JJBS

Volume 4, Number 4, December 2011
ISSN 1995-6673
Pages 205- 212

Jordan Journal of Biological Sciences

Bioactive Compounds from a Polypore Fungus *Ganoderma applanatum* (Per s. ex Wallr.) Pat.

Tawfik M. Muhsin [1,*], Abdul-Hafiz A. Al-Duboon [1] and Kawther T. Khalaf [2]

[1] *Department of Biology, College of Education;* [2] *College of Pharmacology, University of Basrah, Basrah, Iraq*

Received 21 April 2011; received in revised form 17 June 2011; accepted 21 June 2011

Abstract

Bioactive chemical compounds G1 and G2 were isolated, purified and identified from fruit bodies of the wood-rot polypore fungus *Ganoderma applanatum* collected from *Tamarix aphylla* trees in southern Iraq. The identification of these two compounds by using GC-Mass and H^1NMR was confirmed. Solubility of both compounds in different solvents was tested and the toxicity of these two compounds against human blood showed a negative result. The molecular weights of the purified G1 and G2 compounds are 336 kd and 360 kd, respectively. 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. The antimicrobial bioactivities of the purified compounds against bacterial strains *E. coli* and *S. aureus* and against selected dermatophytic fungal isolates were tested using a disc diffusion agar method. The minimal inhibitory concentration (MIC) was also applied. Purified G1 and G2 compounds exhibited good bioactivities against the tested bacteria but did not show any activity against the selected dermatophytes.

Keywords: Bioactive compounds, dermatophytes, *Ganoderma applanatum*, polypore fungus.

1. Introduction

The research interest to explore new antimicrobial agents from fungi is continued. Nonetheless, previous investigations reported that several fungal mushrooms exhibit bioactive chemical components against pathogenic bacteria and fungi (Jonathan and Fasidi, 2003; Anke *et al.*, 2004; Ketting *et al.*, 2005; Al-Fatimi *et al.*, 2006; Jonathan and Awotona, 2010) . *Ganoderma* is a genus of polypore fungi (Basidiomycetes) often grows on tree parks causing white rot (Phillips 1983). Among the *Ganoderma* species, *G. pafeifferi* showed to have an antimicrobial compound known as Ganonycin (Mothana *et al.*, 2000). It has been reported that *Ganoderma* species was used as antitumor and antioxidant, and in some other medicinal therapy (Ulrike *et al.*, 2005). *G. applanatum*, however, is a distinctive species relative to the members of the genus and it grows on parks of *Tamarix aphylla* trees in Southern Iraq. According to our knowledge so far a little information is available about the production of bioactive secondary metabolites by this fungus. This report elucidates interesting chemical compounds extracted, purified and identified from fruit bodies of *G. applanatum* as a bioactive agents tested against a selected isolates of bacteria and dermatophytic fungi.

* Corresponding author: tmuhsin2001@yahoo.com.

2. Materials and Methods

2.1. Fungal Mushroom culture

Fruit bodies of the polypore *Ganoderma applanatum* were collected from the trunks of *Tamarix aphylla* in southern Iraq during spring 2008. In the laboratory, small pieces (0.5 cm long) were cut from the fruit body, surface sterilized with 10 % Sodium hypochlorate for 3 min, washed with sterile distilled water and placed on Malt Extract Agar (MEA) in Petri dishes. Plates were incubated at 25 °C for two weeks. After cultivation, the mycelium was removed from the agar medium surface and amended into a liquid culture medium consisted of (40 g glucose, 10 g malt extract, 4 g yeast extract in 1L distilled water). Then the mycelium culture was transferred into a fermentation medium in 1L volume conical flasks as described by Anke *et al.* (2004) and incubated at 25 °C on a rotary shaker for 3 weeks.

2.2. Extraction, isolation and purification

The fungal culture was filtered on Whatman No. 1 filter paper, the pH was adjusted at 3 using 2N HCl. The filtrate was extracted three times with ethyl acetate (1:1 vol) using a separating funnel. The organic layer was collected and dehydrated with Na_2SO_4 then placed in Petri dishes and dried at room temperature. Thin layer chromatography (TLC) was applied for the isolation of the extracted metabolites using Silica gel of 2x 10 cm (Silica gel GF243, Merck, Germany) and Rf values were measured. Purification of the extracted compounds was made on silica gel column chromatography (Silica gel G-60, Merck, Germany). A further purification of fraction compounds was made by a column chromatography

method **(**Fig.1**)**. The purity of compounds was verified according to the described method (Zure, 2001). The identification of the purified compounds was made by using GC-mass and HNMR techniques.

