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Taxonomy and Bioactive chemicals from Ganoderma and Phellinus of India

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ABSTRACT: Many species of mushrooms have been used in folk medicine for thousands of years. Medicinal mushrooms are now the subject of study for many ethno botanists and medical researchers. The lignicolous fungi were collected from living trees in Andhra Loyola College, Vijayawada, India. They were identified as *Ganoderma lucidum G. applanatum* and *Phellinus noxious*. The lignicolous fungi were analyzed for bioactive compounds in extraction of *Ganoderma* and *Phellinus* based on their polarity solvents like ethanol and methanol. The bioactive compounds like Anthocyanins, tannins, terpenoids, and flavonoids shown excellent concentration in test fungi. The moderate concentration of alkaloids was found in *Phellinus noxious*. For the first time the bio active compounds like Phenols, Alkaloid and Anthocyanins were reported from *G. lucidum G. applanatum*. The wide range of bioactive compounds constituents and these can be useful for drug discovery and development of various new formulations. For the first time the bio active compounds were reported from *G. applanatum* and *P. noxious.*

Keywords: Bioactive compounds, Phenold, Anthocyanins *Ganoderma applanatum, Phellinus noxious*, Taxonomy, India.

INTRODUCTION

The use of plant and plant product as food is as old as human existence of mankind. Ancient man being very close to nature ate raw and fresh produce which contributed to his health living and long-life (Denmeade and Isaacs 2002). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (Abraham and Thomas, 2012). Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system. The herbal medicines serve the health needs of about 80% of the world s population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (Kamaraj *et al*., 2012). Mushrooms are the group of fungi which are widely used as food and medicine in different parts of the world since long time. The history of mushrooms being used as food from has been there for a very long time. An Archaeological survey revealed the association of edible species with people living before 1300 years ago in Chile. But in China eating of wild mushrooms has been noted several hundreds of years ago even before birth of Christ (FAO, 2004).

Many species of mushrooms have been used in folk medicine for thousands of years (Christopher Hobbs 1998). Medicinal mushrooms are now the subject of study for many ethno botanists and medical researchers. It is estimated that there are approximately 1.5 million species of mushrooms in the world of which 70,000 species are described. About 10,000 of the known species belongs to the macro fungi of which about 5000 species are edible and over 1,800 species are considered to have medicinal properties (Chang 1995). There is a constant search for new and effective drugs, which have been driven by the number of pathogenic organism reported to have multi resistant against many of the therapeutic products (Yang *et al.,* 2004)that are currently available in market and toxicity in host organ cells (Prakash, 2006). Besides, medicinal preparations of herbal origin eliminate side effects associated to synthetic drugs (Iwu *et al.,* 1999). Following the discovery of drugs from an array of plants, there has been serious investigations into other various plant extracts as remedies to variety of diseases (Reddy and Jose 2010) with the aim of finding cheaper, readily available, effective and non toxic medications that are herbal based (Rizwan *et al.,* 2012). so Mushroom have been a major focus of investigations for novel biologically active compounds from natural resources and in recent years pharmaceutical companies have spent a lot of time developing these natural products to produce more affordable and cost effective remedies (Farnsworth 1994).

Fungi are well known for the production of important antibiotic compounds such as penicillin; however, the occurrence of antibiotics in the class of fungi known as Basidiomycetes is less well documented (Miles and Chang 1997). Edible mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Lindequist *et al*., 2005). so in the present paper the taxonomy and Phytochemical analysis of bioactive compounds from Ganoderma and Phelinus were studied.

MATERIALS AND METHODS

A. Survey of Krishna district

Vijayawada is famous for educational facilities. By 1950, 5 degree colleges were established in Vijayawada. It is presently a hub of residential Schools, Corporate Junior colleges. Presently we have more than 20 engineering colleges and 4 medical colleges with in radius of 15 km from Vijayawada. It is also a city known for its busy commercial activity. It is called as the Venice of the east. River Krishna flows through the city. It has the privilege of having the famous Krishna dam built across the river in the year 1854 by captain Orr and Sir Arthur Cotton. The city has several historical monuments such as Mughalrajpuram cave, Akkanna madanna cave, Buddha and Jain monuments, Bhavani islands, Gunadala Mary Matha shrine, and the famous Undavalli caves are located on the south bank of the river.

