Original Research Article

GC/MS ANALYSIS AND IN VITRO EFFECT OF *Ganoderma lucidum* SOLVENT EXTRACTS ON MICROORGANISMS ISOLATED FROM THE ARMPIT, SCALP, AND URINARY TRACT

ABSTRACT

Aims: The study was to assess the antibacterial effect of solvent extracts of *Ganoderma lucidum* on isolates from the human scalp, armpit, and urinary tract.
Study design: Descriptive comparative.
Place and Duration of Study: Bells University of Technology, between December 2019 and October 2020.
Methodology: Test organisms isolated from the scalp, armpit, and urine were identified using conventional methods and Analytical Profile Index kits (API). *Ganoderma lucidum* was rinsed with 2.5% potassium hydroxide and sterile distilled water, dried at 50℃ for 72 h, and pulverized. The mushroom powder (10%) was extracted in methanol, acetone, and petroleum ether at 65^{o}C for 48 h. The filtrate was evaporated and two-fold serial dilutions were prepared in Dimethyl sulfoxide (DMSO). Antibacterial activity was done by the agar well diffusion method. Inoculum adjusted to 0.5 McFarland standard was inoculated onto Mueller Hinton agar. Wells of 6mm were filled with 100µl of the extracts. Antibiotic sensitivity was by disc diffusion method. Plates were incubated at 37^{o}C for 24 h. Gas chromatography-mass spectrophotometer (GC-MC) analysis of methanol extract was carried out.
Results: The isolates were identified as follows; scalp (*Corynebacterium kutscheri* and *Enterobacter intermedius*), armpit (*Acinetobacter baumannii*), and urine (*Pseudomonas aeruginosa*). The solvents exhibited varying degrees of inhibition on test organisms. The methanol extract was most inhibitory on all organisms. Petroleum ether showed the least inhibition. *P. aeruginosa* was most resistant to the extracts. Fluoroquinolones and aminoglycoside inhibited all the organisms and recorded a higher zone of inhibition unlike the beta-lactams. GC/MS of methanol extract revealed the presence of 48 compounds amongst them were sugar, alcohols, nitrates, alkaloids, amines, fatty acids, methyl esters, and steroids**.** Also found was Phenol, 2-methoxy butylated hydroxyl anisole (BHA) a**n** antioxidant.
Conclusion: *Ganoderma lucidum* contained bioactive compounds that are antimicrobial and showed utility for use in the medical-pharmaceutical industry.

Keywords: Acetone, Antibacterial, Antibiotics, Ganoderma lucidum, Gas Chromatography-Mass Spectrometry, Methanol, Petroleum ether

1. INTRODUCTION

The human body is a habitat for large numbers of microorganisms that live in various components of the body. The physiological factors of temperature, moisture, presence of certain nutrients, and inhibitory substances make the organism well adapted at these sites [1]. The scalp, armpit, and urinary tract seem to be a conducive environment for the growth of these organisms due to moisture, poor hygiene, and the accumulated number of dead cells which serve as nutrients. Microbes of the normal resident flora are harmless and beneficial in their normal location in the host as they prevent the colonization of other pathogens through bacterial interference. In the absence of coincident abnormalities, they are adapted to a non-invasive mode of life defined by the limitations of the environment [2]. However, some of these resident microbes may produce disease if introduced into foreign locations in large numbers and if predisposing factors are present.

Human-related infections are associated with different pathological conditions. They can cause skin diseases and enter the blood system creating life-threatening diseases, particularly in immunosuppressed people [3]. Antimicrobial drugs have long been used for prophylactic purposes and treatment of such human diseases, but microbes have developed resistance to some of these antimicrobial drugs thereby creating serious treatment problems. The number of multidrug-resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics is continuously increasing [4] due to misuse and overuse of available antibiotics [5] such that the world is facing significant challenges in modern healthcare delivery due to the inefficiency of antimicrobial agents in fighting infections. Therefore, there is a growing need for a search for new and effective antimicrobial substances, especially from natural sources.

Mushrooms are known to contain many bioactive substances like terpenoids, flavonoids, tannins, alkaloids, and polysaccharides and have been used for medicinal purposes since medieval times [6]. The use of mushrooms as a source of biologically active compounds is gaining recognition in recent years. *Ganoderma* is a medicinal mushroom that has a long history of use in traditional medicine and has been reported to possess several bioactive metabolites like glucan and ganoderic acid [7, 8]. Many