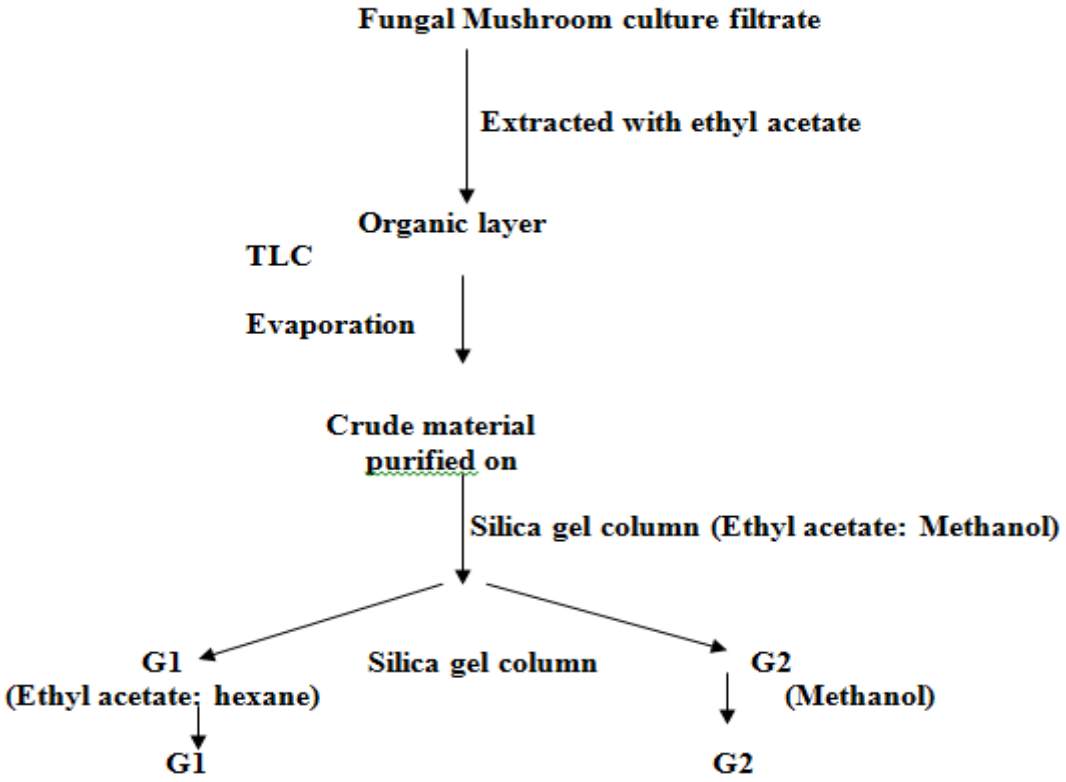


Figure 1. Steps of extraction and purification of bioactive compounds G1 and G2 from *G. applanatum*.

2.3. Bioactivity Test

Discs diffusion agar method (Casals, 1979) was used to examine the antimicrobial activity of the purified compounds. Two strains of bacteria; *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for this purpose. 2.5 mg of the dried fungal extract was dissolved in 1 ml of dimethyl sulfuoxide (DMSO) solvent used as stock solution for this test. Discs of 0.6 mm diameter Whatman No1 filter paper were sterilized and soaked in the fungal extract and placed on plates containing Muller-Hinton Agar (MHA) medium inoculated with 0.1 ml suspension of bacterial strains by streaking method. Similarly, the antifungal bioactivity of these compounds was also tested against three isolates of dermatophytic fungi (*Microsporum canis*, *M. gypseum* and *Trichophyton mentagrophytes*) using Sabouraud dextrose agar (SDA) medium. Bacterial cultures were incubated at 37°C and dermatophytic cultures were incubated at 27°C. Fungal cultures were obtained from the Basrah General Hospital, Dermatology section.

2.4. The minimum inhibitory concentration (MIC) test

The MIC values were determined by the standard serial dilution assay (McGinnis, 1980) using serial dilutions of the fungal extract (100, 50, 25, 12, 6.5, 3.13, 1.56, 0.78, 0.39, 0.2, 0.1, 0.05, 0.025 µg/L). The MIC values in this assay were indicated by the absence of bacterial or fungal growth at the minimal concentration of the compound. Emmons Sabourauds dextrose broth (ESDB) medium was used for this test.

2.5. Cytotoxic test

Cytotoxicity of the purified compounds was examined by using human RBC following a previously described method (Xian-guo and Ursula, 1994).

2.6. Solubility test

The solubility of the bioactive compounds in various solvents (ethyl acetate, ethanol, methanol, chloroform, hexane, DMSO and water) was carried out.

