Original Article

https://napass.org.ng

Antimicrobial Activity of Ethanol Extract of Calocybe indica Purkay & Chandra on Selected Microorganisms

Senjobi C. T.,[1*] Onumah, K. C.,[1] Jimoh M.O., [1] Okechukwu O.C.,[1] Lawal O. I[1]and Ettu A.O[2]

[1]Plant Science Department, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.
[2]Sikiru Adetona College of Education, Science and Technology, Omu-Ajose, Ogun State, Nigeria.

Abstract

The rapid evolution of drug resistant microorganisms has made the absolute treatment of microbial infections difficult and this challenge is of great concern to medical practitioners. Hence, the need for development of novel drugs that are highly potent in combating microbes. This study evaluated the antimicrobial potential of the ethanol extract of *Calocybe indica* on five (5) bacteria strains and two (2) fungi species Phytochemical analysis was carried out to investigate the active constituents of *Calocybe indica* using standard protocols, while gas chromatography mass spectrometry was done to elucidate the active compounds and data were analysed for descriptive statistics. Results revealed the successful eradication of two strains of *Candida* (22.67 ± 3.06mm, 23.33 ± 6.51mm), *S. aureus* (16.33 ± 1.53mm) by the extract of *Calocybe indica*, with *E. coli* having the largest zone of inhibition (18.67 ± 1.15mm) and *Klebsiella pneumonia*, the lowest zone of inhibition (7.33 ± 1.53mm). Phytochemical analysis of *Calocybe indica* unravelled the presence of abundant saponins, moderate alkaloids and steroids, trace flavonoids while tannins and terpenoids were absent. Using gas chromatography mass spectrometry, eleven (11) metabolites such as 1, 12-tridecadiene, tridecanal, 1, 3-cycloheptadiene, hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9, 12-Octadecadienoic acid (Z, Z), methyl ester, amongst other chemical compounds were identified in the *Calocybe indica*. These metabolites might be responsible for the antimicrobial activity of the mushroom, thus the need for further research on the isolation and characterization of the compounds stimulating this activity.

Key words: antimicrobial, *Calocybe indica,* bacteria strains, fungi species, metabolites

OPEN ACCESS
Correspondence:
E-mail:
comfort.senjobi@oouagoiwoye.edu.ng

Phone:
+2347033439472

ORCID No:
0000-0002-9915-9702

Specialty Section;This article was submitted to Sciences a section of NAPAS.

Submitted: *10th August, 2024*
Accepted: *13th November, 2024*

Citation: *Senjobi C. T., Onumah, K. C., Jimoh M.O., Okechukwu O.C., Lawal O. I and Ettu A.O (2024) Antimicrobial Activity of Ethanol Extract of Calocybe indica Purkay & Chandra on Selected Microorganisms*

Effective Date: Vol7(2), 122-132

Publisher:cPrint,Nig.Ltd
Email:cprintpublisher@gmail.com

INTRODUCTION

Microorganisms are causative agents for most infections leading to illness and continue to pose serious hazardous threat to human health. Although, natural and synthetic antimicrobial compounds have been isolated from plants including fungi to effectively eradicate harmful microbes without developing resistance to treatment. Drug resistance is of great concern in disease management. Therefore, new antimicrobial compounds from various biological sources are needed to effectively combat this challenge (Klein *et al.,* 2007).

Mushrooms are increasingly acknowledged in the field of complementary and alternative medicine as functional food due to their immune boosting activity. The milky mushroom, *Calocybe indica* Purkay & Chandra which comes in different sizes has several stems that emerge from a single base. It is totally white from base to cap, does not age or change colour with handling, and does not bruise or change colour. The caps develop into button mushrooms as they become older and can even take on a convex or dome shape. The ease of culture, low cost, beautiful sporocarp, preferred as off white in colour, long shelf-life and nutritional content including the quick growing period of milky mushrooms make them superior to other strains (Chelladurai *et al.*, 2021). The quality of the spawn and substrate are key factors in milky mushroom production, paddy straw has been found to be the optimum substrate for their growth (Maurya *et al*., 2019). *Calocybe indica* has been shown to delay the onset of tumorigenesis, bodily harm, contamination, aging, and blood-vascular issues due to their nutritional composition, this also lessens the damage caused by unconstrained radicals and reactive oxygen species (ROS) (Chelladurai *et al*., 2014; Shashikant *et al*., 2022). Due to its extended shelf life in tropical climates, *C. indica* is therefore a better alternative to *Pleurotus ostreatus* (Chelladurai *et al*., 2014 *C. indica* is a rich source of vitamins, minerals, proteins, amino acids and noted for its low calorie making it a perfect food for people with heart diseases (Anju and Ukkuru, 2016).