published studies that are based on animal, cell culture models, and in vitro assessment of the health effects of *G. lucidum* have corroborated this claim, and there are also some reports of human trials in the field [9, 10, 11]. Extracts of *G. lucidum* had been used in herbal mixtures as an alternative treatment for androgen-dependent and -independent prostate cancer, recurrent oral ulcers, and lung cancer. The treatment showed that the cellular immunity of the patients was significantly enhanced and treatment mediated positive impact on chronic inflammation and oxidative stress [12]. Data from different studies suggested that *G. lucidum* intake helps in modulating blood glucose levels [13]. *G. lucidum* has many health and nutraceutical benefits. It is used for the treatment of bronchitis, allergies, hepatitis, immunological disorders, and cancers [8]. It is also used in syrups, injections, tablets, green teas, coffees, and hot cocoas. The most important bioactive compounds are triterpenoids and polysaccharides which have been reported to possess hepatoprotective, antihypertensive, hypocholesterolemic, antihistaminic, antioxidant, antitumor, and immunomodulatory activities [8] Also, antimicrobial properties of *Ganoderma* mushroom have been reported [14] in literature. Various peptidoglycans and triterpenes with antioxidant and antimicrobial activity had been isolated from *G lucidum* and they include proteoglycan (with antiviral activity) and immunomodulating substance (GLIS) which confer on its various health benefits. Therefore, the study was to assess the antimicrobial activity of methanol, acetone, and petroleum ether extracts of *Ganoderma lucidum* mushroom against microorganisms isolated from the armpit, the scalp, and the urinary tract.

2. MATERIALS AND METHODS

2.1 Materials

This is a descriptive comparative study of the effect of solvent extracts of *Ganoderma lucidum* on human pathogens. The ethics committee was not required. *Ganoderma* mushroom was collected from the wild within the compound of the Bells University of Technology, Ota, Nigeria, and identified by the Mycology Unit of Yaba College of Technology, Lagos Nigeria, as *Ganoderma lucidum*.

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2.2 Isolation and identification of organisms from the scalp, armpit, and urine

The test organisms were isolated from the scalp, armpit, and urine of volunteers according to [15]. A sterile swab stick moistened with sterile distilled water was used to swab the scalp and armpit respectively while 100 µl urine was collected and inoculated onto MacConkey agar, blood agar, and nutrient agar. All inoculations were done in triplicates and incubated for 24 h at 37^{o}C. The typical colonies were aseptically picked and sub-cultured. Identification was based on colonial morphology, gram stain, and biochemical tests. For all the isolations institutional guidelines for biosafety level were observed and, in most cases, SONO disinfecting wipes were employed. Further identification was carried out with the Analysis Profile Index kits (API 20E V4.0, API 20NE V6.0, and API CORYNE V2.0) standardized respectively for identification of non-fastidious gram-negative Enterobacteriaceae, non-fastidious, non-enteric gram-negative rods, and Coryneform bacteria. The kits used miniaturized biochemical tests that generated a numerical profile inputted in a database (API identification software) to identify isolated organisms.

2.3 Preparation of *Ganoderma lucidum* solvent extract

The solvent extraction process for the antibacterial study was done using methanol, petroleum ether, and acetone as solvents according to [16]. Fresh fruiting bodies of *Ganoderma lucidum* were properly rinsed with 2.5% potassium hydroxide and sterile distilled water, cut into smaller pieces, and oven-dried at 50℃ for 72 h. The dried mushrooms were pulverized in a blender and 10% dried powder was stirred into the respective solvent and placed on an orbital shaker at 50^{o}C for 48 h. The mixtures were filtered with Whatman's filter paper (No.1) and the filtrate was evaporated in a water bath. Serial two-fold dilutions (100, 50, 25, 12.5 mg/mL) of the extracts were prepared in Dimethyl sulfoxide (DMSO).

2.4 Screening for antibacterial activity

Screening for antibacterial activity was done using the agar well diffusion method. A 24 h old test organism on nutrient agar was transferred to normal saline and turbidity adjusted to 0.5 McFarland standard. The culture was aseptically inoculated onto Mueller Hinton agar. Six wells were bored with a 6mm sterile cork borer and filled with 100µl of 12.5%, 25%, 50%, and 100% reconstituted extract in DMSO. The solvent and the DMSO served as positive and negative control respectively. Plates were incubated at $37^0C \pm 2^0C$ for 24 h in the incubation chamber. The growth inhibition zone was determined by measuring the zones of inhibition in mm.

2.5 Antibiotic sensitivity of test organisms

Disc diffusion method according to CLSI guidelines was employed. Test organisms' turbidity conforming to 0.5 MacFarland standard were sub-cultured onto Mueller-Hinton agar plates. The antibiotics discs were aseptically embedded onto the agar plates and incubated for 24 h at 37^oC. The antibiotics used for the test were: Ofloxacin, Augmentin, Nitrofurantoin, Ciprofloxacin, Ceftazidime, Cefuroxime, Gentamicin, Cefixime.

2.6 Gas chromatography- Mass Spectrophotometer of methanol extract of *Ganoderma lucidum*

The GC-MC analysis was carried out according to [17]. Dried mushroom powder (10%) in deionized water was prepared, gently mixed, and sonicated in an ultrasonic cleaner at 40 ^oC for 40 mins. The sonicated aqueous extract was centrifuged at 4000 rpm for 10 mins and filtered through Whatman No. 1 filter paper. A gas chromatograph-mass spectrometer (GC–MS) performed at a 1:1 injection ratio (total volume 4 ml) was used to determine the phytochemicals present in the methanolic solution of the aqueous mushroom extract. The phytochemicals were further identified using software with a database incorporated into the GC–MS.

