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EVALUATION OF *IN-VITRO* ANTIFUNGAL POTENTIALITIES AND GC/MS SPECTROSCOPIC APPROACH OF *CLEISTANTHUS COLLINUS* (ROXB.) BENTH. EX. HOOK. F.

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Keywords:

Agar well-diffusion method, Antifungal activity, *Cleistanthus collinus*, GC-MS, Phytochemical profile

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ABSTRACT: India is the home to the familiar plant *Cleistanthus collinus* (Roxb.) Benth. ex Hook. F (Euphorbiaceae) has great ethnomedicinal and therapeutic applications. The present study was designed to assess the phytochemical profile and investigate the antifungal effectiveness of leaf, bark and fruit extracts by using the agar well diffusion method. All crude extracts from leaf, bark, fruit and fractions derived also demonstrated significant antifungal activity (20-80%), but the methanol extracts showed a remarkable antifungal spectrum; as a result, GC-MS analysis was performed on it. From this GC-MS analysis, 14 chemicals, including alkanes, glycosides, phenols, flavonoids and steroids were identified, while leaf methanol extract has high phytochemical diversity with 9 compounds than bark with 6 compounds and fruit with 5 compounds. 1, 2, 3-Benzenetriol, 3-O-Methyl-d-glucose and Undecane are a few significant molecules with larger area percentages that demonstrated biological value. These compounds have been linked to anti-cancer, anti-inflammatory, anti-microbial and antioxidant properties. The current results collectively highlight the appreciable antimicrobial potential of *C. collinus* and its numerous chemical compounds that are physiologically active and offer a thorough understanding of the phytochemical profile, which could be exploited to create plant-based medications.

INTRODUCTION: Rising antibiotic resistance has emerged as a serious hazard to human well-being worldwide [1]. The rising prevalence of drug-resistant diseases necessitates identifying and isolating novel bioactive components from medicinal plant species utilizing standardized contemporary scientific techniques. Medicinal compounds obtained from plants might provide innovative and accessible techniques to pathogenic organisms.

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According to the World Health Organisation (WHO), conventional drugs produced from plants with medicinal properties continue to assist 80% of poor countries. WHO has also documented a list of over 20,000 therapeutic plant species and identified these plants as a possible driver of new medications [2].

The antibiotic era, which has lasted barely 80 years, has recently entered a time of gradual and extensive appearance of drug-resist microbes, threatening to end this era. Microbial diseases, especially fungus, have quick reproductive rates, malleable genes, and the capacity to adapt to environmental conditions containing a high concentration of possible hazardous substances that apply significant selective stresses.

Fungi's eukaryotic chemistry makes them particularly dangerous infections due to a smaller range of selective therapeutic targets over which inhibitors not hazardous to human, animal, and plant hosts may be created. Moreover, there do not exist medicinal vaccination or supplementary immune-mediated therapies present to assist people in their health care, necessitating dependence on a restricted arsenal of antifungal medication classes to combat an increasing wave of fungal diseases [3]. The worldwide stress of fungus-related infections is escalating. Over 150 million severe instances concerning fungal illnesses occur each year worldwide, leading to nearly 1.7 million fatalities. The aforementioned numbers are rapidly increasing, owing to a variety of societal and medical advances in recent decades that have aided the proliferation of fungal diseases [4]. To settle this problem, novel antifungal drugs are required and plants are the main wellsprings of novel drugs.

Due to their vast and varied organic components, which may have an important physiological influence on the human body, medicinal plants have been used to treat human ailments for centuries. Natural assets, such as plants with medicinal properties, which possess a wide range of phytocompounds potential as traditional medicine for treating chronic and infectious illnesses, have long been seen as safer and more efficient options with fewer adverse effects than synthetic drugs [5]. Owing due to their diverse pharmaceutical qualities, plants high in bioactive phytomedicine components such as alkaloids, flavonoids, tannins, and polyphenols have been utilised to treat diseases [6].