It is also a good source of polyphenols (flavonoids, alkaloids, and triterpenoids), which are bioactive polysaccharides like -glucans and polyphenols. The foraging mechanisms, enhanced nutritional activity, and delivery of properties that are anti-diabetic, anti-cancer, and anti-lipid peroxidation could all be attributed to these active components. (Mirunalini *et al.,* 2012; Ghosh, 2022). Pleuran, chitin, schizophyllan, glucans, hemicellulose, lentinan, mannans, galactans and xylans are just a few of the polysaccharides found in *C. indica*, and they all have probiotic properties. (Yu *et al.,* 2018).

Plate 1: Fresh sample of *Calocybe indica*

Plate 2: Dried and shredded *Calocybe Indica*

MATERIALS AND METHOD

Materials

Calocybe indica, ethanol, *Escherichia coli, Staphylococcus aureu, Streptococcus pneumonia, Klebsiella pneumoniae*, and *Salmonella typhi*, *Candida albicana* and *Candida tropicalis*, agar, petri dishes, Whatman filter paper, weighing balance, electric blender and cotton wool.

Source of bacteria

Clinical strains of bacteria were obtained from Pharmaceutical Microbiology Laboratory, Olabisi Onabanjo University, Sagamu Campus Ogun State, Nigeria.

Collection and identification of mushroom

Calocybe indica was collected from the Waste Utilization and Fermentation division under the Biotechnology Department of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos state and also authenticated at the institute.

Drying and storage

Fresh samples of *C. indica* were shredded, exposed to open sunlight to reduce water content, dried in a cool dry room for seven (7) days, after which they were grounded into coarse form using an electric blender, weighed and packed in an airtight container for further use.

Phytochemical Screening

Powdered sample of *C. indica* was screened for phytoconstituents to determine the presence of tannins, saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, and anthraquinones using standard protocols proposed by Trease and Evans (1989) and Sofowora (1993).

Alkaloid

Powdered sample of *C. indica* (1 g) was extracted with 10ml of 10% hydrochloric acid (HCl) on a water bath for 5 min. The extract was filtered, allowed to cool and the pH was adjusted to about 6 -7 by adding 10% ammonia and also using litmus paper. The filtrate (5mL was taken into separate test tube, small quantity each of Wagners and Dragendoff reagents were added and observed. The presence of turbidity and precipitation indicated the presence of alkaloid.

Anthraquinone glycoside

Powdered sample of *C. indica* (1 g) was extracted with 2mL of 10% of Hydrochloric acid by boiling for 5 min and filtered while still hot, then allowed to cool. The filtrate was partitioned with equal volume (aliquot) of chloroform and shaken gently. The chloroform layer (lower layer) was transferred to a clean test tube and aliquot 10% ammonia solution was added and the shaken gently. The presence of delicate rose pink layer on the test solution indicated the presence of anthraquinone glycoside.

Cardiac glycoside

Powdered sample of *C. indica* (1 g) was extracted with water and added into 2mL of glacial acetic acid containing a drop of ferric chloride reagent. Then 1mLof concentrated tetraoxosulphate (vi) acid H_2SO_4 was gently added to form an under layer. A brown/purple/reddish-brown ring formed at the interface and green colour in the acetic acid layer indicated cardiac glycoside present.

Flavonoid

a. Powdered sample of *C. indica* (1 g) was added to 10mL of ethanol and 3 drop of ferric chloride ($FeCl_3$) solution was added. A dark green colour observation indicated the presence of flavonoid.

b. Powdered sample of *C. indica* (1 g) was extracted with ethylactate and heated for 3 min. the residue

was treated with 10% ammonia (NH_3). A yellow colour observed indicated the presence of flavonoid.

c. Powdered sample of *C. indica* (1g) was extracted with distilled water and heated for 3 min. It was allowed to cool and 2ml concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added, a yellow colour appearance which disappeared on standing observed for positive results.