3. RESULTS AND DISCUSSION

3.1 Identification of organisms isolated from the scalp, armpit, and urine of volunteers

The morphological parameters and biochemical tests of the isolates from the scalp, armpit, and urine are shown in Table 1. The analytical profile index further confirmed the identity of the microorganisms from the scalp, armpit, and urine as *Acinetobacter baumannii*, *Corynebacterium kutscheri,* *Enterobacter intermedius*, and *Pseudomonas aeruginosa* respectively. Earlier workers had reported these organisms are human pathogens; *Acinetobacter baummanii* was detected in the human body and head lice in Ethiopia [18]. *Corynebacterium kutscheri* was reported in the skin and soft tissue following rat-bite [19]. *Pseudomonas aeruginosa,* an opportunistic human uropathogenic organism, was incriminated in severe urinary tract infections (UTIs) [20, 21] while Enterobacter was associated with nosocomial infections was present on human skin surfaces of patients. These reports corroborated the presence of these organisms in the human body as pathogens.

Table 1: Morphological and biochemical identification of isolates from the scalp, armpit and urine of volunteers

Tests	Results	Results	Tests	Results	Tests	Results

Gram's reaction	-	-	Gram's Reaction	+	Gram's Reaction	-
ONPG	-	+	NIT	+	NO_3	+
ADH	-	-	PYZ	+	TRP	-
LDC	-	-	PyrA	+	GLU	-
ODC	-	+	PAL	-	ADH	+
CIT	+	-	βGUR	-	URE	-
H_2S	-	-	βGAL	-	ESC	-
URE	-	-	αGLU	+	GEL	+
TDA	-	-	βNAG	-	PNG	-
IND	-	-	ESC	+	GLU	+
VP	-	-	URE	+	ARA	-
GEL	-	-	GEL	-	MNE	-
GLU	-	+	O	+	MAN	+
MAN	-	+	GLU	+	NAG	+
INO	-	+	RIB	+	MAL	-
SOR	-	+	XYL	-	GNT	+
RHA	-	+	MAN	-	CAP	+
SAC	-	+	MAL	+	ADI	+
MEL	+	+	LAC	-	MLT	+
AMY	-	+	SAC	+	CIT	+
ARA	+	+	GLYG	-	PAC	-
OX	-	-	CAT	+	OX	+
NO_2	-	+				
N_2	-	-				
MOB	-	+				
M_eC	+	+				
OF-O	+	+				
OF-F	-	+				
Probable Organism	*Acinetobacter Baumannii*	*Enterobacter intermedius*		*Corynebacterium kutscheri*		*Pseudomonas aeruginosa*
% Identity	99.9%	92.1%		99.9%		99.5%

Legend: ONPG- Ortho-Nitrophenyl-β-galactoside, **ADH**- Arginine DiHydrolase, **LDC**- Lysine Decarboxy, **ODC**- Ornithine DeCarboxylase, **CIT-** Citrate, **H_2S**- Hydrogen Sulphide Production, **URE**- Urease, **TDA**- Tryptophane DeAminase, **IND**- INDole Production, **VP**- Voges Proskauer, **GEL**- Gelatinase, **GLU**- D-Glucose, **MAN**- D-Mannitol, **INO**- Inositol, **SOR**- D-Sorbitol, **RHA**- L-Rhamnose, **SAC**- Saccharose (D-Sucrose), **MEL**- D-Melibiose, **AMY**- Amygdalin, **ARA**- L-Arabinose, **OX**- Cytochrome-Oxidase, **NO_2-** Nitrogen dioxide, **N_2**- Nitrogen, **MOB**- Motility, **M_cC-** MacConkey medium, **OF-O**- Fermentation- under mineral oil, **OF-F-** Oxidation- exposed to the air. **NIT**- Potassium nitrate, **PYZ**- Pyrazinamidase, **PyrA**- pyrrolidinyl/ Arylamidase, **PAL**- Alkaline phosphatase, **βGUR**- β-Glucuronidase, **βGAL-** β-Galactosidase, **αGLU-** **α**-Glucosidase, **βNAG**- N-Acetyl-β-Glucosamidase, **ESC-** Esculin, **O-** Negative control, **RIB-** Ribose, **XYL-** Xylose, **LAC-** Lactose, **GLYG-** Glycogen, **CAT-** Catalase. A, **NO_3-** Potassium nitrate, **TRP-** Tryptophan **PNPG-** Para-Nitrophenyl-βD-Galactopyramosidase, **MNE-** D-Mannose, **NAG-** N-acetyl/ glucosamine, **GNT**- Potassium gluconate, **CAP**- Capric-acid, **AD-** Adipic acid, **MLT**- Malic acid, **PAC**- Phenylacetic acid.

3.2 Antibacterial potential of methanol, acetone, and petroleum ether extracts of *Ganoderma lucidum*

The solvent extract of *Ganoderma lucidum* inhibited the growth of the isolated microorganisms, each exhibiting varying zones of inhibition. Methanol extract inhibited the growth of all the isolated microorganisms, *Acinetobacter buamannii*, *Corynebacterium kutscheri, Enterobacter intermedius*, and *Pseudomonas aeruginosa* at different concentrations (Fig.1).

