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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Phytochemical and Antimicrobial Properties of *Ganoderma Lucidum* using Different Extracts

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ABSTRACT
To determine the various bioactive components and secondary metabolites found in Ganoderma lucidum extract, researchers employed the Maceration extraction method (which works based on the degree of polarity of the solvents used). Following their identification through phytochemical analysis, the compounds were tested for antifungal and antibacterial activity against bacteria E. coli and B. subtilis, as well as fungus C. albicans. The antibacterial activity of polar extracts was higher than that of other extracts, with a larger zone of inhibition and the lowest minimum inhibitory concentration against microorganisms.
KEYWORDS: *Phytochemical analysis, Anti-fungal property, Antibacterial property, G. lucidum*

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INTRODUCTION

Bioactive compounds are those that can regulate specific metabolic functions, resulting in beneficial benefits, and are isolated from plant-based foods, as well as other edible substances or by-products. Secondary metabolites discovered in modest amounts in many plants are known as bioactive substances [1]. (a) Terpenes and terpenoids (about 25,000 varieties), (b) alkaloids (roughly 12,000 types), and (c) phenolic compounds are the three major classes of bioactive substances (about 8000 types) [2] Immunomodulating, anti-inflammatory, anti-tumor, chemo- and radio preventive, enzyme inhibitory, and mitogenic activities have all been described for *G. lucidum*. There were also reports of effects on effector cells such as macrophages, natural killer cells, and mast cells. Antibacterial, antiviral, and antifungal activities were also discovered [3]. Control of blood glucose levels, immune system regulation, hepatoprotection, bacteriostasis, and other applications have been attributed to the health advantages of lingzhi [4]. Direct cytotoxicity and anti-angiogenesis were also discovered in *In vitro* tests, indicating that it has anti-cancer characteristics [5].

Antimicrobial activity refers to the ability of any element or molecule to inhibit the growth of microorganisms (most commonly bacteria). Fungi, viruses) or kills, deactivates, or disrupts their dangerous metabolic activities. The antimicrobial activity of plants or microbial extracts is commonly assessed using the agar well method [6]. Bacteria, viruses, and fungi all play a role in human health and disease epidemiology. They cause dangerous sickness, symptoms, or side effects, and they may grow resistant to current antimicrobial medicines after a period of time.

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and non-albicans species remaining the most common pathogens, with an increasing incidence of clinical and/or microbiological resistance to numerous antifungal medications [6]. The goal of this study is to conduct in vitro tests to evaluate the antibacterial and antifungal activities of several extracts of commercially grown and harvested *Ganoderma lucidum* at various doses. Its biopharmaceutical properties are shown in this study.

MATERIALS AND METHODS

The mushrooms utilised in this study (*Ganoderma lucidum*) were obtained by placing an order through a commercially selling industrial agent in Malaysia, where these mushrooms are farmed and harvested for various purposes. The dried mushrooms are roughly 25g in weight (Approx.). The mushrooms were carefully cleaned with a dry cloth and dried in the shade for another 2-3 days. The mushrooms were then split into small bits and coarsely pulverised using a mortar and pestle. Nothing was left out when the samples were ground whole, including the fruiting body and little stout stalk.

EXTRACT PREPARATION

The weighing machine was used to weigh the ground mushroom sample. 20 g of ground material was added to a 500 mL sterile, clean conical flask, which was then filled with 200 mL of non-polar solvent (chloroform) measured with a measuring cylinder. As a result, it was 1:10 (Sample: Solvent) accordingly. To prevent evaporation of the solvent, the lip of the conical flask was wrapped with aluminium foil and fastened with a rubber band. For the next 48 hours, it was left undisturbed to allow optimal diffusion of bioactive chemicals into the solvent.After 2 days of soaking, the extracts containing bioactive chemicals were filtered using Whatman's No 1 filter paper held over a funnel, the filtrate was transferred to another conical flask labelled and maintained separately, and the filter residue (sample) retained in filter paper was allowed to dry. The dry residues were transferred into a clean conical flask containing a 1:10 (sample:solvent) ratio of solvent (mid polar) as Ethyl acetate.After 48 hours, collect the filtrate, label it, and store it for later use. After Ethyl acetate treatment, the sample residue was dried and placed in a conical flask containing another mid-polar solvent, acetone, in a 1:10 ratio. After 48 hours of acetone treatment, the sample residues were filtered and dried. The filtrate was collected and placed in a second conical flask. The polar solvent was used to dissolve the dried filter remnants (Ethanol 1:10 ratio).

