Bulk density is a measure of the heaviness of a powder sample, which determines the relative volume of the packaging material required. The dispersibility of powder in water indicates its reconstitutability (Kulkarni et al., 1991 Singh et al., 2014a). In the present study the bulk density was high for applanatum so it is used in packaging materials. The presence of these primary and secondary metabolites points to the high nutritional and medicinal values of G. lucidum and G. philippii (Singh et al., 2014a). In the present study also shown different

concentrations of primary and secondary metabolites in both G. lucidum and G. applanatum. Hence, we conclude that the present study provides useful standards that will help to identify the genuine species and check for adulteration of intact fruit bodies and powder available commercially. The preliminary chemical tests are helpful in finding the chemical constituents that may have medicinal properties and can be utilized for the treatment of various diseases.

Effects of extraction method on total phenolics content

Phenolic compounds were found to be a major class of phytochemicals, which are responsible for inhibiting the oxidative damage caused by free radicals generated inside our body (Ferguson 2001). In the present study also both white rot fungi have an excellent concentration of phenols. The total phenolic content in methanolic extracts of Ganoderma lucidum clearly exhibits that it can be considered as a better source of polyphenols (kumara et al. 2016). In the present paper the white rot fungi G. lucidum is able to produce secondary metabolites like phenols and polyphenols. Heleno et al. (2012) report a value of 47 mg GAE/g for the hydroalcoholic extract of G. lucidum and Cilerdzic et al. (2014) report values from 33.42 to 52.15 mg GAE/g of G. lucidum ethanolic extracts. In the present paper, the ethanolic extract prepared by maceration water bath have 100.59 mg GAE/g of sporophore powder. 70% ethanol was used as the extraction solvent and an 18% yield of extracted material was obtained from *G. lucidum* fungus. Further, hydro-ethanolic extract of *G. lucidum* (HEGL) prepared by accelerated solvent Extraction method was found to be rich in total phenolics contents (Rathor et al., 2014). In the present paper also total phenolic content in the extract of G. lucidum prepared by maceration water bath showed high concentration. Ethanol extract of G. lucidum prepared by stirred apparatus using a rotary shaker shows the TPC were ranged from 8.6 ± 1.0 (E4) to 13.9 ± 0.3 (E6) g/100 g gallic acid equivalents (GAE) (Veljovic et al., 2017) in the present paper the highest concentration of phenolic compounds is observed in 50% hydroethanolic extract. TPC of hot water extracts of G. lucidum and G. appalantum was found to be 3.3 and 4.7 g/100 g, respectively (Kozarski et al. 2012). In the present paper water extracts prepared by all three methods showed high total phenolic content. The quantitative determination of phytochemicals of methanol extract of G.applanatum prepared by soxhlet apparatus shows that good amount of phenols and flavonoids followed by steroids and tannins and a very low amount of alkaloids and saponins (Nagaraj et al 2014). In the present study, both white rot fungi showed a good amount of total phenolic content in all four extracts prepared by maceration water bath. The obtained results showed that the extraction procedure had a significant effect on the TPC, wherein the highest concentration of phenolic compounds

was achieved in extracts produced from ground mushroom with the extraction time of 24 h (Veljovic et al 2017). In the present study also the method of extraction and solvent used for extraction showed the effect on the yield of total phenolic content from white rot fungi G. lucidum and G. applanatum.

Effects of extraction method on total flavonoids content

Flavonoids are also very important dietary biochemical agents, which are very effective for the cardiovascular system and work as cardioprotective agents (Barros et al., 2007). The total flavonoid contents in methanolic extracts of G. lucidum prepared by stirring were found to be 1.253 mg catechin/ g extract (kumara et al. 2016). In the present study the total flavonoid content of white rot fungi G. lucidum and G. applanatum showed 39.28 mg of rutin/ g of fungal powder of applanatum and for lucidum is 25.61 mg of rutin/g hydro-ethanolic extract of *G. lucidum* (HEGL) prepared by accelerated solvent Extraction method was found to be rich in total flavonoid contents (Rathor et al., 2014). In the present study also the G. lucidum showed high total flavonoid content in all extracts. So the extraction method showing effect.