2.7. 2.7 Identification of bioactive compounds

Ultra violet (UV) spectrum (LKB-Sweeden UV), Infra-red spectrum (IR) (Pye-Unicam sp 3-3005 UK), Gas chromatography Mass (GC) and H^1NMR methods were applied for the identification and determination of the molecular weights, chemical formula and structure of the purified bioactive compounds. .

3. Results

Fungal extract showed two spots on TLC referred as G1 and G2 with Rf values of 0.70 and 0.50, respectively. Solubility test of G1 and G2 indicated that both components are insoluble in the water but they are soluble or partially soluble in most of the other examined solvents **(**Table 1).

Table 1. Solubility test of purified compounds G1 and G2 in various solvents

Purified component	Ethyl acetate	Methanol	Ethanol	Chloroform	Hexane	Water	DMSO
G1	Soluble	Partially soluble	Soluble	Soluble	Partially soluble	Non-soluble	Soluble
G2	Partially soluble	Soluble	Soluble	Soluble	Partially soluble	Non-soluble	Soluble

Ultra violet (UV) spectra showed that the absorbency value of G1 compound was about three fold higher than G2 compound (Fig. 2).

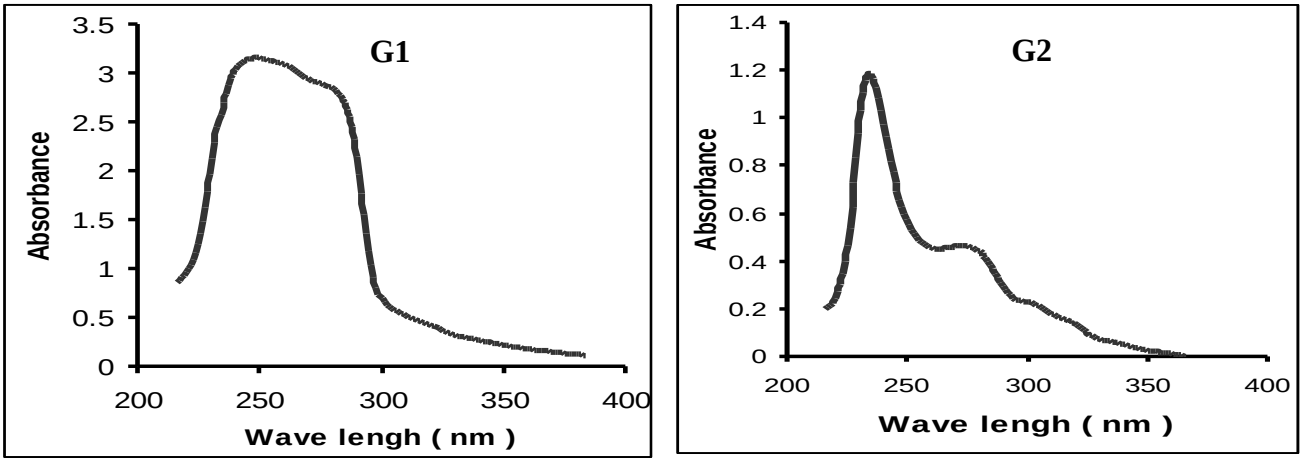


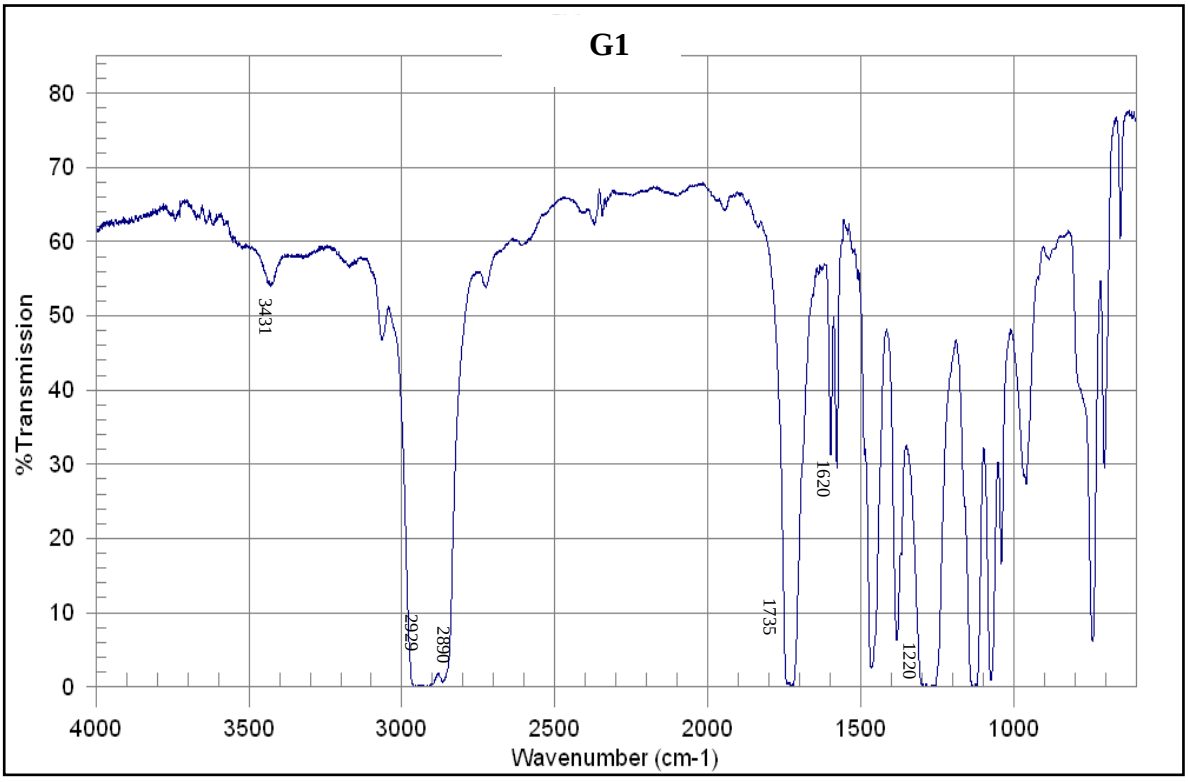
Figure 2. Absorbency of purified compounds (G1 and G2) from *G. applanatum.*