The Maceration extraction method is the process of treating sample residues in order to determine their degree of polarity. This extraction procedure adheres to industry standards [9]. To obtain concentrated extracts, chloroform, ethyl acetate, acetone, and ethanol extracts were subjected to rotary evaporator vacuum. The pure extract was stored in Eppendorf containers with the appropriate labels.

PHYTOCHEMICAL ANALYSIS

The extracted material was employed in a variety of phytochemical experiments. Test for alkaloids, phenolic chemicals, flavonoids, saponins, steroids, sugars, tannins, triterpenoids, and coumarin in this phytochemical examination. These tests are carried out in accordance with the guidelines. [10].

ANTIMICROBIAL TEST METHOD

The excellent diffusion method was used to perform the antibacterial activity. The well diffusion test is straightforward, affordable, and simple to read and understand [6]. To avoid contamination, all of the procedure's equipment must be sanitised before use. In sterile Petri plates, around 25ml of molten Mueller Hinton agar was created. The agar plates were left to harden. Bacillus subtilis (MTCC 441) and Escherichia coli (MTCC 443) are bacterial strains, whereas *Candida albicans* is a fungal strain (MTCC 227).Microbial Type Culture Collection and Genbank, IMTECH, Chandigarh, provided these test microorganisms for in-vitro antimicrobial activity. Using a sterile L-rod spreader, pathogenic bacteria such as E. coli and Bacillus subtilis, as well as fungi such as *Candida albicans*, were inoculated on the plate after being cultivated for 18 hours. A sterile cork borer was used to form a 5mm well on the agar after 5 minutes of pathogenic microorganisms settling. The test material (multiple *Ganoderma lucidum* extracts) was diluted in sterile saline and placed into the wells at varied concentrations, including 50 µg/well, 75 µg/well, 150 µg/well, and 200 µg/well.The solvent saline-loaded well serves as a negative control for bacteria and fungi, while the Streptomycin (30µg/ml) and Clotrimazole (30µg/ml) wells serve as positive controls. At 37°C, the plates were incubated for 24 hours. To determine antibacterial and antifungal activity, the diameter of the zone of inhibition around the well was measured using the antibiotic zone scale.

RESULTS

PHYTOCHEMICAL ANALYSIS

The below-mentioned table indicates the presence and absence of various compounds present in four different extracts (Chloroform, Ethyl Acetate, Acetone, Ethanol).

TABLE 1: RESULTS OF PHYTOCHEMICAL ANALYSIS

S.NO	COMPOUNDS	CHLOROFORM	ETHYL ACETATE	ACETONE	ETHANOL
1.	ALKALOID	-	-	+	+
2.	FLAVONOID	-	-	-	-
3.	PHENOLIC COMPOUND	+	+	+	+
4.	SAPONIN STEROIDS	+	-	+	+
5.	STEROIDS	+	+	+	+
6.	SUGAR	+	+	+	+
7.	TANNIN	-	-	+	+
8.	TRITERPENIOD	-	-	-	-
9.	COUMARIN	-	-	+	+

ANTIMICROBIAL ACTIVITY: The results of antimicrobial activity of different extracts of concentration 50µg /well, 100µg/well, 150µg/well, 200µg/well, 30µg/well (standard) of *Ganoderma lucidum* were determined by agar well diffusion method.