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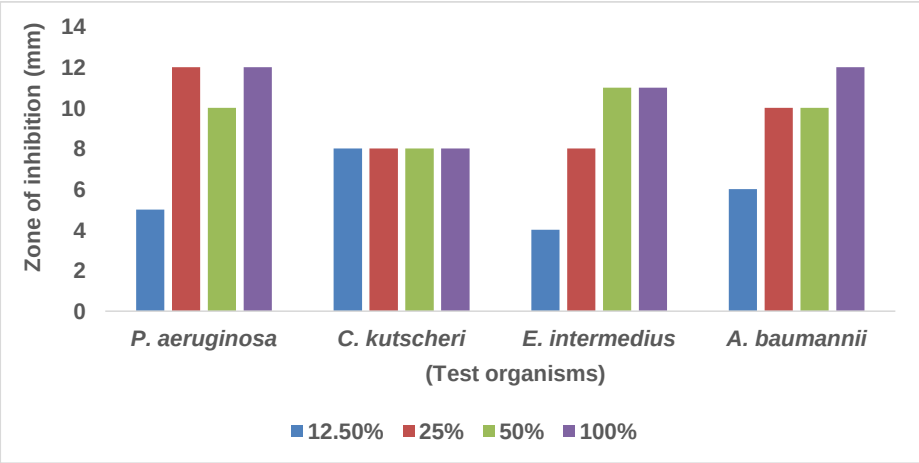

Fig. 1. Antibacterial effect of methanol extract of *Ganoderma lucidum* on *Pseudomonas aeruginosa, Corynebacterium Kutscheri, Enterobacter intermedius,* and *Acinetobacter baumannii*

Acetone extract of *G. lucidum* inhibited *Enterobacter intermedius, Corynebacterium kutscheri, and Acinetobacter baummanii* except *Pseudomonas aeruginosa* Fig. 2).

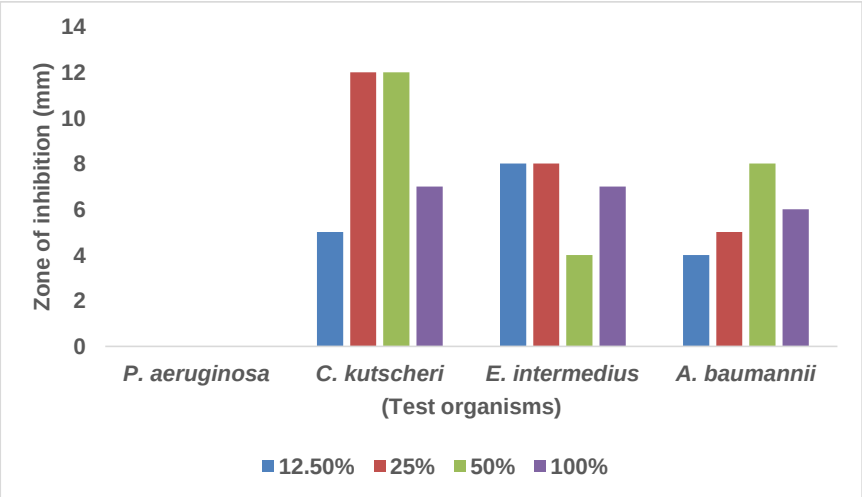

Fig. 2. Antibacterial effect of acetone extract of *Ganoderma lucidum* on *Pseudomonas aeruginosa, Corynebacterium Kutscheri*, *Enterobacter intermedius*, and *Acinetobacter baumannii*

Petroleum ether was the least sensitive as it only inhibited *Corynebacterium kutscheri* and *Enterobacter intermedius, while Pseudomonas aeruginosa* and *Acinetobacter baumannii* were not inhibited (Figure 3). *Pseudomonas aeruginosa* from the urine was the most resistant organism as it showed no inhibition by acetone and petroleum ether extracts of *Ganoderma lucidum* (Figures 2 and 3).

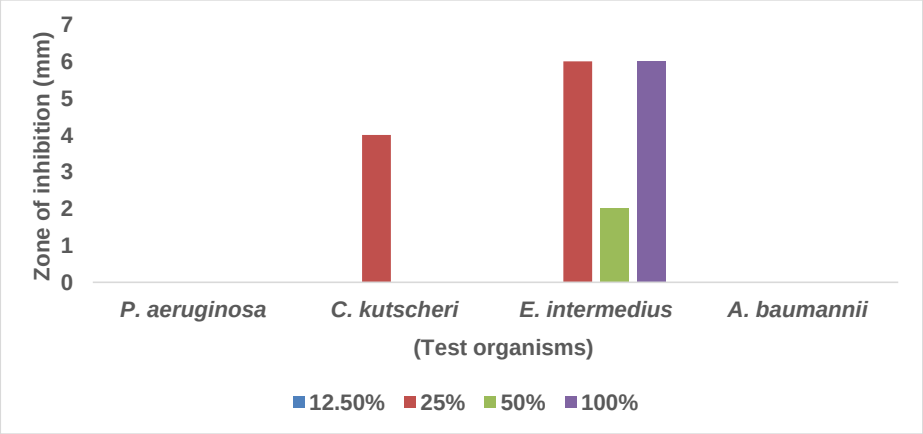

Fig. 3. Antibacterial effect of Petroleum ether extract of *Ganoderma lucidum* on *Pseudomonas aeruginosa, Corynebacterium Kutscheri, Enterobacter intermedius,* and *Acinetobacter baumannii*