Both G1 and G2 components did not show any toxicity by using human RBC test. IR spectrum revealed that G1 and G2 composed of various functional molecules structure (Table 2).

Table 2. Infra Red spectra showed the absorbency bands of different chemical functional groups composed the purified G1 and G2 components from *G. applanatum*

Functional groups	G1	G2
N-H , O-H	3431	3400
	Weak band	Strong band
CH,CH2 ,CH	2890-2929	2900-2950-
	Strong band	Weak band
C=O	1735	1650
	Strong band	Strong band
C=C	1620	1400
	Very strong band	Weak band
C-O	1220	1260
	Very strong band	Weak band

Each of the purified compound exhibited different spectra bands representing different chemical functional groups (Fig. 3).



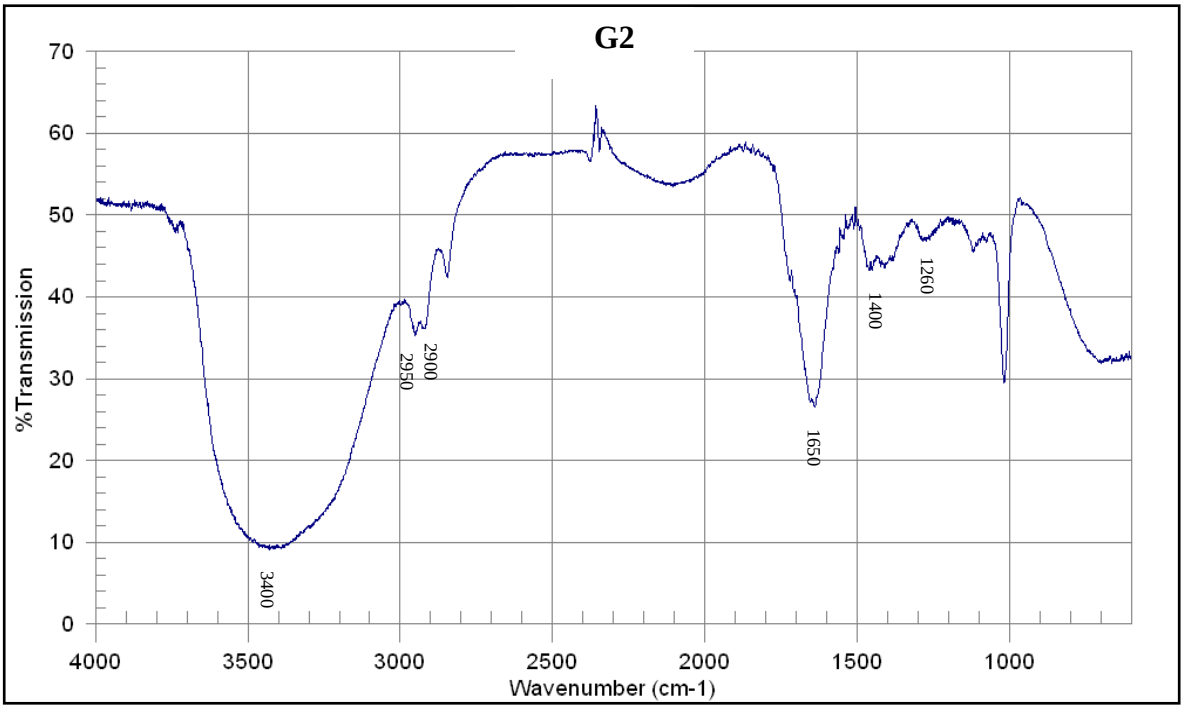


Figure 3. Infra Red spectra of the purified G1 and G2 compounds from *G. applanatum*