Around 25% of the active ingredients of medicinal plants that have been used as legal drugs have been discovered [7]. *Cleistanthus collinus* is an essential medicinal tree that has piqued the interest of researchers owing to its potential for medication development. *C. collinus*, a member of the Euphorbiaceae family, is a small tree featuring ellipse foliage and its silken villous spike. It tends to occur in South Indian deciduous dry highland forests, Sri Lanka and Malaysia [8]. Cleistanthus A and Cleistanthus B, diphyllin glycosides, are cytotoxic to a variety of cancerous cell lines [9]. Because of its unique pharmacological active principles, the isolation of independent bioactive chemicals of *C. collinus* might be widely exploited in the pharmaceutical industry. Despite being a toxic plant, its active constituents have been investigated for medicinal qualities such as antihypertensive, diuretic, and anticancer properties. Active compounds in *C. collinus* that show ability will have a good probability of being developed into drug use [10]. The plant's stem bark can be used as an antiseptic in skin illnesses and to treat bovine hoof sores [11]. A detailed examination of the literature reveals that two have been discovered in the assay of anti-bacterial activity reports [12, 13] and one in antifungal activity [14] from this plant. The primary goal of this study was to appraise the GC-MS analysis study and examine the antifungal potentiality of diverse plant solvent extracts.

MATERIALS AND METHODS:

Sample Collection: The plant *C. collinus* was handpicked from the vicinity of Gopavaram village, Andhra Pradesh, India (17˚22’09.16” N 81˚47’48.42” E), in January 2022 and authenticated by Prof. N. Savithramma, Sri Venkateswara University, Botany Department, Tirupati, Andhra Pradesh, India, with accession number SVUH: 1226.

Extraction of Plant Material: Plant parts were harvested, dried in the shade at 32^{o}C and ground into a fine powder. The powder was then sieved through a 0.5 mm sieve to maintain consistent molecule size and stored in sterile containers until usage. For the extractions of leaves, bark and fruit, the following solvents were selected: methanol, ethyl acetate and chloroform in the ascending order of polarity. Using a soxhlet extractor, the phytochemicals from the plant material were isolated using the aforementioned solvents, which were then stored for 72 hours in sealed jars. A thick concentration was generated when the extracts were collected in a rotary evaporator at 45°C under reduced pressure. The crude extracts were kept in a 4°C refrigerator for future usage.

Collection of Test Pathogens: Four test fungal species; two non-filamentous human pathogens *Saccharomyces cerevisiae* (MTCC 4742), *Candida albicans* (MTCC 227) and two filamentous plant pathogens *Aspergillus niger* (MTCC 282) and *Aspergillus flavus* (MTCC 277) were utilized in

this work; they were acquired from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Preparation of Test Sample: The methanol, ethyl acetate and chloroform extracts were dissolved in 10% aqueous DMSO (Himedia) to get different concentrations (10 mg, 5 mg and 2.5 mg per 20µL). 10% aqueous DMSO was utilized as the negative control (solvent control). As positive reference standards, Fluconazole (Central Drug House P. Ltd., India) was employed for fungal strains at a dosage of 30µg/mL.

Antifungal Activity Assay: The agar well diffusion method was used to assess the antifungal potentialities of plant extracts in a Potato Dextrose Agar (PDA) medium. A sterilized L-shaped bent rod was used to disperse the 100 µL fungal inoculums evenly and allowed it to harden in the Petri plates.

With the aid of a sterile cork borer, 5mm-diameter wells were made on the plates and filled with various solvent extracts (10 mg, 5 mg and 2.5 mg per 20µL in 10% aqueous DMSO) positive and negative controls of 20 µL each.

After a 48-hour incubation period at 26°C, the inhibitory zone's diameter was determined. The usual positive and negative controls were fluconazole 30 µg/mL and DMSO (10%) and the results of each test were averaged after being repeated three times. A strict aseptic environment was maintained during the entire microbiological experiment.

GC-MS Analysis: The extracts were examined using the Agilent Technologies GC-MS (GC-8890, GC/MS 5977 MSD). With an 18 mL/min split flow and a 3 mL/min purge flow in the injection mode. The temperature of the oven was programmed to range from 75°C to a maximum of 360°C.