Andhra Loyola College was founded in December 1953 and established in 1954. Today it is one of the premier institutions of higher learning in southern India. It spread over a sprawling campus of 100 acres with gothic structures in the heart of Vijayawada city (Fig. 1). The different plants like *Peltophorum rouxbergii, Ficus bengalensis, Dalbergia sisso, Polyalthia longifolia, P. pendula, Eucalyptus, Syzygium commune* and *Albezia lebica* were planted at the starting of the college so the age of the plants is around 50 years. Recently teak plantation is done which is having the age around 10 to 13 years. Different medicinal plants are preserved in herbal garden and *Santalam album* is also present in Botanical garden maintained by the college. Different lignicolous fungi were found associated 50 years of age old plants so the fungi were collected, identified, their taxonomical and bioactive compounds are described.

Fig. 1. Aerial view of the Andhra Loyola College with different plants.

B. Identification of fungi

Stains and Mounting medium.

Lactic acid in glycerol: Although less effective than lacto-phenol for cleaning plant tissues, lactic acid has similar properties and can be used neat or combined with glycerol and water for routine examinations of many fungi. (Composition for 100 ml.){Lactic acid: 25 ml, Glycerol: 50 ml and Distilled water: 25 ml}

Cotton blue in lactic acid: This is widely used as standard mordant which rapidly stains the cytoplasm of fungal cells. Permanent mounts can be prepared by sealing with nail varnish. Add cotton blue powder to lactic acid. Heat in a glass beaker and stir until dissolved. Add to cool then filter to remove any sediment. (Composition for 100 ml.){Cotton blue: 0.01 gm and Lactic acid (85%): 100 ml}

Potassium hydroxide (KOH): Used at 5-10 % potassium hydroxide is useful for softening the fungal tissue. After clearing, the KOH can be washed off with water using a dropper or pipette and replaced with stain. A 5 % solution is prepared as follows: Weigh out 5gms of potassium hydroxide pellets.

Add to distilled water in a glass beaker. Stir until dissolved. (Composition for 100 ml.){Potassium hydroxide: 5 gm, Dist. water: 100 ml.

Melzer's regent: Mainly used for testing the amyloid reaction in sporulating structures of Basidiomycetes. Also clears and stains the fungal tissues including cell walls and can be used for general examination of fungal structures. Prepare in a fume cupboard. Dissolve potassium iodide in distilled water. Add iodine and leave to dissolve for 24 hours. Add chloral hydrate and stir until dissolved. Mix thoroughly. (Composition for 100 ml.){Cholral hydrate: 100 gm, Iodine: 1.5 gm, Potassium iodide: 5 gm and Distilled water: 100 ml}

Microscopic observation

Fruiting bodies of the lignicolous fungi were collected from its natural habitat, growing on wooden logs. Standard methods were followed for collection, preservation, macroscopic and microscopic studies (Kumar *et al* 1990 Atri *et al* 2003). The morphological features of the fungi were recorded from fresh specimen. Materials were collected in clean polythene bags from different locations and brought to the laboratory. Basidiomes were studied using macroscopic (eg. size, colour, number of pores/mm, length of tubes) and microscopic (presence or absence of structures, dimensions, vegetative and reproductive characters (Ryvarden 1991). To observe basidia and setae, free hand sections were taken. For the clear observation of setae, trammel setae and setal hyphae, lacto phenol cotton blue was used as staining and mounting medium. Xanthochoric reaction was also tested using potassium hydroxide solution. The various details of specimens were compared with Hymenochaetaceae of India (Sharma 1995), Indian Polyporaceae (Bakshi 1971), CBS Aphyllophorales database, New Zealand Fungi database, and Species Fungorum. Certain specimens were sent to the Royal Botanical Garden, Kew for final confirmation. These fungi are kept in fungal collection of Botany Department, Andhra Loyola College, Vijayawada, Andhra Pradesh, India.

Extraction of bioactive compounds

The fruiting bodies of *G. lucidium G. apllanatum* and *P. noxious* was initially rinsed thrice in distilled water and dried on paper toweling and samples was cut into fine pieces and powdered. For preparing the extracts methanol, ethanol was used as solvents. For every 1 gram of powder, 50 ml of solvent was used and was subjected to extraction using a reflux apparatus. After the completion of extraction, 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.