Based on GC- Mass and H^1 NMR methods, apparently that the molecular formula of G1 compound is $\mathbf{C_{20}H_{34}O_4}$ (Fig. 4) and its chemical structure is: **[**(19,19a-dihydroxy-2-methyl-2,3,4,5,6,7,8,9,10,11,12,13,14,15 tetradecahydrobenzo[b] [1] oxacycloheptadecin-17(19aH) with a molecular weight 334 Kd. This indicated that G1 compound is more related to Tanin group by comparing its spectroscopic data with available literature (Al-Fatimi *et al.*, 2006; Mothana *et al.*, 2000) (Fig. 5).

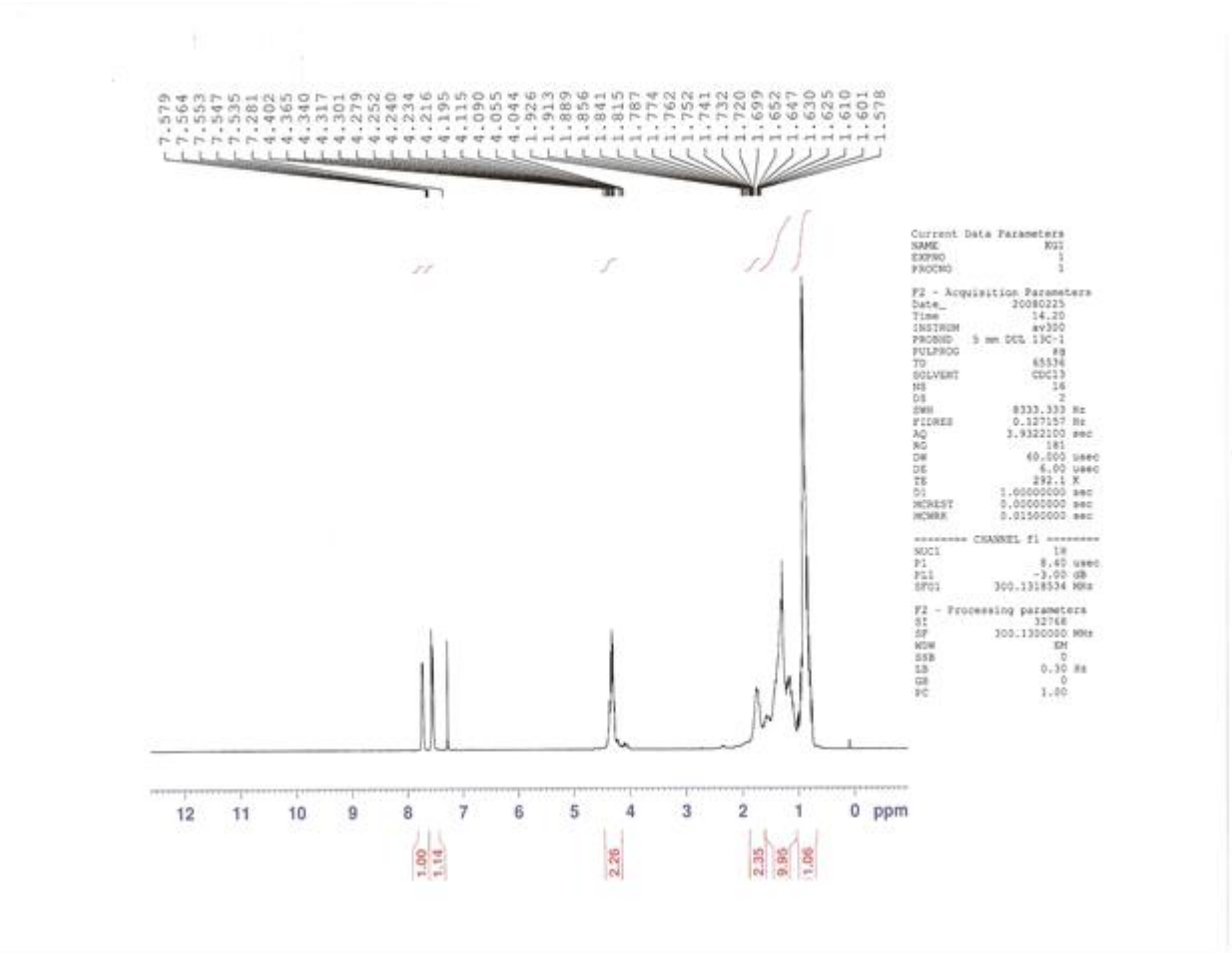


Figure 4. H^1NMR spectra of the purified compound G1 from *G.applanatum*

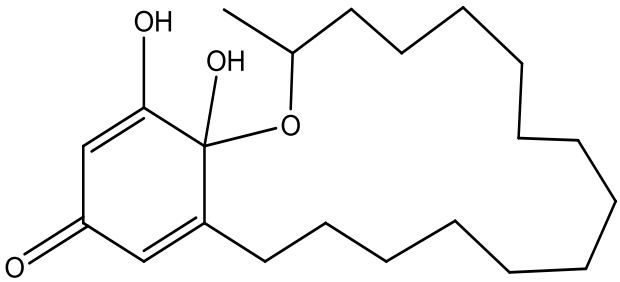


Figure 5. Chemical structure of G1 compound isolated from *G. applanatum.*

While G2 chemical formula is $\mathbf{C_{21}H_{28}O_2}$ (Fig. 6) with a molecular weight 360 kd. Its chemical structure is: **[**(2-(2-(2,5-dihydroxyphenyl) ethylidene)- 11-hydroxy-6,10-dimethylundeca-5,9-dienoic acid and its belongs to Terpenoides group (Fig. 7).