Tannin

Braemer's test

Powdered sample of *C. indica* (1g) was decocted with 10ml of distilled water by boiling for 10 min and filtered while hot and allowed to cool. ferric chloride (0.1%) was added to the filtrate. A blue-black, green or blue green precipitate indicated the presence of tannin.

Saponin

Frothing test

Powdered sample of *C. indica* (1 g) was transferred into a test tube containing 10ml of distilled water and the boiled for 5 min and then filtered. The filtrate was shaken vigorously and observed. The presence of froths indicated the presence of saponin.

To the frothing was added three drop of olive oil and shaken vigorously; the formation of emulsion confirms the presence of saponin.

Phenol

Powdered sample of *C. indica* (1 g) was added to 10mL of ethanol and 3 drops of phenol solution was added. A dark green coloration indicated the presence of phenol.

Steroids

Acetic anhydride (2mL) was added to 0.5 g of ethanol extract of *C. indica* with 2mL H_2SO_4. The colour changed from violet to blue of green in some sample indicating the presence of steroids.

Terpenoids

The ethanol extract of *C. indica* (5mL) was mixed with 2mL of chloroform and 3mL of concentrated H_2SO_4 was added carefully form a layer. A reddish-brown coloration of the inter-phase formed indicated a positive result for the presence of terpenoids.

Phloba-tannin

Powdered sample of *C. indica* (1 g) was transferred into a test tube containing 10ml of distilled water and then boiled for 5 min and filtered. To the filtrate, 5mL of 1%of HCl was added and boiled for 5 min. The presence of yellow precipitate is a positive result.

Extract preparation

The powdered sample of *C. indica* is extracted by cold extraction method, using ethanol as solvent. The powdered sample (50 g) was weighed and poured into a flat bottom flask which contains 450mL of ethanol for 7 days with frequent shaking. Defatted cotton wool and Whatman filter paper was used for the filtration process. The defatted cotton wool was first used before finally using the filter paper. The extract was then subjected to rotary evaporator to separate the filtrate (mushroom) from the solvent used for the extraction after which the filtrate was concentrated to dryness using water bath (Jonathan and Fasidi, 2003; Balakumar *et al.*, 2011).

Assay for antimicrobial activities and minimum inhibitory concentration

The antimicrobial activity was screened through agar disc diffusion method using the standard procedure of (Hemashenpagam and Selvaraj, 2010)

and Minimum Inhibitory Concentration assay (MIC) using tube Dilution Technique according to Iheukwumere and Umedum (2013). The Minimum Bactericidal Concentration (MBC) was determined through the modified methods of Iheukwumere and Ubajekwe (2012).

Antibacterial Screening of *Calocybe indica* extracts

Calocybe indica mushroom extracts (ethanol) was screened using the agar well diffusion method. The medium was put into Petri dish and autoclaved at 121.6 ˚C for 30 min. For 24 hrs., the bacteria was grown in nutrient broth. With the aid of a sterilized stainless steel cork borer, five 8 mm diameter agar wells were created in each Petri dish. Each plate's wells contained different concentrations of prepared *C. indica* extracts at the rate of 25%, 50%, 75%, and 100%. Only pure solvent was present in the control.. The plates were incubated in the incubation chamber for 24 hrs. at 37 ± 2ºC. The diameter (mm) of the inhibition zone surrounding the well, including the well diameter, was used to compute the zone of growth inhibition. In all three replicates, readings were obtained perpendicularly, and the average results were also be tabulated. After removing the control, the percentage of bacterial microbe growth suppression was calculated based on the inhibition diameter values with the control as the reference (Hemashenpagam and Selvaraj, 2010).

Percentage of growth inhibition= (Control-Test/Control) x100

Where: Control=average diameter of bacterial colony in control. Test=average diameter of bacterial colony in treatment sets.

Determination of minimum inhibitory concentration (MIC)

According to Iheukwumere and Umedum (2013), the minimum inhibitory concentration (MIC) was performed using the tube dilution technique. By employing the two-fold serial dilution procedure, various extract concentrations were achieved. The test tubes were filled with 1 mL of each of the test organisms before being incubated at 37 °C for 24 hrs. The extract's minimum inhibitory concentration (MIC), or greatest dilution, at which the test organism's growth was inhibited, was recorded.