The column was a Polar Columns (DB-WAX) & HP-5 MS UI with a 1.21 mL/min flow rate and helium gas (99.99%) as the carrier gas. A temperature range of 60°C to 325^{o}C was set for the column. The GC-MS lasts for a total of 53.5 minutes. The sample pieces were ionized using the EI mode (70 eV). The spectra of the unknown components were compared to the spectrum of the known components using the Spectral Library and Database (licensed NIST 2017 Library; Software: Open Lab CDS 2.5 version).

RESULTS AND DISCUSSION:

Anti-fungal Activity: The antifungal bioassay findings against pathogens using crude extracts of the *C. collinus* plant (10 mg, 5 mg, and 2.5 mg of each) were described regarding the zone of inhibition's diameter. According to the observed inhibitory zones, which were also correlated with their tested doses, the entire extract and various fractions of *C. collinus* displayed strong antifungal activity against *C. albicans*, considerable anti-yeast ability against *S. cerevisiae* and moderate activity against *A. flavus* and *A. niger* **Table 1**.

The majority of the studied substances had strong inhibitory effects on *C. albicans*, among them fruit methanol crude extract showing the highest activity (25mm), followed by leaf methanol extract (21mm), bark ethyl acetate (19mm) and bark methanol (17.3mm). Methanol and ethyl acetate extracts showed high to moderate activity against test fungal pathogens while chloroform extracts had the lowest activity.

Here, *A. flavus* shows the strongest resistance to crude extracts of *C. collinus*, whereas *C. albicans* is the most vulnerable. It was discovered that the fractions containing the highest secondary metabolites concentration had higher levels of antifungal activity.

Moreover, the highest inhibitory properties against *A. flavus* were exhibited by the fruit methanol extract (12.3mm) followed by bark ethyl acetate (10.3mm) and bark methanol (10mm) extracts.

Whereas both ethylacetate and chloroform extracts of leaf and fruit did not show any activity. Contrary to *A. flavus*, *A. niger* was more susceptible to bark methanol extract (14mm) followed by fruit methanol extract (11.3mm).

The effectiveness of fluconazole has consistently outperformed that of plant solvent extracts, which has made it a recognized measure for comparing their effects. The DMSO containing well (negative control) exhibited no bioactivity.

TABLE 1: ANTIFUNGAL ACTIVITY OF DIFFERENT EXTRACTS (ME, EA &CH) AT THE DOSAGES OF 10mg, 5mg, AND 2.5mg

Plant part	Extract	*S. cerevisiae*			*C. albicans*			*A. flavus*			*A. niger*		
		10mg	5mg	2.5mg	10mg	5mg	2.5mg	10mg	5mg	2.5mg	10mg	5mg	2.5mg
Leaf	ME	8.6± 0.5	8±0	8±1	21±1	13.6± 0.5	12±1	6.6±. 0.57	6±0	-	7.6± 0.57	6.5± 0.7	-
	EA	8±1	-	-	15±1	12±1	10.3± 0.5	-	-	-	-	-	-
	CH	10±1	-	-	17±1	13±1	8±0	-	-	-	-	-	-
Bark	ME	13±1	9 ±1	8±1	17.3± 0.5	15±1	11.6± 0.5	10±1	8±1	6.6±.0 .57	14.3± 0.57	12± 1	9±1
	EA	8.3±0.5	7.3± 0.5	-	19.3± 0.5	14±1	10±1	10.3± .0.57	9.6± .0.5 7	7.6±.0 .57	9±1	7.6± 0.57	6.3±0. 57
	CH	7.6± 0.5	7.6±0 .5	-	11±1	9±1	8±1	-	-	-	-	-	-
Fruit	ME	10.6± 0.5	7.6± 0.5	7.6±0.5	25±1	22±1	21±1	12.3 ±.0.5 7	10± 1	6±0	11.3± 0.57	10± 1	7±1
	EA	8±1	7±0	-	13±1	11.3± 0.5	8±0	-	-	-	-	-	-
	CH	11±1	8.6±0 .5	7.6±0.5	16±1	12±1	8.6±0. 5	-	-	-	-	-	-

Values represent mean ± standard deviations; ME stands for methanol extract, EA for ethyl acetate, CH for chloroform, and "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive. Source: authors.