Phytochemical Screening of Bio active compounds

(Evans W.C., Trease 1989, Gokhale S.B 1993, Trease, G.E, Evans 1996, Harborne JB,1973 and Shanmugam, S., Kumar, 2010).

Detection of Alkaloids:

Mayer's Test: One or two drops of Mayer's reagent are added to a few ml of extract by the sides of the test tube. White precipitate indicates the presence of alkaloids.

Wagner's test: One or two drops of Wagner's reagent is added to a few ml of extract by the sides of the test tube. Reddish brown precipitate indicates the presence of alkaloids.

Hager's Test: One or two drops of Hager's reagent is added to a few ml of extract. Prominent yellow precipitate indicates the presence of alkaloids.

Dragen darf: One or two drops of Dragen darf 's reagent is added to a few ml of extract by the sides of the test tube. Orange precipitate indicates the presence of alkaloids.

Detection of Tannins:

Ferric chloride test: To the extract few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicates the presence of tannins.

Detection of flavonoids:

The extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Lead acetate solution Test: The extract was treated with few drops of 10% lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Detection of Phenols:

Lead acetate test: To the extract 3 ml of 10% lead acetate solution was added. Formation of a bulky white precipitate indicates the presence of phenols.

Ferric chloride test: the extract was treated with 5% ferric chloride. Formation of intense colour indicates the presence of phenols.

Detection of Terpenoids:

0.5ml of extract was mixed with 2ml of chloroform and concentrated H_2SO_4 is added carefully. Formation of red brown color at the interface indicates the presence of terpenoids.

Detection of Di Terpenoids:

The extract was treated with few drops of 1% lead copper acetate solution. Formation of green precipitate indicates the presence of Di Terpenoids**.**

Detection of Anthocyanins:

The extract was added to 2 ml of 2N HCl and ammonia. Initial appearance of pink-red colour turning into blue-violet indicates the presence of anthocyanin

RESULTS AND DISCUSSION

A. Survey of Krishna District

A survey was conducted during 2014-2016 for the collection of lignicolous fungi from living trees in Andhra Loyola College, Vijayawada, India.

The two species of Ganoderma and one species of Phelinus was identified as *Ganoderma lucidum* (Curtis) P. Karsten, *Ganoderma applanatum* (Per.) Pat. and *Phelinus noxious* (Corner) G. Cunningham. *G. lucidum* was found on the living trees of *Syzygium cumini* and dead wood of *Azadirechta indica, G. applanatum* was found on the living trees of *Peltophorum rouxbergii* and *P. noxious* was found on the living trees of *Delonix regia* from Andhra Loyola College, Krishna district, Andhra Pradesh, India.

Identification and taxonomy of fungi

***Ganoderma lucidum* (Curtis) P. Karsten Rev. Mycol. (Toulouse) 3(9): 17, 1881**

Basionym: *Boletus rugosus* Jacquin, Flora Austriaca 2: 44, f. 169, 1774, *Boletus lucidus* W. Curtis, Flora londinensis f. 4, pl. 224, 1781 (typus iconog.)., *Polyporus lucidus* Fr.. Syst. Mycol. 1: 353, 1821., *Ganoderma tsugae* Murrill, Bull. Torrey bot. Cl. 29: 601, 1902.

The sporophores perennial, stiptiate (Fig. 2 a, b), corky becoming woody later, 12-15 x 8-12 x 1-3 cm. many grow up to 30cm. stalk lateral, varnished and encrusted, up to 8 cm long and 0.5 to 4.5 cm thick, upper surface shiny with laccate crust, reddish brown, smooth. Cutis thin, a fraction of 1 mm, Cutis of hymenioderm type composed of anticline inflated extremities of hyaline context, hyphae swollen by melanoid substances leaving usually a central lumen; the melanoid substances are easily saponifed by KOH; Context brown, 2-8 mm thick, pores small, brown, 90-250 µ diameter, pore tubes 6-7 mm long, hyphal system timitic; Basidiospores brown, thick walled, minutely verrucose, truncate at base, 9.33 – 14.25 x 5 - 7.25 µm.

Habitat: Found on the living trees of *Syzygium cumini* and dead wood of *Azadirechta indica* from Andhra Loyola College, Krishna district, Andhra Pradesh, collected by N. Praveen Kumar, Accession no: ALC 2. 20- 9-2015.