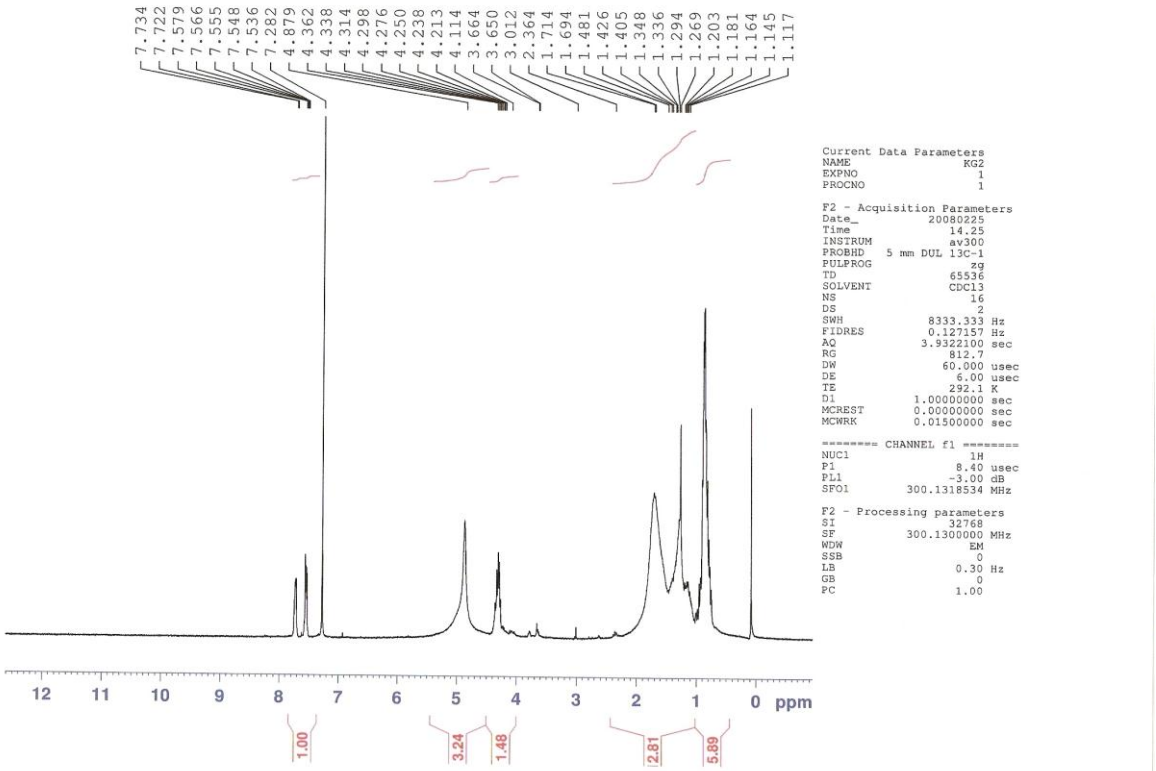


Figure 6. H[1]NMR Spectra of the purified compound G2 from *G. applanatum*

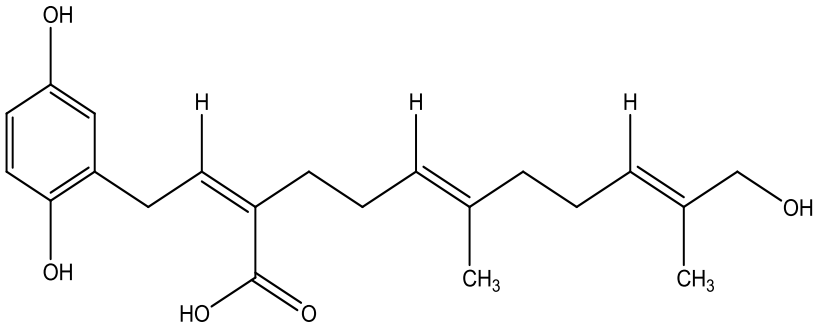


Figure 7. Chemical structure of G2 compound isolated from *G. applanatum*

Both G1 and G2 components did not show any toxicity by using human RBC test. The MIC value of the bioactive compounds G1 and G2 was 6.25 µg/L for both *E. coli* and *S. aureus*. While the two compounds were not active against the selected dermatophytic fungal isolates as they did not show a growth inhibition at any of the concentrations used. A clear zone inhibition of 23 mm diameter was observed for the crude extract against both bacterial strains E. *coli* and *S. aureus.* The inhibition zones diameters, however, exhibited by the purified G1 and G2 compounds were higher reaching to 26 mm and 29 mm for *E. coli* and *S. aureus*, respectively (Fig. 8).

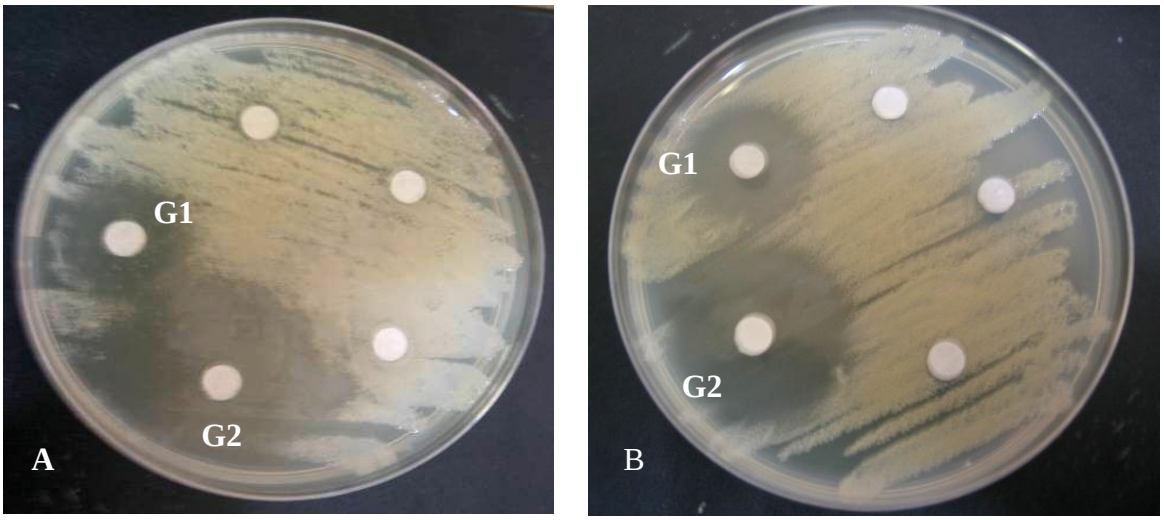


Figure 8. Inhibition zones by the purified G1 and G2 compounds against. *E. coli* (A) and *S. aureus* (B).

4. Discussion

Fungi in general and mushrooms in particular are a good source for antimicrobial products (Janes *et al.,* 2007). A few reports on the bioactive secondary metabolites of *G. applanatum* are available. This basidiomycetes species is not a rare polypore fungus living on trunks of some trees over the world (Phillips, 1983) and in southern Iraq, however, no phytochemical investigation so far has been carried out on mushrooms in this region. Our present study revealed that purified extract of *G. applanatum* exhibited an inhibitory action against both *E. coli* and *S. aureus.