Furthermore, the antifungal activities of higher concentration (10mg) plant extracts were compared to the positive control fluconazole's inhibition capacity **Fig. 1**.

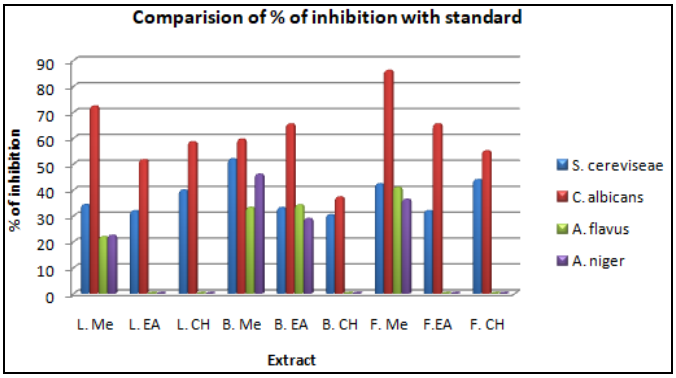


FIG 1: ANTIFUNGAL ACTIVITY (% OF INHIBITION) WITH RESPECT TO +' VE CONTROL

The highest percentage of inhibition (86.2%) against *C. albicans* was found in the fruit methanol extract *of C. collinus*, followed by leaf methanol (72.4%) and bark ethyl acetate (65.5%). The remaining eight plant extracts, except bark chloroform extract, demonstrated more than 50% inhibitory effect against *C. albicans* compared to the standard. All plant extracts inhibited *S. cerevisiae* growth in a medium-to-high range (30-55%). *S. cerevisiae* and *C. albicans* were more susceptible and all plant extracts tested inhibited their growth, whereas *A. flavus* and *A. niger* were more resistant and only methanol extracts with moderate activity, i.e., 20-45% when compared to standard, inhibited their growth. The antifungal activity of leaf acetone, water and benzene extracts from *C. collinus* studied by Majiet al., 2010 against *C.albicans* ranged from 13 to 8 mm[12] however in my study Methanol, Ethyl acetate and chloroform extracts of leaves showed higher activity ranged from 21 to 15 mm. Previous research employed aqueous and ethyl acetate solvent-based *C. collinus* leaf extracts against *C. albicans*, *C. tropicalis*, and *C. glabrata*, in which the ethyl acetate extract only showing action against *C. albicans* and other species being resistant [15]. In addition to previous data, the current investigation demonstrates that *C. collinus* methanol and ethyl acetate extracts are more efficacious than the chloroform extract. This might be because the kind of solvent utilized in the extraction technique substantially influences the subsequent characterization of phytochemicals. 1,2,3 Benzenetriole has strong antimicrobial properties [25, 26] and GC-MS analysis revealed that it was found in higher concentrations in all leaf, bark, and fruit methanol extracts; this may be a reason behind the results obtained in antifungal assays.

The antifungal screening supports *C. collinus* potentiality as a traditional cure for several fungal illnesses, including its usage as an antiseptic.

GC-MS Chemical Profiling: Methanol extracts were chosen for gas chromatography and mass spectrometry (GC-MS) analysis to examine the phytochemical composition because they displayed the highest antifungal efficacy compared to the other solvent extracts. This study demonstrates that a variety of physiologically active compounds are present in leaf, bark and fruit methanol extracts at various concentrations and also offers a thorough understanding of the phytochemical profile that may be exploited to create plant-based therapeutics.

GC/MS spectrum data of the methanol extracts showed several peaks **Fig. 2, 3** & **4** that indicated the presence of 14 different chemicals (9 from leaf, 6 from bark and 5 from fruit). Each of these chemicals was recognized and described using the mass fragmentation patterns and retention indices from the Spectral Library and Database (NIST 2017 Library). In **Table 1**, the active ingredients are listed in chronological order of retention durations, their peak area percentage, molecular formula, molecular weight, structural features and biological activities. To look at the biological effects of important substances, a review of the literature was conducted.