P. aeruginosa was the most resistant of all the organisms tested being only sensitive to methanolic extract. *P. aeruginosa* had been known as an opportunistic pathogen with remarkable multidrug-resistant ability. Its drug resistance mechanism has been characterized and was attributed to biofilm–mediated resistance and the formation of multidrug tolerant persister cells [22]. Petroleum ether extracts exhibited scarcely very low zones of inhibition on *Corynebacterium kutscheri* and *Enterobacter intermedius*. This could be attributed to the low polarity index of petroleum ether which is 0.1 as against 5.1 for methanol. Polarity plays important role in solubility as it is a measure of the degree of interaction of solvents with various polar test solutes. The higher the polarity the higher the extractability. Hence, the low polarity index of petroleum ether could have contributed to the lower dissolubility of bioactive substances. Methanol with a high polarity index recorded high inhibitory activity. This could mean that petroleum ether may not be a good solvent for the

extraction of bioactive components of *Ganoderma lucidum.* In addition, methanol has a shorter chain length, and such solvents have a higher solubility than longer chains. Therefore, methanol with a higher solubility ratio and improved extraction property extracted more bioactive compounds. The antibacterial activity of *Ganoderma lucidum* extracts agrees with [20]. Other reports have confirmed the high content of bioactive compounds in *Ganoderma lucidum* which made it suitable for medicinal purposes [23]

3.3 Antibiotic effect of standard antibiotics on test organisms

The result of the effect of standard antibiotics on test organisms showed that gentamicin, an aminoglycoside, ofloxacin (OFL) and ciprofloxacin (CPR), fluoroquinolones inhibited all the test organisms (Figure 4).

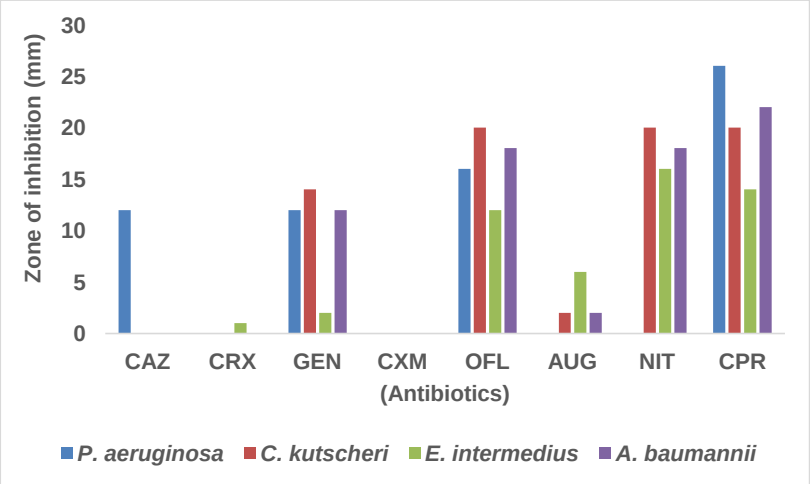

Fig. 4. Antibiotic sensitivity of *Pseudomonas aeruginosa, Corynebacterium Kutscheri, Enterobacter intermedius* and *Acinetobacter baumannii*

Antibiotics: CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime,

OFL- Ofloxacin, AUG- Augmentin, NIT- Nitrofurantoin, CPR- Ciprofloxacin.

Gentamicin (GEN) inhibits protein synthesis in bacteria by binding irreversibly to the 30s ribosomal subunit while Ofloxacin (OFL) and Ciprofloxacin (CPR), fluoroquinolones inhibit enzymes involved in bacteria DNA synthesis. Nitrofurantoin and Augmentin inhibited three organisms except for *Pseudomonas aeruginosa*. Nitrofurantoin is a sulphonamide that acts as a competitive inhibitor of enzymes involved in the growth and multiplication of bacteria. Augmentin is made of amoxicillin (Beta-lactam antibiotic) and clavulanic acid (Beta-lactamase inhibitor). The incorporation of clavulanic acid counters Beta-lactamase enzymes and also serves as an antagonist so that amoxicillin is not affected by Beta-lactamase enzymes [24]. However, cefixime (CXM), cefuroxime (CRX), and Ceftazidime (CAZ), Beta-lactam antibiotics, had little or no effect on test organisms. Beta-lactam antibiotics act by inhibiting the synthesis of the peptidoglycan layer of the bacterial cell walls. But most bacteria often develop resistance to beta-lactam antibiotics by synthesizing a beta-lactamase, an enzyme that attacks the beta-lactam ring. In order to overcome this resistance, beta-lactam antibiotics are given with beta-lactamase inhibitors like clavulanic acid or boronic lactamase inhibitors as mentioned with Augmentin. This could explain the inhibition of the three organisms by augmentin. However, Pseudomonas aeruginosa was most sensitive to CIP (26mm) and OFL (16mm) while Corynebacterium kutscheri was most sensitive to CPR (20mm), OFL (20mm), and NIT (20mm).