TABLE 2: RESULTS OF ANTIMICROBIAL ACTIVITY

EXTRACTS	ZONE OF INHIBITION (mm)					MICROBES
CONCENTRATIONS	50µg	100µg	150µg	200µg	30µg (std)	
CHLOROFORM	15	16	17	19	26	*Bacillus subtilis*
	nil	nil	7	9	16	*Escherichia coli*
	7	8	9	10	13	*Candida albicans*
ETHYL ACETATE	7	10	14	15	25	*Bacillus subtilis*
	nil	7	9	13	20	*Escherichia coli*
	7	8	10	12	16	*Candida albicans*
ACETONE	nil	nil	nil	8	26	*Bacillus subtilis*
	nil	nil	nil	9	16	*Escherichia coli*
	8	9	11	12	13	*Candida albicans*
ETHANOL	9	12	14	16	25	*Bacillus subtilis*
	12	16	19	24	17	*Escherichia coli*
	11	14	16	18	14	*Candida albicans*

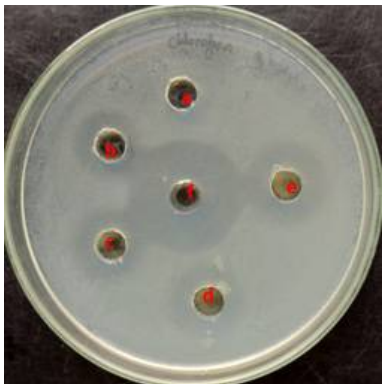


Fig .1a *Bacillus subtilis*

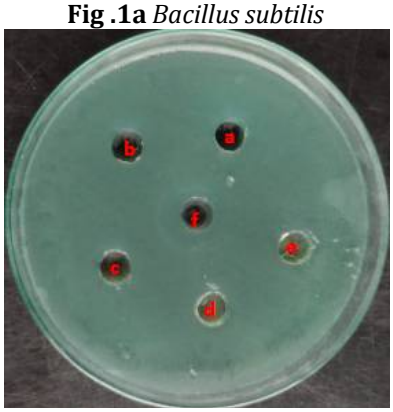


Fig .1b *Escherichia coli*

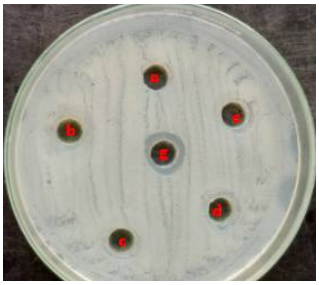


Fig.1c: *Candida albicans*

FIGURE 1: ANTIMICROBIAL ACTIVITY OF CHLOROFORM EXTRACT
a: 0 µg/well; b: 50 µg/well; c: 100 µg/well; d: 150 µg/well; e: 200 µg/well; f: 30 µg/well (Strepotomycin); g: 30 µg/well (Clotrimazole)

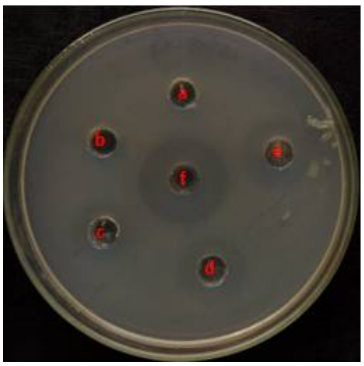


Fig 2a. Bacillus subtilis

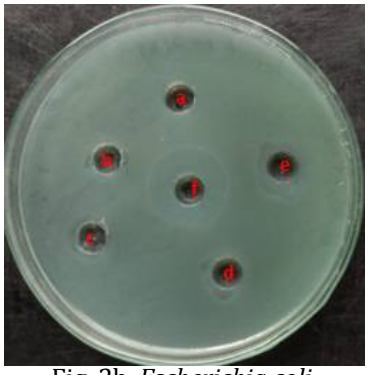


Fig. 2b. *Escherichia coli*

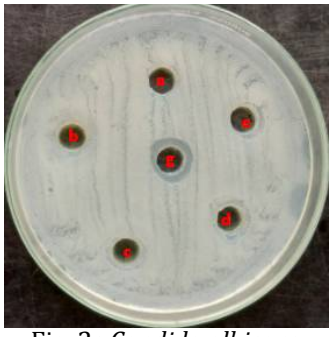


Fig. 2c *Candida albicans*

FIGURE 2: ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACT
a: 0 µg/well; b: 50 µg/well; c: 100 µg/well; d: 150 µg/well; e: 200 µg/well; f: 30 µg/well (Strepotomycin); g: 30 µg/well (Clotrimazole)