Medicinal uses: *G.lucidum*, was uses in Antimicrobial activity including antibacterial, antifungal, antiparasitic anti- hydrogenic, and antiviral agents. *G.lucidum*, through a lactase, showed a potent inhibitory activity against HIV-1 reverse transcriptase. *Garnodema lucidium* also called reshi used as an antioxidant mushroom due to its phenolics, terpenoids and polysaccharide and polypeptide contents. It was used for the treatment of diabetes, stress, altitude sickness, Allergies, cardiovascular disease, AIDS and Cancer. it posses the ability to strengthen human body immune system

***Ganoderma applanatum* (Per.) Pat. Bull. Soc. Mycol. France 5: 67, 1889.**

Basionym: *Boletus applanatus* Pers. 1799, *Polyporus applanatus* (Pers.) Wallr., 1833,

Fomes applanatus (Pers.) Gillet, 1878, *Placodes applanatus* (Pers.) Quel., 1886, *Phaeoporus applanatus* (Pers.) Schroet., 1888,*Ganoderma megaloma* (Lev.) Bres., 1912.

Sporophore perennial, sessile, applanate, single, corky soon becoming hard and woody in dry condition, 10 - 16 X 8 - 10 X 2 - 4 cm sometimes very large, upper surface brown, zoned, uneven (Fig. 2 e, f), crusty; context light brown, interspersed with white lint material, fibrous, with silky shine, 2-3.5 cm thick. Hymeneal surface white when fresh turning light brown on drying, pores round, 4-5 per mm, pore wall thick, pore tube distinct from context, generally with a distinct white region bordering pore surface stratified, Cuticle hard, less than 0,5 mm thick, context is 2 mm broad, hyphal system trimitic, Basidiospores brown, broadly ellipsoid, thickwalled with outer wall smooth, inner wall echinulate, truncate 5.25- 8.33 (10) X 4.1 – 6.8 (8) µm.

Habitat: Found on the living trees of *Peltophorum rouxbergii* from Andhra Loyola College, Krishna district, Andhra Pradesh, collected by N. Praveen Kumar, Accession no: ALC 10. 20- 9-2015.

Medicinal uses: The fruit body of *G. applanatum* has been used as traditional medicine for anti-cancer in China and reported to have diverse physiological activities including antitumor, anti-virus, immuno-stimulation and in treatment of chronic diseases such as hepatitis, bronchitis, asthma and haemorrhoids.

***Phelinus noxious* (Corner) G. Cunningham** Bull. New Zealand Dept. Sci. Industr. Res. 164: 221, 1965.

Basionym: *Fomes noxius* Corner 1932

Basidiocarps perennial, imbricate, effused-reflexed, woody hard, light in weight when dry, 18 cm wide 10 cm broad, 6 cm thick near the base, upper surface black, glabrous, with a hard crust, azonate, frequently nodulose at the center, margin, obtuse, entire, yellowish brown; pore surface reddish brown, tubes distinctly layered, 2 mm deep in each layer, pores small 6-8 per mm, angular, moderately thickwalled entire, darker than the context (Fig. 2 c, d); context 2 cm thick at base, zoned, pale brown, radially fibrous; hyphal system dimitic, setal hyphae present, 6.25 – 15.6 µm wide, up to 4.25 µm long, rare and narrow in context, frequently projecting into the lumen of tubes, dark reddish brown, tips obtuse; hymenial setae absent, Basidia hyaline, 5.25 -8.33 x 3.12 -5.25 µm, Basidiospores hyaline, subglobose, smooth, thinwalled, 2.12 – 3.89 (5) X 2.12 µm.

Habitat: Found on the living trees of *Delonix regia* on road in front of Andhra Loyola College, Krishna district, Andhra Pradesh, collected by N. Praveen Kumar, Accession no: ALC 25. 20- 9-2015.

Medicinal uses: The extracts from *Phellinus sp* contain potential anti-cancer agents so used for cancer, Antibactrial, diarrhea, haemorrhage, Gastroenteric dysfunction.