Conclusions

The present results revealed that the choices of solvent employed for extraction and extraction methods play an important role in the mycochemical composition of Ganoderma lucidum G. applantum powder extract. Total phenolics and flavonoids content of Ganoderma lucidum and G. applantum powder extract are varying with the extraction method. Maceration water bath method has produced higher phenolics in both Ganoderma lucidum and G. applantum extracts compared to maceration and reflux method, and for water bath method was shown the high amount of flavonoids in G. applanatum and for G. lucidum reflux method was better compared to maceration. The results showed that the water bath assisted extraction method is a preferred extraction method compared to maceration and Reflux extraction methods. The morphological or phenotypical identification of lignicolous fungi was important to prepare different formulations for treating certain Skin diseases.

References

[1] Abdurrahman A, Gokhan Z, Gokalp OG, Yavuz SC, Ahmet D. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. Food and Chemical Toxicology 2013; 55: 290-296

[2] Aremu MO, Olaofe O, Akintayo ET. Functional properties of some Nigerian varieties of legume seed flour and flour concentration effect on foaming and gelation properties. Journal of Food Technology, 2007; 5: 109–115.

[3] Asatiani MD, Elisashvili V, Songulashvili G, Reznick AZ, and Wasser SP. Higher Basidiomycetes mushrooms as a source of antioxidants. In: M. Rai & G. Kövics, eds. Progress in Mycology. Jodhpor, India: Scientific Publishers/Springer; 2010. pp 311–326

[4] Asuquo JE, Etim EE. Phytochemical and antinutrients evaluation of *Oxyporus populinus*. J Emerg Trends Eng Appl Sci 2011; 2: 817–820.

[5] Barros L, Baptista P, Ferreira ICFR. Effect of Lactarius piperatus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food and Chemical Toxicology. 2007; 45(9):1731-1737.

[6] Chen YF, Yang CH, Chang MS, Ciou YP, Huang YC. Foam properties and detergent abilities of the saponins from *Camellia oleifera*. Int J Mol Sci 2010; 11: 4417–4425.

[7] Ćilerdžić J, Jelena V, Mirjana S, Tatjana S, Jasmina G. Biological activity of *Ganoderma lucidum* basidiocarps cultivated on alternative and commercial substrate. Journal of Ethnopharmacology. 2014; 155:312-319.

[8] Ede SO, Olaniru E, Oteminyn S, Aguiyi JC, Ekwere EO. Analgesic and anti inflammatory activities of the ethanolic extract of the mushroom Ganoderma applanatum. Int J Res Rev Appl Sci 2012; 13: 349–352.

[9] Evans WC, Trease GE, Pharmacognosy 13th ed., Bailliere Tindall, London. 1989.

[10] Ferguson LR. Role of plant polyphenols in genomic stability. Mutation Research. 2001; 475(2):89-111.

[11] Ferreira ICFR, Vaz JA, Vasconcelos MH, Martins A. Compounds from wild mushrooms with antitumor potential. Anti-cancer Agents in Medicinal Chemistry 2010; 10:424-436.

[12] Gaithersburg MD, AOAC. Official methods of analysis of the Association of Official Analysis Chemists (17th ed.), AOAC International, 2000.

[13] Heleno SA, Lillian B, Anabela M, João RP, Maria Q, Celestino S, et al., Fruiting body spores and in vitro produced mycelium of *Ganoderma lucidum* from Northeast Portugal:

A comparative study of the antioxidant potential of phenolic and polysaccharidic extracts. Food Research International. 2012; 46:135-140.

[14] Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Vol.1. New Delhi: Government of India Press. 2007.