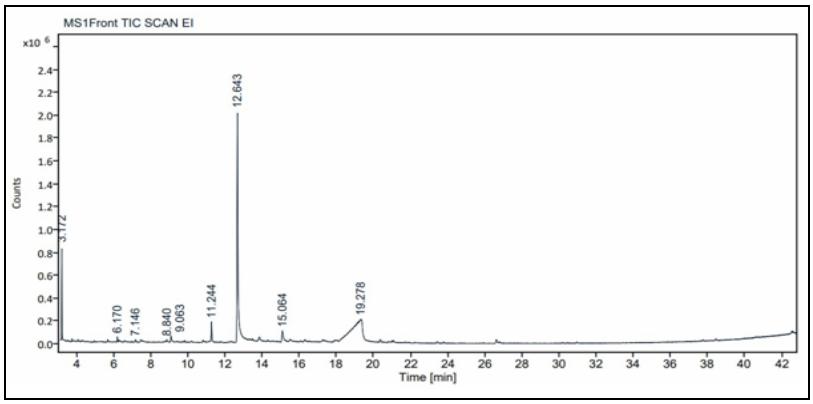


FIG. 2: GC-MS CHROMATOGRAM OF *C. COLLINUS* LEAF METHANOL EXTRACT

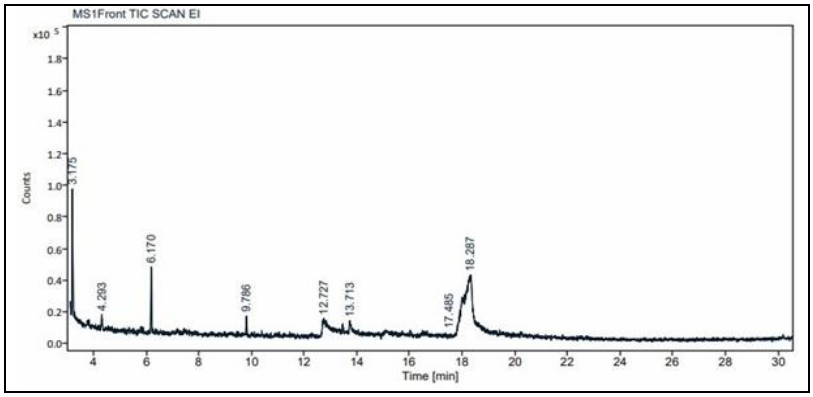


FIG. 3: GC-MS CHROMATOGRAM OF *C. COLLINUS* BARK METHANOL EXTRACT

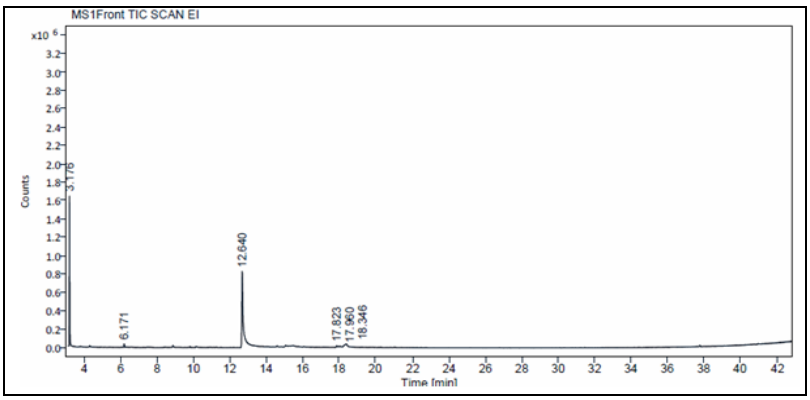


FIG. 4: GC-MS CHROMATOGRAM OF *C. COLLINUS* FRUIT METHANOL EXTRACT

We discovered that most of the constituents in the *C. collinus* leaf methanol extract exhibit a variety of advantageous pharmacological and therapeutic activities. The leaf methanol extract provides a rich source of significant bioactive molecules. Ten different chemicals, including hydrocarbons, phenols, terpenes and glucose derivatives, were found to be present. Undecane, 1, 2, 3-Benzenetriol and 3-O-Methyl-d-glucose were discovered in all three plant sections, whereas fruit and bark extracts contained N-[3-[N-Aziridyl] propylidene] hexylamine. A little amount of the following compounds were detected only in the leaf methanol extract: 4H-Pyran-4-one, 2, 3-dihydro - 3, 5 – dihydroxy – 6 - methyl, 6-Methyl-enebicyclo [3.2.0] hept-3-en-2-one, 5-Hydroxymethylfurfural, Phenol, 5-ethenyl-2-methoxy and D-Allose. Only bark extracts included decane, 4(3H)-Pteridinone, 2-amino-7, 8-dihydro-8-methyl-6, 7-diphenyl and fruit extracts had trace amounts of phthalic acid, 5-methylhex-2-yl heptadecyl ester.