Determination of minimum bactericidal concentration (MBC)

The modified (Iheukwumere and Ubajekwe, 2012) procedures were used to establish the Minimum Bactericidal Concentration (MBC). Following the measurement of the MIC concentration, the minimum bactericidal concentration (MBC) was carried out. Equal amounts of different concentrations that failed to cause any discernible growth in the MIC were sub cultured into nutrient agar for the bacteria and incubated at 37 °C for 24 hrs. The MBC was noted as the least amount of extracts necessary to kill the test organism in the allotted amount of time.

Statistical analysis

Data was statistically analyzed by using One-way analysis of variance (ANOVA). The values are reported as mean ± SD using SPSS statistical package.

RESULTS

Phytochemical analysis of *Calocybe indica* unravelled the presence of abundant saponins, moderate alkaloids and steroids, trace flavonoids while tannins and terpenoids being absent (Table 1).

The diameter of zones of inhibitions of ethanolic extract of *Calocybe indica* against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Streptococcus, Klebsiella pneumoniae, Candida tropicalis* and *Candida albicans* are shown in table 2. It was revealed that the inhibition zones were between 7.33±1.53 and 23.33±6.51 mm. The inhibition zones of ethanolic extract of *Calocybe indica* were (16.33±1.53mm, 13.00±1.00 mm, 13.00±1.00 mm, 18.67±1.15 mm, 15.00±2.65 mm, 22.67±3.06 mm, 23.33± 6.51 mm)100% on (*Staphylococcus aureus, Salmonella, streptococcus, Echerichia coli, Klebsiella pneumoniae, Candida. albicans, Candida. Tropicalis*) respectively. A larger inhibition zone indicates higher antimicrobial activity of the extract against the tested microbial species.

A peak in the GC-MS spectra of the ethanol extract of Calocybe indica's GC fractions is shown in Table 6 along with the number of chemicals present. Eleven bioactive substances such as 1,12-Tridecadiene, Tridecanal, 1,3-Cycloheptadiene, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 10-Octadecenoic acid, methyl ester, 9-Octadecenoic acid, methyl ester, Methyl stearate, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Eicosanoic acid, methyl ester were discovered during the analysis.

Table 1: Phytochemical constituents of ethanol extract of *Calocybe indica*

Phytochemicals	Inference
Alkaloid	++
Saponins	+++
Flavonoid	+
Tannins	-
Terpenoids	-
Steroids	++

Keys: - = absent **+** = trace **++** = moderate **+++** = abundant

Table 2: Antimicrobial activity of ethanol extract of *Calocybe indica* against different bacteria and fungi strain

Test organisms	Zones of inhibition			
	12.5%	**25.0%**	**50.0%**	**100%**
Staphylococcus aureus	10.33± 1.53	11.33 ± 0.58	12.33 ± 1.53	16.33 ± 1.53
Salmonella typhi	7.67 ± 1.53	9.67 ± 0.58	11.33 ± 1.15	13.00 ± 1.00
Streptococcus pneumonia	7.67 ± 1.53	9.67 ± 0.58	11.33 ± 1.15	13.00 ± 1.00
Escherichia coli	9.67 ± 2.52	14.33 ± 1.15	16.67 ± 1.53	18.67 ± 1.15
Klebsiella pneumoniae	7.33 ± 1.53	10.00 ± 2.00	11.33 ± 1.53	15.00 ± 2.65
Candida albicans	11.33 ± 2.08	14.33 ± 3.21	18.00 ± 1.00	22.67 ± 3.06
Candida tropicalis	13.00 ± 4.36	15.00 ± 4.36	19.33 ± 3.79	23.33 ± 6.51

*Value are given in mean ± standard deviation in triplicate values

Table 3: Activities of Standard Drugs against Bacteria Strains

Drugs	Test organisms				
	Sa	**St**	**Str**	**Kp**	**Ec**
Ciprofloxacin	20	16	20	20	21
Amoxicillin	12	11	17	18	19
Augmentin	16	20	19	19	20
Gentamycin	19	20	22	10	17
Pefloxacin	18	21	20	14	18
Tarivid	19	19	10	16	20
Streptomycin	13	11	18	19	16
Chloramphenicol	17	10	10	16	13
Septrin	10	10	15	18	20
Spafloxacin	17	21	16	19	15

Key: Sa= *Salmonella typhi***,** St= *Staphylococcus auerus***,** Str= *streptococcus pneumonia***e**
Kp= *Klebsiella pneumoniae***,** Ec= *Escherichia coli*

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extract *Calocybe indica* against bacteria strain