3.4 Gas chromatography-Mass Spectrophotometer of methanol extract of *Ganoderma lucidum*

The GC/MS analysis of methanol extract of *G. lucidum* revealed the presence of 48 organic compounds mostly sugar, sugar alcohol, alcohols, esters, alkaloids, amines, unsaturated fatty acids, steroids, sulfur compounds, and phenol (Figure 5). The most abundant compound in the methanol extract was arabinitol (29.66%, peak 30), a low-calorie sweetener

used in diet and health-oriented foods (Table 2). Other compounds detected were phenol 2-methoxy (3.36%, peak 4), known as Butylated hydroxyl anisole (BHA), a known antioxidant used as a preservative in food, packaging animal feed, and cosmetics. Antioxidants prevent oxidative damage related to aging and diseases.

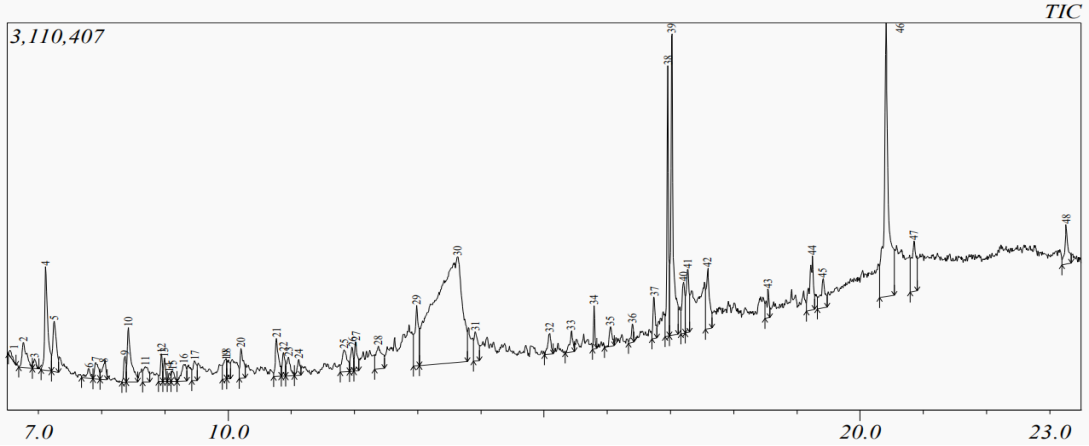

Fig. 5. GC/MS analysis of a methanol extract of Ganoderma

Table 2. GC-MS of the methanol extract of *Ganoderma lucidum* shows the phytochemicals present and their corresponding retention time and peak area

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Peak	Retention time (min)	Peak area (%)	Name of compound
1	6.551	0.17	(Z)-1-Chloro-2-(methylbulfonyl)ethylene
2	6.762	1.24	(Z)-1-Chloro-2-(methylbulfonyl)ethylene
3	6.933	0.26	(Z)-1-Chloro-2-(methylbulfonyl)ethylene
4	7.111	3.36	Phenyl, 2-methoxy-
5	7.250	1.88	1H-Azepin-1-amine, hexahydro-
6	7.794	0.39	(1-bromo-ethanesulfunyl)-thane
7	7.900	0.73	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
8	8.037	0.67	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-

9	8.364	0.68	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
10	8.423	2.09	Isosorbide
11	8.689	0.78	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
12	8.945	0.58	Lup-20(29)-ene-3,21,28-triol, 28-acetate, (3-
13	8.992	0.47	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
14	9.065	0.20	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
15	9.119	0.34	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
16	9.298	0.91	2R,3S-1-{[1,3,4-Trihydroxy-2-butoxymethyl
17	9.467	0.75	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
18	9.965	0.67	2-Deoxy-D-galactose
19	9.980	0.56	2-Deoxy-D-galactose
20	10.202	0.97	1,3,5-Triazine-2,4-diamine, N,N-bis(1-methyl-
21	10.766	1.31	1,2-Epoxynonane
22	10.873	0.63	Trans-2,3-Epoxyoctane
23	10.950	0.73	Trans-2,3-Epoxyoctane
24	11.115	0.45	Acetic acid, chloro, decyl ester
25	11.834	1.13	Trans-2,3-Epoxynonane
26	11.962	0.71	[1,1′-Bicyclopropyp]-2-octanoic acid
27	12.018	0.78	Pyridine, 1,2,3,6-tetrahydro-1,2-dimethyl-
28	12.374	1.50	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
29	12.984	2.18	Isosorbide Dinitrate
30	13.632	29.66	D-Arabinol
31	13.911	1.15	n-Propylnonyl ether
32	15.088	0.69	[1,1′-Bicyclopropyp]-2-octanoic acid
33	15.431	0.68	[1,1′-Bicyclopropyp]-2-octanoic acid
34	15.793	0.55	Cyclopentanetridecanoic acid, methyl ester
35	16.052	0.59	Cis-Z-alpha-Bisabolene epoxide
36	16.395	0.44	Cis-Z-alpha-Bisabolene epoxide
37	16.737	0.98	Cis-Z-alpha-Bisabolene epoxide
38	16.958	4.26	9,12-Octadecadienoic acid, methyl ester
39	17.021	7.36	6- Octadecenoic acid, methyl ester, (Z)-
40	17.207	1.53	Cyclopropaneoctanoic acid, 2-{[2-[(2-ethyl
41	17.271	1.85	Z,Z-3,13-Octadecedien-1-ol
42	17.589	2.25	Ergosta-7,22-dien-2-ol, (3.beta,22E)