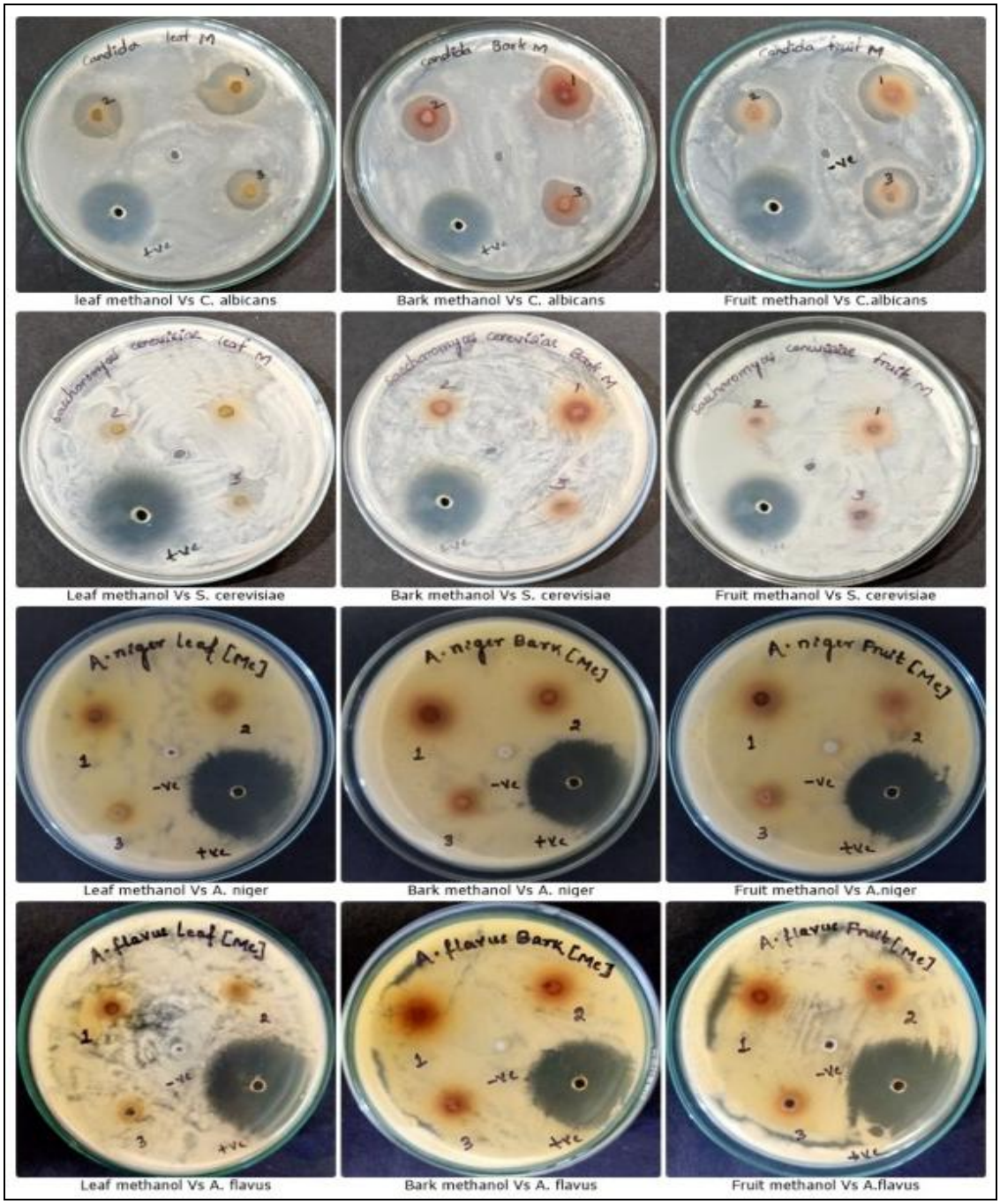
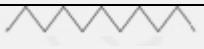
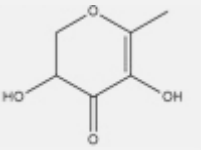
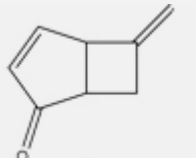
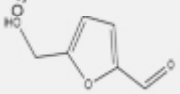
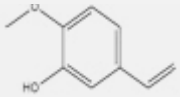
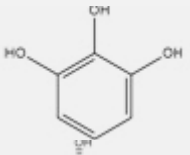
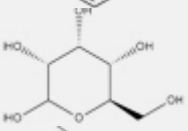
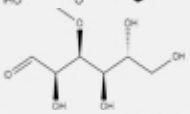
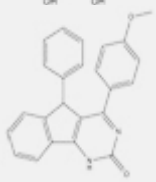
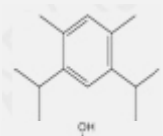
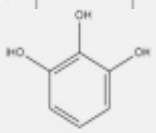
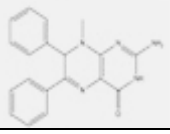
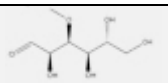
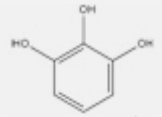
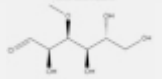