Bacteria	MIC (mg/mL)	MBC (mg/mL)
Salmonella typhi	6.25	>100
Staphylococcus auerus	6.25	>100
Streptococcus pneumonia	6.25	>100
Klebsiella pneumonia	6.25	>100
Escherichia coli	3.13	>100

Table 5: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extract *Calocybe indica* against fungi strain

Fungi	MIC (mg/mL)	MBC (mg/mL)
Candida albicana	25	20
Candida tropicalis	9	13

Table 6: GC-MS analysis of ethanolic extract of *Calocybe indica*

S/N	RT	Name of compound	Molecular formular	Molecular weight
1	13.73	1,12-Tridecadiene	$C_{13}H_{24}$	180.32
2	13.80	Tridecanal	$C_{13}H_{26}O$	198.34
3	14.17	1,3-Cycloheptadiene	C_7H_{10}	94.15
4	16.62	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
5	14.99	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
6	16.24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47
7	16.29	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48
8	16.35	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48
9	16.53	Methyl stearate	$C_{19}H_{38}O_2$	298.50
10	16.68	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47
11	18.30	Eicosanoid acid, methyl ester	$C_{21}H_{42}O_2$	326.55

Key: RT = retention time

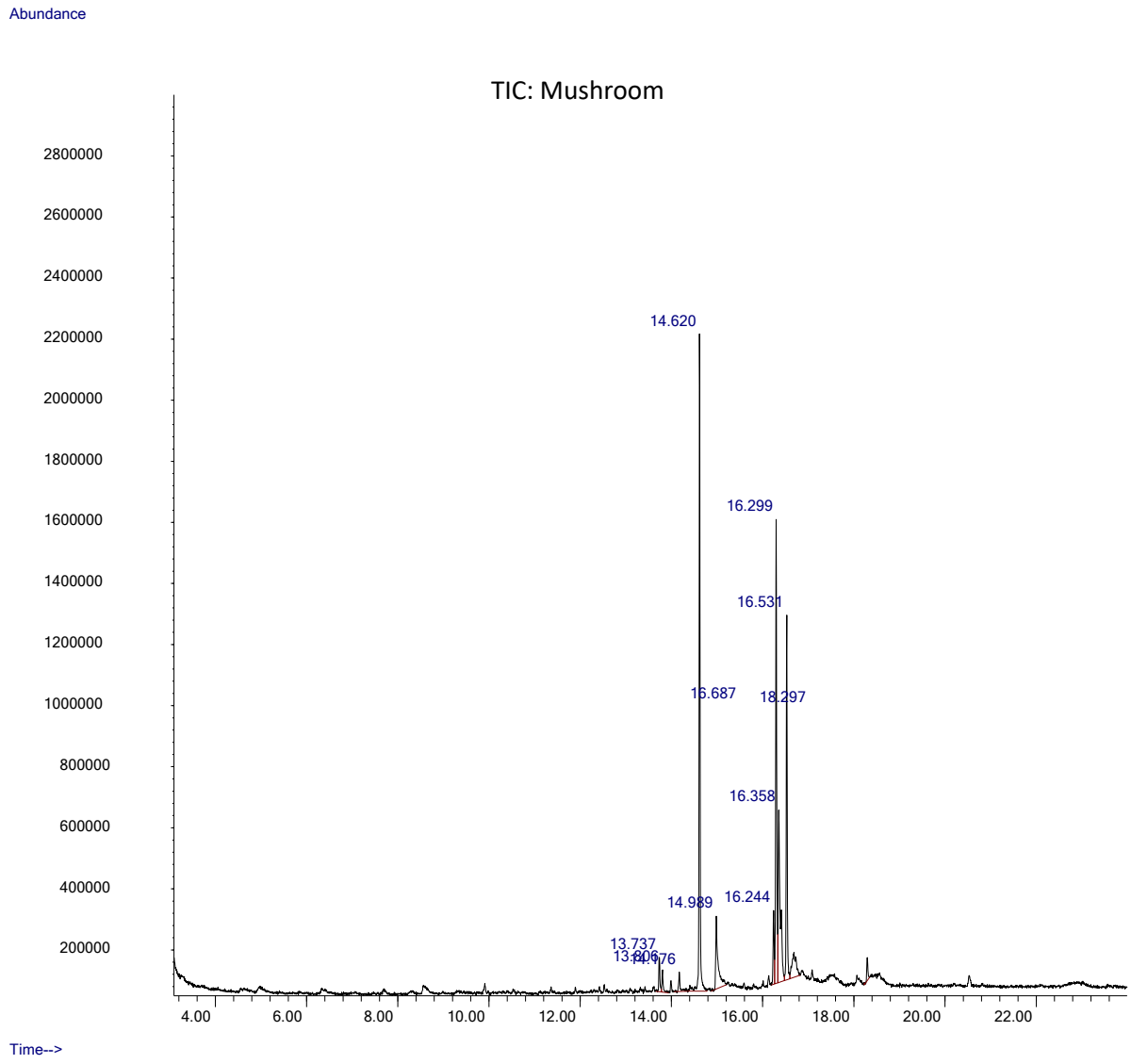

Figure 1: GC-MS chromatogram of ethanolic extract of *Calocybe indica*

DISCUSSION

Calocybe indica is one of the most valuable mushrooms, rich in nutrients and utilized in traditional medicine. The qualitative phytochemical analysis of the ethanolic extracts of *C. indica* as shown revealed secondary metabolites such as steroids, alkaloids, flavonoids, saponins, and flavonoids. The mushroom was discovered to contain saponins in appreciable amount while flavonoid was in trace amount. The presence of these phytochemicals could be responsible for the anti-bacterial activity of *Calocybe indica*.

Using agar well diffusion to test the ethanolic extracts of *C. indica* for antimicrobial activity, it was discovered that the two (2) strains of *Candida* as well as *S. aureus* and *E. coli* were all successfully eradicated by the extract. *E. coli* demonstrated the largest zone of inhibition (18.67 ± 1.15 mm), while

Klebsiella pneumoniae had the lowest zone of inhibition (7.33 ± 1.53mm), according to the results from table 2. The two (2) fungi, *Candida albicans* and *Candida tropicalis* both displayed a sizable zone of inhibition (22.67 ± 3.06 mm, 23.33 ± 6.51 mm) respectively. Greater antimicrobial activity of the tested microbial species' extraction was indicated by the larger inhibition zone. The results of Bains and Tripathi (2015) support the sensitivity of *S. aureus, E. coli,* and *K. pneumoniae* to *C. indica* extracts. In contrast to Gram negative bacteria, Gram positive bacteria have an outer membrane and