FIGURE 3: ANTIMICROBIAL ACTIVITY OF ACETONE EXTRACT

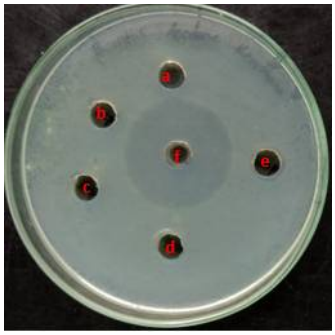


Fig. 3a *Bacillus subtilis*

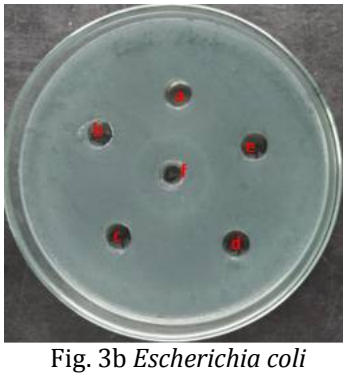


Fig. 3b *Escherichia coli*

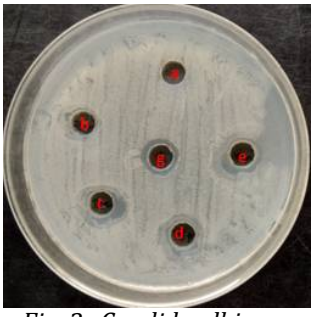


Fig. 3c Candida albicans

a: 0 µg/well; b: 50 µg/well; c: 100 µg/well; d: 150 µg/well; e: 200 µg/well; f: 30 µg/well (Strepotomycin); g: 30 µg/well (Clotrimazole)

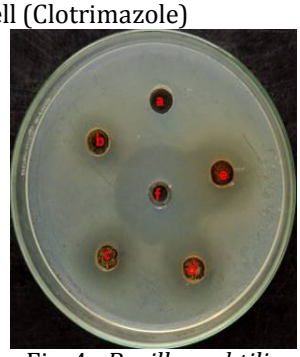


Fig. 4a *Bacillus subtilis*

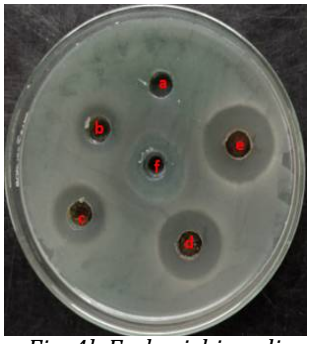


Fig. 4b Escherichia coli

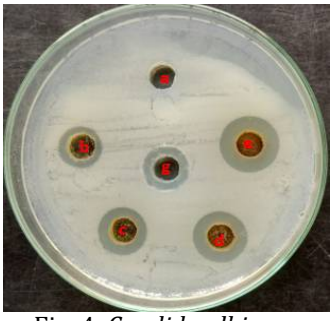
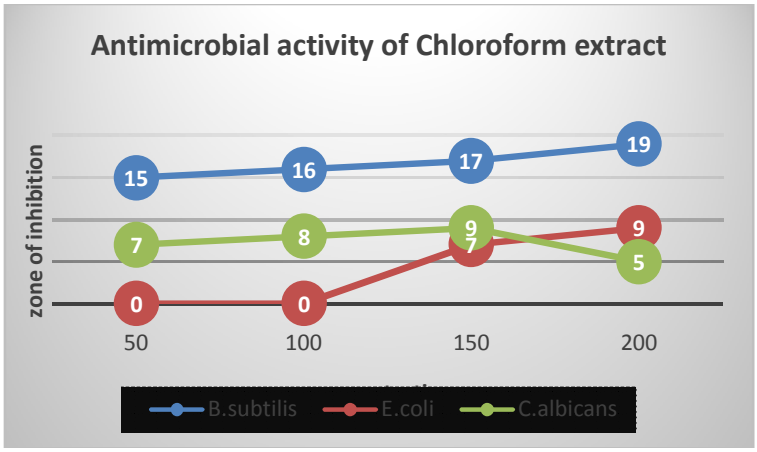