43	18.545	0.83	Z-8-Pentaecen-1-ol acetate
44	19.247	2.27	13-Oxabicyclo[9.3.1]pentadecane, 15-chloro-
45	19.414	1.42	i-Propyl 9-tetradecanoate
46	20.408	12.41	13-Oxabicyclo[9.3.1]pentadecane, 15-chloro-
47	20.853	2.51	1,2-Bis(trimethylsilyl)benzene
48	23.260	1.40	1,2-Bis(trimethylsilyl)benzene
		100	

Nitrogen-containing compounds like 1H-azipin-1-amine, hexahydroxy-(1.88%, peak 5), Isosorbide (2.09%, peak 10), Isosorbide dinitrate (2.18%, peak 29), and their derivatives were detected and the compounds can be used to nature protein hence have antimicrobial potential. Lup-ol (0.58%, peak 12) a triterpene with antiprotozoal, antimicrobial, anti-inflammatory, antitumor, and chemo-preventive properties was identified. Fatty acids found include methyl ester of linoleic acid (4.26%, peak 38) and oleic acid (7.36%, peak 39). Monounsaturated and polyunsaturated fats are known to lower the risk of heart diseases and stroke, lower bad low-density lipoprotein (LDL) cholesterol levels while increasing good high-density lipoprotein (HDL). Fatty acids are also reported to influence other disease conditions such as type 2 diabetes, inflammatory disease, and cancer [29]. 3,13-Octadecedien-1-ol (1.85%, peak 41) is fatty alcohol produced from the hydrogenation of fatty acid methyl ester. Alcohol is a major contributor to odor in mushrooms. Other identified compounds include: epoxynonane (1.31%, peaks 21 and 25), trans-2,3-epoxyoctane (2.49%, peaks 22, 23 and 25). These are three-membered oxygen heterocycles known as oxiranes with a pleasant odor. Ergosta 7, 22-dien-3-one (2.25%, peak 42) is a steroid that shows pro-inflammatory properties. Inflammation is an essential component of the host response to infection or injury. However, excess chronic inflammation is responsible for chronic diseases like cardiovascular disease. Studies showed that natural substances and medicinal plants due to their active ingredients and medicinal and antioxidant compounds have beneficial effects on human health and have a therapeutic effect on various organs of the body and various diseases (26-30). Using 80% methanol, 39 compounds, mainly alkenes and fatty acids were

detected by [5]. The differences in the number of extracted products could be due to the solvent concentration and polarity.

4. CONCLUSION

The study has shown the biopharmaceutical potential of *Ganoderma lucidum* for use in medicine as the solvent extract contained bioactive compounds that had in-vitro antimicrobial activity against human pathogens. The methanol extract exhibited the highest level of inhibition while petroleum ether showed the least and *Pseudomonas aeruginosa* was the most resistant compared to other organisms. Fluoroquinolones (ofloxacin and ciprofloxacin) and aminoglycoside (gentamicin) antibiotics were most inhibitory compared to beta-lactams antibiotics.

CONSENT (WHERE EVER APPLICABLE)

All authors declare that informed consent was obtained from the volunteers before samples were taken and the volunteers wished to remain anonymous.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

THIS IS NOT APPLICABLE

REFERENCES

Comment [DGM5]: They didnt make refernce accroding to journal format, they wrote just carelessely

1. Callewaert C, Kerckhof FM, Granitsiotis MS, Van Gele M, Van de Wiele T, Boon N. PloS one. 2013;8:e70538. (Doi Link: https://doi.org/10.1371/journal.pone.0070538).

2. Brooks GF, Karen C, Butel J, Morse S. Normal microbial flora of the human body. 24th edition Lange Medical/McGraw-Hill Medical 2004, (Doi Link: http://doi.org/10.1036/0071476660).