periplasmic space that surround the cell wall. The inner leaflet of this outer membrane is made up primarily of phospholipids, whereas the outside leaflet is primarily made up of lipopolysaccharides (Holst *et al.,* 2007). Antibiotics, chemotherapeutic drugs and the attack of mushroom polysaccharides all penetrate gram positive bacteria more readily. It is possible that the organisms have a detoxification system for the active ingredients, which explains why the extracts are unable to stop the growth of gram-negative bacteria like *P. aeruginosa*. (Chika *et al.*, 2007). These current findings are in line with those of earlier fungi researchers (Nasim and Ali, 2011; Kamra and Bhatt, 2012), who also noted a significant antibacterial effect of the methanol extract of *Ganoderma lucidum* against gram-negative bacteria *(E. coli)* and a relatively low antibacterial effect against gram-positive bacteria *(S. aureus).* Earlier investigations on other fungus, (Sagar and Thakur, 2012) have demonstrated that methanol extracts of *Lactarius, Sparassis crispa, Morchella esculenta, and Ganoderma lucidum* all have comparable antibacterial activity against *S. aureus* and *E. coli*. Ramesh and Pattar (2010) found that extracts of *Clavaria vermicularis* and *Marasmium oreades* were more effective at inhibiting gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa* than gram-positive bacteria like *Bacillus sutilis* and *Staphylococcus aureus*. From this study, *Calocybe indica,* an edible mushroom had varying degrees of antibacterial activity in several solvents.

Klebsiella pneumonia has reduced minimum inhibitory concentration (MIC) compared to *Salmonella typhi, Staphylococcus aureus, Streptococcus,* and *Echerichia coli* as shown in table 4. This means that extract from *Calocybe indica* cannot kill *Klebsiella pneumoniae* and cannot be employed as a potential antibacterial candidate. According to reports by Al-Sa'ady (2020) and Atata *et al.* (2003), *K. pneumoniae* also exhibits resistance to plant extracts with antibacterial potential. The above result also validates the resistance of *Klebsiella pneumoniae* to *C. indica.* However, some studies have noted antimicrobial activities of various plant extracts in various places around the world (Pallavali *et al.*, 2019). It is possible to accept the fact that *C. indica* has very little antibacterial potential or none at all against *K. pneumoniae* yet having a strong antimicrobial potential against *S. aureus* and *E. coli.* Both of these organisms have a high resistance-ability, which may be related to the fact that they are more prone than other gram-negative bacteria to develop multi-drug resistance to well-known antibiotics (Sah *et al.*, 2020).