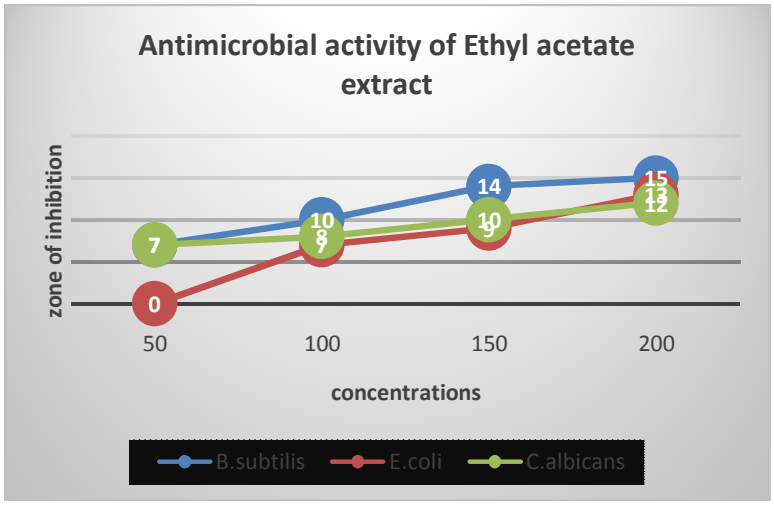
Fig. 4c*Candida albicans*

a: 0 µg/well; b: 50 µg/well; c: 100 µg/well; d: 150 µg/well; e: 200 µg/well; f: 30 µg/well (Strepotomycin); g: 30 µg/well (Clotrimazole)

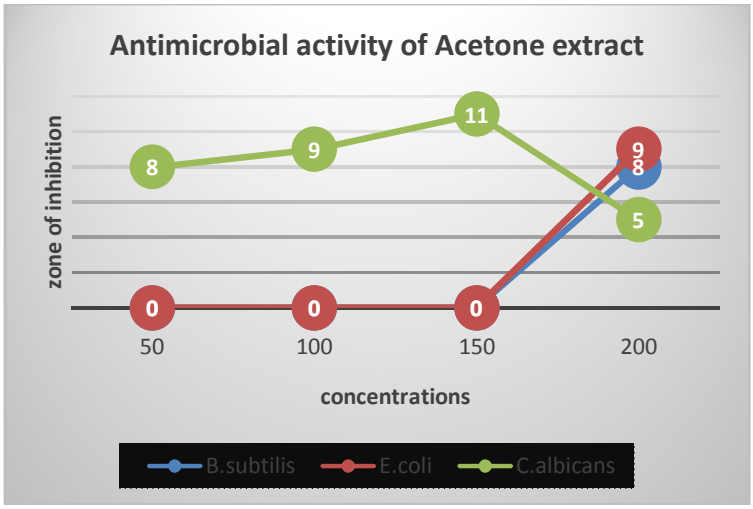
FIGURE 4: ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT



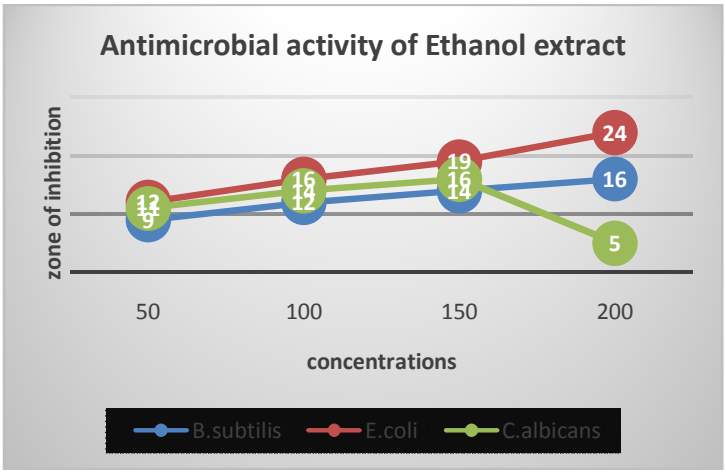
GRAPH: 1 ANTIMICROBIAL ACTIVITY OF CHLOROFORM EXTRACTOF *Ganoderma lucidum*



GRAPH 2: ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF *Ganoderma lucidum*



GRAPH 3: ANTIMICROBIAL ACTIVITY OF ACETONE EXTRACT OF *Ganoderma lucidum*



GRAPH 4: ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT OF *Ganoderma lucidum*

DISCUSSION

PHYTOCHEMICAL ANALYSIS:

The extracts of *Ganoderma lucidum* contain Alkaloids, Steroids, Phenolic compounds, Sugars, Coumarin, Saponins, Tannins, and other phytochemicals, according to the phytochemical screening. An alkaloid is a nitrogen-containing chemical that piqued researchers' interest because the nitrogen-rich plant also has numerous therapeutic benefits [11]. Phenolic Compounds are found in all plants and serve an important

role in human nutrition. Their antioxidant qualities have sparked curiosity [12].According to the above-mentioned reference, *Ganoderma lucidum* is expected to have antioxidant characteristics, which have been described in other *Ganoderma* species in the literature. Mushroom *Ganoderma lucidum* hot water extract has been found to have an antioxidative action against heart toxicity [13]. Coumarin is a secondary metabolite generated from plants that has anti-inflammatory, antibacterial, antifungal, antioxidant, anticancer, anticoagulant, and antihypertensive activities [14].

Steroids were found in all of the *Ganoderma lucidum* extracts, as shown in the preceding tabular column (Table 2), however Triterpenoids were absolutely lacking. Other *Ganoderma* species, however, are said to contain both Triterpenoids and Steroids. The anti-inflammatory effects of Triterpenoids and Steroids were discovered in both *Ganoderma lucidum* and *Ganoderma Tsuage*. It had a substantial inhibitory effect on rat neutrophil superoxide anion production caused by Fmlp/CB[15].

Because of its low polarity, the extracts derived from Acetone and Ethanol were shown to be the most active components in the phytochemical investigation of *Ganoderma lucidum*. Similar findings have already been reported in the literature [16] and [17].

ANTIMICROBIAL ACTIVITY:

Ganoderma lucidum extracts contain phenols, alkaloids, sugars, steroids, and other phytochemicals, according to preliminary phytochemical study. The antibacterial activity of various extracts of *Ganoderma lucidum* at concentrations of 50µg/well, 100µg/well, 150µg/well, and 200µg/well was assessed using the agar well diffusion method.

The lowest concentration of a substance, generally a medicine, that limits observable growth of a bacterium or bacteria is known as the minimum inhibitory concentration (MIC) in microbiology. To confirm resistance and to check the in vitro activity of any novel antimicrobials, as well as to calculate MIC breakpoints, mostly in diagnostic laboratories [17]. The antimicrobial activity of Chloroform extract demonstrated that at a low concentration of roughly 50µg of extract, it inhibited the growth of Bacillus subtilis with a high zone of inhibition (15mm) and increased activity as concentration was raised.At 150µ g and 200µ g, the growth inhibition zone for *Escherichia coli* was discovered. As a result, increasing the content of Chloroform extract improved the activity against gramme negative bacteria. From a minimal concentration of 50g, antifungal activity was seen. The antifungal activity of chloroform extract was raised when the concentration was increased. (Graph 1 in Table 2 and Fig. 2). The most active *Achillea* species (A. teretifolia, A. multifida) demonstrated selective activity against the studied bacteria isolates, with MICs ranging from 50 to 75 µg/mL against S. aureus, S. epidermidis, and S. typhymurium [19].