[15] Islam MdS. Rahi Md.S, Koli HK, Jerin I, Sajib SA, Hoque KMdF, and Reza Md.A. Evaluation of phytochemical, antioxidant, cytotoxicity and in vitro antibacterial activity of aqueous extract of *Ganoderma lucidum* cultivated in Bangladeshi habitat. Malaya Journal of Biosciences 2018; 5(1):1-13

[16] Jafari S, Saeidnia S, Ardekani MRS, Hadjiakhoondi A, Khanavi M. Micromorphological and preliminary phytochemical studies of *Azadirachta indica* and *Melia azedarach.* Turk J Bot 2013; 37: 690–697.

[17] Joseph S, Sabulal B, George V, Smina T, Janardhanan KK Antioxidative and antiinflammatory activities of the chloroform extract of *Ganoderma lucidum* found in South India. Sci Pharm 2009; 77: 111–121.

[18] Kadiri M, Fasidi IO. Secondary plant products in some Nigerian mushrooms. Niger J Bot 1992; 5: 187–192.

[19] Khan BA, Akhtar N, Khan HMS, Waseem K, Mahmood T, Rasul A, Iqbal M, Khan H. Basics of pharmaceutical emulsions: a review. Afr J Pharm Pharmacol 2011; 5: 2715–2725.

[20] Kornerup and Waanscher JH. Metheun's handbook of colors. 3rd ed. Metheun and Co. Ltd. London, 252. 1978.

[21] Kozarski M, Klaus A, Niksˇic´ M, et al Antioxidative activities and chemical characterization of polysaccharide extracts from widely used mushrooms *Ganoderma applanatum, Ganoderma lucidum, Lentinus edodes* and *Trametes versicolor*. J Food Compos Anal 2012; 26:144–153

[22] Kulkarni DK, Kulkarni DN, Ingle UM. Sorghum malt-based weaning food formulations. Preparation, functional properties and nutritive value. Food Nutr Bull 1991; 13: 14–16.

[23] Kulkarni DK, Kulkarni DN, Ingle UM. Sorghum malt-based weaning food formulations. Preparation, functional properties and nutritive value. Food Nutrition Bulletin, 1991; 13, 14–16.

[24] kumari K, Prakash V, Rana S, Sagar A In Vitro antioxidant activity of methanolic extract of *Ganoderma lucidum* (Curt.) P. Karst. International Journal of Advanced Science and Research 2016; 1(5): 51-54

[25] Manasseh AT, Godwin J. TA, Ubleni EE, Borisde OO. Phytochemical properties of *Ganoderma applanatum* as potential agents in the application of nanotechnology in modern day medical practice Asian Pacific Journal of Tropical Biomedicine 2012; Suppl: 580-S583

[26] Nagaraj K, Mallikarjun N, Naika R, Venugopal TM. Antioxdative Activities Of Wild Macro Fungi *Ganoderma Applanatum* (Pers.) Pat. Asian J Pharm Clin Res, 2014; 7(2): 166-171

[27] Nagaraj K, Mallikarjun N, Naika R, Venugopal TM: Phytochemical analysis and in vitro antimicrobial potential of *Ganoderma applanatum* (Pers.) Pat. of Shivamogga district-Karnataka, India. Int J Pharm Sci Rev Res 2013; 23: 36-41.

[28] Nithya M, Ambikapathy V, Panneerselvam A.. Collection, Identification, Phytochemical analysis and Phyto toxicity test of Wood inhabiting Fungi *Ganoderma lucidum* (Curt.Fr.)P.Karst. Hygeia.J.D.Med. 2014; 6 (1),: 31-39

[29] Odoemelam SA. Functional properties of raw and heat processed jackfruit (*Atrocarpus heterophyllus*) flour. Pakistan J Nut 2005; 4: 366–370.

[30] Ogbe AO, Ditse U, Echeonwu I, Ajodoh K, Atawodi SE, Abdu PA. Potential of a wild medicinal mushroom, *Ganoderma* sp., as feed supplement in chicken diet: effect on performance and health of pullets. Int J Poultry Sci 2009; 8: 1052–1057.