The MUFA (monounsaturated fatty acid) 9-octadecenoic acid has been proven to have anticancer effects in vitro mutant as well as anti-

inflammatory, antibacterial, and cancer-preventive properties (Lokesh *et al.*, 2017; Rajeshwari *et al.*, 2022). Reports from earlier literature have indicated that the discovered bioactive components are responsible for the pharmacological properties of *C. indica* (Yu *et al.*, 2005). The presence of 1, 12-Tridecadiene is an indicator of defence potential of *C. indica* which corroborate its antimicrobial activities observed in the present work, the presence of this compound could be used as a marker for disease resistance in plants.

CONCLUSION

In conclusion, this present study focused on the phytochemical components of the mushroom. It was reported that *Calocybe indica* had abundant Saponins, moderate quantity of alkaloid and steroids, trace amount of Flavanoids, Tanins and Terpenoids were completely absent. This study further investigated the antimicrobial activity of *C.indica* ethanol extract. The ethanolic extract showed varying susceptibility, followed by *Klebsiella pneumoniae*, *staphylococcus aureus* and the two fungi strains used (*Candida albicana and Candida tropicalis*) were also susceptible. All Organisms used exhibited high Minimum Inhibition Concerntration (MIC) except *E.coli*. The result from the study was consistent with various works that has been done by some researcher.

The Gas Chromatography Mass Spectrometry result revealed the presence of numerous amounts of chemical compounds embedded in the mushroom. This chemical compounds found present are responsible for their health related actions.

Hence, further research should be carried out on the isolation and characterization of these bioactive compounds to produce potent drug that would break through the defence mechanisms of microorganisms and also provide more health related information about *Calocybe indica*.

Conflict of Interest

The author clearly declares that there is no conflict of interest with the co-authors.

REFERENCES

Al-Sa'ady AT, (2020). Antibacterial screening for five local medicinal plants against nosocomial pathogens: Klebsiella pneumoniae and Staphylococcus epidermidis. Eurasian Journal of Biosciences 14(1):553-559.

Anju RP, Ukkuru M, (2016). Health impact and medicinal properties of nutritionally edible milky mushroom (Calocybe indica). International Journal of Advance Engineering and Resource Science 3:236-932.

Atata RF, Sani A, Ajewole SM, (2003). Effect of Stem Bark Extracts of Enantia chloranta on Some Clinical isolates. Biokemistri 15(2):0795-8080.

Bains A, Tripathi A, (2015). Antibacterial and antioxidant properties of wild mushrooms collected from Himachal Pradesh. International Journal of Biology Pharmacy and Allied Sciences 4 (10):6161-6170.

Balakumar R, Sivaprakasam E, Kavitha D, Sridhar S, Kumar JS, (2011). Antibacterial and antifungal activity of fruit bodies of

Phellinus mushroom extract. International Journal of Biology 1:72-77.

Chelladurai G, Mohan, JR, Sasirekhamani M, (2014). In vitro cultivation technology and nutritional status of milky mushroom (Calocybe indica). African Journal of Biotechnolog, 13:3901-3906.

Chelladurai G, Yadav TK, Pathak RK, (2021). Chemical Composition and Nutritional Value of Paddy Straw Milky Mushroom (Calocybe indica). National Environmental Pollution Technology 20:1157-1164.

Chika CO, Jude NO, Beatrice NA, (2007) .The effect of ethanolic and boiling water extracts of root barks and leaves of Uvaria chamae on some hospital isolates. Journal of America Science 3(3):68-73.

Ghosh S, Sarkar T, Pati S, Kari ZA, Edinur HA., & Chakraborty R, (2022). Novel bioactive compounds from marine sources as a tool for functional food development. Frontiers in Marine Science, 9, 832957.