3. Cogen AL, Nizet V, Gallo RL. Brit J Derma. 2008;158:442 (Doi Link: https://doi.org/10.1111/j.1365-2133.2008.08437.x)

4. Davies J, Davies D. Micro Mol Biol Rev. 2010;74: 417 (Doi Link: https://doi.org/10.1128/MMBR.00016-10)

5. Elkhateeb WA, Daba GM, Sheir D, El-Dein AN, Fayad W, Elmahdy EM, et al. Egypt Pharma J. 2019;18:102 (Doi Link: 10.4103/ep.epj_17_19)

6. Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Evid Based Compl Alt Medi. 2019;2019. (Doi Link: https://doi.org/10.1155/2019/6212673)

7. Tang W, Liu JW, Zhao WM, Wei DZ, Zhong. J Life Science. 2006;23:80: 205 (Doi Link: https://doi.org/10.1016/j.lfs.2006.09.001)

8. Hsu H, Hua K, Lin C, Lin C, Hsu J, Wong C. J Immunol. 2004;173: 5989 (Doi Link: (https://doi.org/10.4049/jimmunol.173.10.5989)

9. Ofodile L, Ofodile N, Ogbe A, Olufunke O. Inter J Biol. 2011;3:111. (Doi Link: https://doi.org/10.5539/ijb.v3n2p111)

10. Gao YH, Zhou ST, Chen GL, Ye XH, Gao H. Inter J Med Mush. 2002;4:323. (Doi: Link: https://doi.org/10.1615/IntJMedMushr.v4.i4.50)

11. Gao Y, Tang W, Dai X. J Med Food. 2005;8:159. (Doi Link: http://doi.org/10.1089/jmf.2005.8.159)

12. Wachtel-Galor S, Yeun J, Buswell JA. Herbal Medicine: Biomolecular and clinical **a**spects. 2nd ed. Taylor and Francis Group, LLC; 2011.

13. Nahata A. *Ganoderma lucidum*: A potent medicinal mushroom with numerous health benefits. Pharma Anal Acta. 2013;4(159). Doi: 10.4172/2153-2435.1000e159

14. Karwa AS, Rai MK. Int Med Mushrooms. 2012;14:481 (Doi Link: https://doi.org/10.1615/IntJMedMushr.v14,i15.60.

15. Christie S, Syahril R, Lubis SR, Ariyati Yosi A, Dian D, Wahyuni DD. Int J Sci Res. 2020; 10:569 (Doi link: https://doi.org/10.29322/IJSRP.10.01.2020.p9785)

16. Ambika J, Suresh N, Elavarise A, Kalaiselvam M. World J Pharma Res. 2017;6:884. (Doi Link: https://doi.org/10.20959/wjpr201716-10247)

17. Essien RE, Atasie VN, Udobang E, Umanu G. J Nano Chem. 2019;9:259 (Doi: https://doi.org/10.1007/s40097-019-00316-x.)

18. Kempf A, Abdissa G, Diatta JF, Trape E, Angelakis O, Mediannikov. Int J Infect Dis 2012;16:680 (Doi: Link: https://doi.org/10.1016/j.ijid.2012.05.1024)

19. Natasha EH, Korman TM. J Clin Microbiol. 2007:45:3468 (Doi Link: https://doi.org/10.1128/JCM.00607-07)

20. Narten M, Rosin N, Schobert M, Tielen P. Curr Microbiol. 2012:64:7 (Doi link: https://doi.org/10.1007/s00284-011-0026-y).

21. Zhang X, Niu S, Zhang L. Urol Inter. 2014:93:464. (Doi Link: https://doi.org/10.1159/000358493).

22. Zheng P, Renee R, Bernard RG, Tong-Jun L, Zhenyu C. Biotech Adv. 2019:37:177. (Doi Link: https://doi.org/10.1016/j.biotechadv.2018.11.013)

23. Wachtel-Galor S, Tomlinson B, Benzie IF. Br J Nutr. 2004:91:263 (Doi Link: https://doi.org/10.1079/BJN20041039)

24. Leonard DA, Bonomo RA, Powers RA. Acct Chem Res. 2013;46:2407. (Doi Link: https://doi.org/10.1021/ar300327a)

25. Manouchehri A, Shakib P, Biglaryan F, Nazer M, Darvishi M. Uludag Aricilik Dergisi. 2021:21:91 (Doi Link: https://doi.org/10.31467/uluaricilik.887370)

26. Zhang Y, Mahdavi B, Mohammad Hosseini M, Rezaei-Seresht E, Paydarfard S, Qorbani M. et al. Arab J Chem. 2021:14:103105. (DOI Link: https://doi.org/10.1016/J.Arabjc.2021.103105)

27. Sedighi M, Sewell RDE, Nazari A, Abbaszadeh S, Cheraghi M, Amini A. Curr Pharma Des. 2019:25:352. (Doi Link: https://doi.org/10.2174/1381612825666190329144016)

28. Hussain T, Tan B, Yin Y, Blachier F, Tossou Myriene CB, Rahu N. Oxid Med Cell Longev. 2016:2016:9 Doi Link: https://doi.org/10.1155/2016/7432797

29. Solati K, Karimi M, Rafieian-Kopaei M, Abbasi N, Abbaszadeh S, Bahmani M. Mini-Rev Med Chem. 2021:21:500 (Doi Link: https://:doi.org/10.2174/1389557520666201119122608)

30. Abbasi N, Khalighi Z, Eftekhari Z, Bahmani M. Adv Anim Vet Sci. 2020;8:647 (Doi Link: http://dx.doi.org/10.17582/journal.aavs/2020/8.6.647.652)