In all concentrations, the ethyl acetate extract was shown to have good antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*, with the exception of the low concentration (50µg) of ethyl acetate, which showed no inhibitory zone against Escherichia coli. (See Table 2, Graph 2, and Figure 9 for further information.) By using the cup plate diffusion method, the antimicrobial activity of various parts of *Croccus sativus* L. (saffron), including stigma, stamen, leaves, and colora, extracted with various solvents, was tested against different bacteria (Microccucos luteus, Staphylococcus epidermitis, Staphylococcus aureus, and E. coli) and fungi (*Candida albicans, Aspergillus*). The values of each active extract's Minimal Inhibitory Concentration (MIC) were determined. The results reveal that the ethyl acetate extract of diverse plant parts (excluding leaves) has a good antibacterial and antifungal activity against the bacteria and fungi used as test organisms [20].

Table 2 shows that, despite having active biomolecules and showing positive results for phytochemical analysis, the extract obtained from acetone had more effective antifungal activity in all concentrations than antibacterial activity on gramme positive and gramme negative bacteria only at high concentrations of about 200µg and not at low concentrations of about 50µg, 100µg, and 150µg.

The Zone of Inhibition is a circular area surrounding the antibiotic's active site where bacteria colonies do not develop. The zone of inhibition can be used to determine a bacteria's sensitivity to antibiotics [21]. Table 2 shows that ethanol extracts' antimicrobial activity was efficient against all three bacteria at all four concentrations (50µg, 100 µg, 150 µg, and 200 µg). In comparison to the other three extracts, the ethanol extract extracted the majority of the bioactive chemicals that can prevent microbial development. When tested against *E.coli*, the maximum millimetre of zone of inhibition found in ethanol extract at 200 microgram of sample was 24mm. (Figures 5).

Ethanol extract, which is a full polar solvent, exhibited the highest antibacterial action, and ionic liquids (ILs) are becoming increasingly essential in separation research. ILs are used in separation procedures including as extraction, gas chromatography, and supported liquid membrane (SLM) separation [22]. Following ethanol, ethyl acetate, and chloroform, ethyl acetate and chloroform have shown to have superior antibacterial action. Acetone extract, which is a midpolar solvent, has the lowest antibacterial action. Antimicrobial activity increases with increasing concentration in all of the extracts, as seen in the graph.

CONCLUSION

The extracts of *Ganoderma lucidum* were tested for phytochemical and antibacterial activities in this study. According to other studies, not only *Ganoderma lucidum* but also other *Ganoderma* species have high levels of bioactive chemicals and secondary metabolites. As a result, it can be employed in the pharmaceutical and food industries all over the world. Because phytochemical research reveals the amount of bioactive substances found in *Ganoderma lucidum*, it is thought to be a good candidate for usage as a medicine for a variety of ailments.Due to its ability to predict the binding confirmation of molecule ligand to its binding sites, GCMS can be used to conduct extensive research on various compounds such as steroids, ethers, fatty acids, and terpenes, and further molecular docking can be performed using bioinformatics tools to perform structure-based drug design. Comparative phytochemical research of different *Ganoderma* species can identify which species is better for drug creation and other pharmaceutical needs.

Ganoderma lucidum extracts were reported to have effective antibacterial action against two pathogenic bacteria species and one fungus species. According to the findings, the activity of Ganoderma lucidum polar extract (ethanol extract) was found to be more effective than non-polar and midpolar extracts. As a result of the in vitro antibacterial activity, *Ganoderma lucidum* can be used as an antimicrobial agent in a variety of industrial applications. According to the findings, the extracts are far more efficient as an antifungal agent than they are as an antibacterial agent. *Ganoderma lucidum*'s antifungal qualities can be further investigated and tested on a variety of additional fungus species, as well as used in pharmaceutical firms.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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