* These findings are in concomitant with the previous study (Roberts, 2004) who reported that *G. applanatum* produces steroides compounds which are active against G-negative and G-positive bacteria. The purified G1 compound is more likely to be chemically related to tannin group while G2 compound is more related to terpenoides group based upon their chemical structural verification by using HNMR and GC-mass spectra and agreed with the previous studies (Al-Fatimi *et al.,* 2006; Mothana *et al.,* 2000). It has been stated that ascomycetes and basidiomycetes fungi often produce terpenoides compounds (Anke, 1989). The bioactivity of G1 and G2 against the test bacteria can be attributed to the presence of tannin and terpenoide compounds. Also various chemical compounds have been screened in some species of *Ganoderma* (Bojana *et al.,* 2000). Apparently, the purified G1 and G2 compounds are not effective against the tested dermatophytic fungal isolates since no growth inhibition was observed. These results support the earlier findings (Roberts, 2004; Samania *et al.,* 2001). Although, Smala *et al*. (2003) stated that *G. annulare* produces applanoxidic acid compound which showed a weak activity against the dermatophyte *T. mentagrophyes.* The antibacterial activity of the purified compounds in this study was significantly increased compared with the activity of crude extract. A similar results were reported for some other mushrooms extract (Jonathan and Fasidi, 2003; Jonathan *et al.,* 2008; Olawuyi *et al.,* 2010). Seemingly, the isolated G1 and G2 compounds from *G. applanatum* are a good bioactive agents and promising to be used as an antibacterial. Nevertheless, there is still more fungi need to be examined for their potentiality against bacteria and pathogenic fungi.