Holst O, Muller –Loennies S, (2007). Microbial polysaccharide structure. In J. P. Kamerling, GJ. Boons, YC, Lee, A Suzuki, N. Taniguchi and AGJV oragen Eds. Comprehensive glycoscience. From chemistry to System biology. Amsterdam Elsevier Inc 1:123-179.

Hemashenpagam N, Selvaraj T, (2010). Antibacterial potential of different extracts of Solanum xanthocarpum Chard and Wendt. Plant Arch, 387-390.

Iheukwumere IH, Ubajekwe CC, (2012). Antibacterial activity of Bafia nitida leaf extracts against phytopathogenic pseudomonas species. Journal of Applied Sciences 15 (3):10741-10749.

Iheukwumere I H, Umedum CU, (2013). Effects of Gossypium hirsutum leaf extracts on gram negative bacteria isolated from the cervix of females with unexplained infertility. African Journal of Science 14 (1):3261-3270.

Jonathan SG, Fasidi IO, (2003). Antibacterial activities of Nigerian edible macrofungi-Lycoperdon pusilum (Bat. Ex.) and Lycoperdon giganteus (Pers.). African Journal of Biomedical Research 6:85-90.

Kamra A, Bhatt AB, (2012). Evaluation of antimicrobial and antioxidant activity of Ganoderma lucidum extracts against human pathogenic bacteria. International Journal of Pharmacy and Pharmaceutical Science 4:359-362.

Klein E, Smith DL, Laxminarayan R, (2007). Hospitalizations and deaths caused by methicillin-resistant S. aureus, United States, 1999–2005. Emerging Infectious Diseases, 13:1840–1846.

Lokesh R, Kannabiran K, (2017). Cytotoxic Potential of N-hexadecanoic Acid Extracted from Kigelia pinnata Leaves. Asian Journal of Cell Biology 12:20-27.

Maurya AK, John V, Murmu R, Simon S, (2019). Impact of different substrates for spawn production and production of milky mushroom (Calocybe indica). International Journal of Pharmaceutics and Biological Science 10:5-10.

Mirunalini S, Dhamodharan G, Deepalakshmi K, (2012). Antioxidant potential and current cultivation aspects of an edible milky mushroom-Calocybe indica. International Journal of Pharmacy and Pharmaceutical Science 4:137-143.

Nasim, G, Ali, M, (2011) Estimation of antimicrobial potential of Ganoderma lucidum (Leyss. Ex. Fr.) Karst. Extracts. Pakistan Journal of Botany, 43:183-189.

Pallavali RR, Avula S, Degati VL, Penubala M, Damu AG, Prasad-Durbaka VR, (2019). Data of antibacterial activity of plant leaves crude extract on bacterial isolates of wound infections. Data in brief, 24.

Rajeshwari T, Suresh R, Sudhakar M, (2022). Phytochemicla investigation and antioxidant potential of two Amaranthaceae plants: Achyranthes aspera Linn and Chenopodium album Linn. International Journal on Pharmaceutical Sciences and Research 13(2):902-911.

Ramesh C, Pattar MG, (2010). Antimicrobial properties, antioxidant activities and bioactive compounds from six wild edible mushrooms of Western Ghats of Kamataka. Indian Pharmacognosy Research 2:107-112.

Yu Y, Shen M, Song Q, Xie J, (2018). Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. Carbohydrate Polymer 18(3): 91-101.

Sagar A, Thakur K, (2012). Study on antibacterial activity of Lactarius deliciosus (L.) Gray. Indian Journal of Mushroom 30:10-14.

Sah S. K, Rasool U, Hemalatha S, (2020) Andrographis paniculata extract inhibit growth, biofilm formation in multidrug resistant strains of Klebsiella pneumoniae. Journal of Traditional and Complementary Medicine 10(6):599-604.

Shashikant M, Bains A, Chawla P, Fogarasi M, & Fogarasi S, (2022). The current status, bioactivity, food, and pharmaceutical approaches of Calocybe indica: A review. Antioxidants, 11(6), 1145.

Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993, 289.

Trease GE and Evans WC, (1989). Pharmacognosy, 13th Ed. ELBS/BailliereTindall, London. Pp 345-773.

Yu FR, Lian XZ, Guo HY, McGuire PM, Li RD, Wang R, Yu FH, (2005). Isolation and characterization of methyl esters and derivatives from Euphorbia kansui (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. Journal of Pharmacy and Pharmaceutical Science 8(3):528-535.