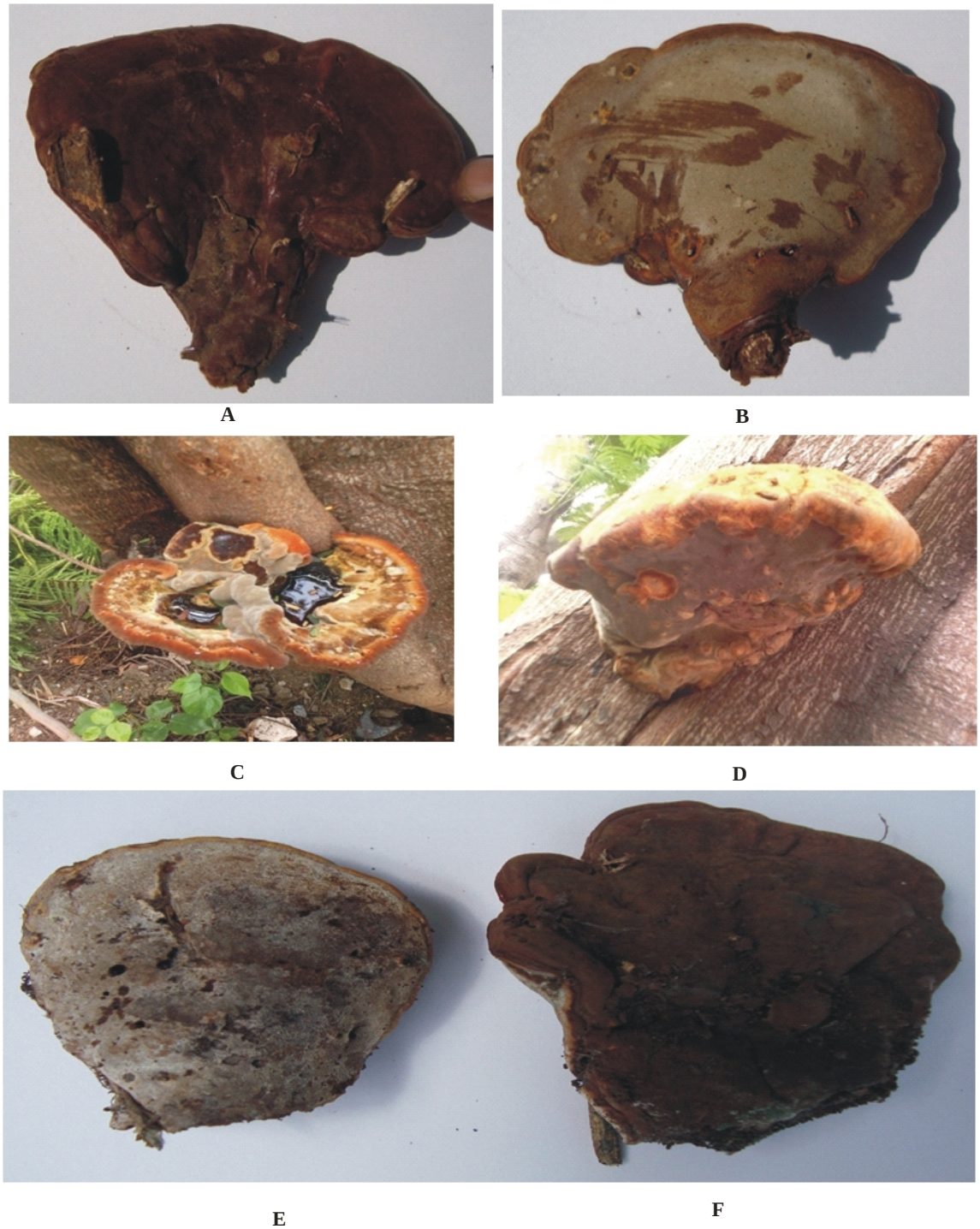

Fig. 2. (A) upper surface of *G. lucidum*, (B) lower surface of *G. lucidum* (C) upper surface of *P. noxious* (D) lower surface of *P. noxious* (E & F) lower surface and upper surface of *G. apllanatum.*

It improves blood circulation, enhancing detoxication and hepatoprotection of human body, combating allergy and diabetes, curing oral ulcer and alleviating lymphatic disease.