Acknowledgments

We are thankful to the authorities of the University of Basrah, College of Education (Iraq) for the financial support for this work. Thanks are due to the staff at the Chemistry Department for providing spectral data analysis.

References

Al-Fatimi MA, Julich WD, Jansen R and Lindequist U. 2006. Bioactive components of the traditionary used mushroom *Podaxis pistillaris. eCAM* . **3**: 87-92.

Anke T. 1989. Basidiomycetes: A source for new bioactive metabolites. *Prog. Ind. Microbiol*. **27**: 51-66.

Anke T, Werle A, Kappe R and Sterner O. 2004. Laschiatrion, a new antifungal agent from a *Favolaschia* species (Basidiomycetes) active against human pathogen. *J. Antibiot*. **57**: 496- 501.

Bojana B, Dolncar D, Berovic M and Ponleven F. 2000. Isolation and quantification of terpenoid acids from *Ganoderma applanatum* of Istrain origin. *Food Technol. Biotech.* **38:** 11-18.

Casals JB. 1979. Tablet sensitivity of pathogenic fungi. *J.Clin. Pathol*. **32**: 719- 722.

Janes D, Kreft S, Jurc M, Seme K and Strukelj B. 2007. Antibacterial activity in higher fungi: Mushrooms and endophytic fungi from Slovenia. *Pharma Biol*. 2007, **45**: 700-7006.

Jonathan SG and Fasidi IO. 2003. Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata. Afric. J.Biomed. Res*. **6**: 85-90.

Jonathan SG, Kigigha ,LT and Ohimain E. 2008.Evaluation of the Inhibitory potentials of eight edible higher Nigerian fungi against pathogenic Microorganisms.*Afric J Biomed Res*, **11**:195-200

Jonathan SG and Awotona FE 2010. Studies on Antimicrobial Potentials of three G*anoderma* species. *Afric J Biomed Res*, **13(2):**119-125

Kettering M, Valdivia C, Sterner O, Anke T and Thines E. 2005. Heptemerones A- G, seven novel diterpenoids from *Coprinus heptemerus*; producing organism, fermentation, isolation and biological activities. *J Antibiot*., **58**: 390-396.

McGinnis M R. 1980). **Laboratory Handbook of Medical Mycology**, Academic Press, New York.

Mothana RA, Jansen R, Julich WD and Lindequist U. 2000. Ganomycin A and B, a new antimicrobial farnesyl hydroquinones from the basidiomycetes *Ganoderma pfeiffer. J. Nat. Produc*. **63**: 416-418.

Olawuyi OJ, Jonathan SG, Aina DA and Baysah GI. 2010. Studies on antibacterial activities of *Fomes lignosus*-A Nigerian mushroom. *Niger J Microbiol*., 24 (1): 2153-2159.

Phillips R. 1983. **Mushrooms and other fungi of Great Britain and Europe**, Pan Roberts LM. 2004. Australian *Ganoderma* identification, growth and antibacterial properties. Ph.D. Thesis, Swinburne University of Technology. Australia.

Smala EF, Monache DF, Smania JA and Cuneo YR. 2003. Antifungal activity of sterol and triterpenes isolated from *Ganoderma annulanane. Fitoterapia.* **74**: 375-377.

Smania A, Monache FD, Logueric C, Smania EF and Gerber AL. 2001. Antimicrobial activity of basidiomycetes. *Int .J. Med. Mush*.**, 3**: 87.

Ulirike L, Timo HJ and Julich WG. 2005. The pharmacological potentials of mushrooms. *eCAM* **2**: 285-299.

Xian-guo H and Ursula M. 1994. Antifungal compound from *Solanum nigrescens*. *J. Enthopharm*., **43:** 173-177.

Zur E. 2001. Isolation, structure determination and biological activity assessment of secondary metabolites from marine derived fungi. Ph.D. Thesis, Von Claudia Osterhage. 186 pp.