[31] Rathor R, Tulsawani R, and Misra K. Hydro-Ethanolic Extract Of *Ganoderma Lucidum* (Hegl) Shows Antiinflammatory Activity On Thp1 Cytokines And Nf- B P65 Response. IJPSR 2014; 5(6): 2337-2348

[32] Russell R, Paterson M. Ganoderma: a therapeutic fungal biofactory. Phytochemistry, 2006; 67:1985-2001.

[33] Shanmugam S, Kumar TS, Selvam KP. Laboratory hand book on biochemistry, PHI learning Private limited, New Delhi, India.pp.129- 133, 2010.

[34] Sheena N, Ajith TA, Mathew AT, Janardhanan KK. Antibacterial Activity of Three Macrofungi, *Ganoderma lucidum, Navesporus floccosa* and *Phellinus rimosus* Occurring in South India. Pharmaceutical Biology 2003; 41 (8): 564–567.

[35] Sheikh IA, Vyas D, Ganaie MA, Dehariya K, Singh V HPLC determination of phenolics and free radical scavenging activity of ethanolic extracts of two polypore mushrooms. Int J Pharm Pharm Sci 2014, 6:679–684

[36] Singh R, Dhingra GS, Shri R, A comparative study of taxonomy, physicochemical parameters, and chemical constituents of *Ganoderma lucidum* and *G. philippii* from Uttarakhand, India. Turk J Bot 2014a; 38: 186-196

[37] Singh R, Singh AP, Dhingra GS, Shri R. Taxonomy, physicochemical evaluation and chemical investigation of *Ganoderma applanatum* and *G. brownie* International Journal of Advanced Research 2014b; 2 (5):702-711

[38] Soni N, Lal VK, Agrawal S, Verma H. Pharmacognostical and phyto physico-chemical profile of *Curculigo orchioides* (Gaertn). Adv Res Pharm Biol 2011; 1: 130–138.

[39] Terangpi R, Basumatary R, Tamuli AK, Teron R. Pharmacognostic and physicohemical evaluation of stem bark of *Acacia pennata* (L.) wild., a folk plant of the Dimasa tribe of Assam, Journal of Pharmacognosy and Phytochemistry, 2013; 2, 134–140.

[40] Trease G. E., Evans W. C. A Textbook for pharmacognosy, 14th Eds. Saunders, W.B (Ed), London. 13-53, 1997.

[41] Udensi EA, Okoronkwo KA. Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate. Afr J Biotechnol 2006; 5: 896–900.

[42] Usman SB, Kyari SU, Abdulrahman FI, Ogbe AO, Ahmad GY, Ibrahim UI, Sakuma AM. Proximate composition, phytochemical and elemental analysis of some organic solvent extract of the wild mushroom - *Ganoderma lucidum.* J Nat Sci Res 2012; 2: 24–35.

[43] Veljovic S., Veljovic M, Nikicevic N, Despotovic S, Radulovic S, Niksic M., et al., Chemical composition, antiproliferative and antioxidant activity of differently processed *Ganoderma lucidum* ethanol extracts. J Food Sci Technol 2017; 54(5):1312–1320

[44] Wachtel-Galor S, Buswell JA, Tomlinson B, Benzie IFF, Lingzhi polyphorous fungus. In: Herbal and Traditional Medicine: Molecular Aspects of Health. New York: Marcel Dekker Incpp; 2004, 179-228.

[45] Wasson RG. Soma: Divine mushroom of immortality. Harcourt Brace Jovanovich New York; 1968.

[46] WHO Quality Control Methods for Medicinal Plant Materials. Geneva: World Health Organization Press; 1998.

[47] Yatsumatsu K, Sawuda K, Moritaka S, Miscki M.,Whipping and emulsifying properties of soybean products. Journal of Agaricultural and Biological Chemistry, 1972; 36, 719–726.

[48] Yogita C, Singhal RS. Ultrasound-assisted extraction (UAE) of bioactives from arecanut (*Areca catechu* L.) and optimization study using response surface methodology. Innovative Food Science and Emerging Technologies 2013; 17:106-113.