Phytochemical Screening of Bioactive compounds

The Phytochemical Screening of two different extracts showed great variation in terms of bioactive compounds. The extracts of *G. lucidum, G. applanatum,* and *P. noxious* contain Alkaloid, Tannins,

Flavonoids, Phenols, Terpenoids, Di Terpenoids, and Anthocyanins compounds. The bioactive compounds like Anthocyanins, tannins, terpenoids, and flavonoids shown excellent concentration in all fungi in test. The moderate concentration of alkaloids was found in *Phellinus noxious*. For the first time the bio active compounds like Phenols, Alkaloid and Anthocyanins were reported from *G. lucidum G. applanatum.* For the first time the *P. noxious* showed positive reactions to bioactive compounds like Alkaloid, Tannins, Flavonoids, Phenols, Terpenoids, Di Terpenoids, and Anthocyanins in both Methanol and Ethanol extracts (Table 1). Methanol extract were found to extract the maximum active components being solvents that have low polarity (Pooja Shah *et al* 2014). This result was in accordance to the previously reported literature (Shamaki *et al*., 2012). But in the present paper the ethanol extract showed maximum bioactive components. The genus *Ganoderma* has More than 140 biologically active triterpenoids and 200 polysaccharides, as well as proteins. Miscellaneous metabolites have also been isolated from the fruiting bodies, mycelium and spores of different species of *Ganoderma* (Trigos, and Suárez Medellín. 2011**)**.

Table 1: Bioactive compounds from *G. lucidum*, *G. apllanatum* and *P. noxious* in Methanol and ethanol extracts.

		G. lucidum		***G. apllanatum***		***P. noxious***	
S.No	**Test**	Methanol	Ethonol	Methanol	Ethonol	Methanol	Ethonol
1	**Alkaloid**	+	+	+	+	+	+
	Mayer's test	++	++	++	+	+++	+++
	Wagner's test	-	+	-	+	+	-
	Dragen darf test	++	++	+	+	+	+
2	**Tannins**	+++++	+++++	++++++	++++++	++++++	++++++
3	**Flavonoids**	+++++	+++++	+++++	+++++	++++++	++++++
4	**Phenols**	+++	++++	+++	++++	++++	+++
	Lead acetate test	+++	+++	+++	+++	+++	+++
	$FeCl_3$ test	+++	+++	+++	+++	+++	+++
5	**Terpenoids**	+++	+++++	++	++++++	++++++	++++++
6	**Di Terpenoids**	++++	+++++	+++	++++++	++++	++++
7	**Anthocyanins**	+++	+++	++++	++++	++++	++++

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

The major bioactive polysaccharides isolated from *Ganoderma* species are glucans, β-1-3 and β-1-6 D-glucan (Sakai and Chihara1995). Sheena *et al.* (2003) reported that the major secondary metabolites of *G. lucidium* are ganodermic acid, triterpenes and carcinostatic polysaccharides, tripenes, sterols, lectins and proteins (Sakai and Chihara1995). But in the present paper also different bio active compounds like Terpenoids, Di Terpenoids, Alkaloid, Tannins, Flavonoids, Phenols, and Anthocyanins was present. Bioactive chemical compounds G1 and G2 were isolated, purified and identified from fruit bodies of the wood-rot polypore fungus *G. applanatum* collected from *Tamarix aphylla* trees in southern Iraq. The chemical formula of G1 is $C_{20}H_{34}O_4$ which belongs to Tanin group while G2 is $C_{21}H_{28}O_2$ and belongs to terpenoides group (Muhsin *et al* 2011). But in the present paper also *G. applantum* showed Tannins, Terpenoids, Di Terpenoids, and others bio active compounds are also present i.e. Flavonoids, Phenols, Alkaloid, and Anthocyanins. Biological active compounds were found to be associated with isolated polysaccharides like Protein-bound polysaccharides, proteoglycans like acidic proteoglycans and other organic compounds in *Phelinus linteus* (Kang *et al* 2013). *Phelinus linteus* also contain bioactive substances like cyclophellitol, furan derivatives, hispidin, hispolon, were thus identified and their bioactivities verified *in vivo* or *in vitro*. It also has caffeic acid, davallialactone, interfungins A and inoscavin A (Kim *et al* 2003). But in the present paper the *P. noxious* has bio active compounds like Alkaloid, Tannins, Flavonoids, Phenols, Terpenoids, Di Terpenoids, and Anthocyanins. The wide range of bioactive compounds constituents were observed in *Ganoderma* and *Phelinus* from India, so these can be useful for drug discovery and development of various new formulations. For the first time the bio active compounds were reported from *G. applanatum* and *P. noxious.*

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