FIG 5: PICTORIAL DATA OF ANTIFUNGAL ACTIVITY OF *C. COLLINUS* (LEAF, BARK & FRUIT) METHANOL EXTRACTS

TABLE 2: BIOACTIVE CHEMICAL PROFILE OF *C. COLLINUS* THROUGH GC-MS

S. no.	Name of the compound	RT minutes	Mol. weight	Mol. formula	Area %	Structure	Importance
				Leaf			
1	Undecane	6.169	156.31	$C_{11}H_{24}$	0.55		Mild sex attractant[20]anti-inflammatory and antioxidant effects[21]
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.144	144.12	$C_6H_8O_4$	0.35		Antimicrobial, anti-inflammatory, Anti-proliferative[22]
3	6-Methylenebicyclo[3.2.0]hept-3-en-2-one	8.838	120.15	C_8H_8O	0.19		Bacteriostatic, fungistatic, anti-parasitic activity[23]
4	5-Hydroxymethylfurfural	9.063	126.11	$C_6H_6O_3$	0.43		Anti-oxidant activity[24]
5	Phenol, 5-ethenyl-2-methoxy-	11.245	150.17	$C_9H_{10}O_2$	2.00		Aromatic, flavoring, cytotoxic, anti-inflammatory, germination inhibitor, insect repellent[19]
6	1,2,3-Benzenetriol	12.645	126.1100	$C_6H_6O_3$	35.95		Anti-microbial, Antibiofilm and Antivirulence activity[25,26]
7	D-Allose	15.064	180.16	$C_6H_{12}O_6$	1.72		Anti-oxidant, Anti-tumour activity[27,28]
8	3-O-Methyl-d-glucose	19.277	194.18	$C_7H_{14}O_6$	25.33		Anti-tumor activity[29]
9	2-Oxo-5-phenyl-4-(4-methoxyphenyl)-1,2-dihydro-5H-indeno[1,2-d]pyrimidine	49.245	366.4	$C_{24}H_{18}N_2O_2$	27.54		-
				Bark			
1	Decane	4.294	142.28	$C_{10}H_{22}$	2.09		cocarcinogenic and tumor-promoting activities[30]
2	Undecane	6.169	156.31	$C_{11}H_{24}$	7.42		Mild sex attractant[20]anti-oxidant and anti-inflammatory activities [21]
3	Benzene, 1,5-dimethyl-2,4-bis(1-methylethyl)-	9.788	190.32	$C_{14}H_{22}$	2.89		-
4	1,2,3-Benzenetriol	12.726	126.1100	$C_6H_6O_3$	5.10		Anti-microbial, Apoptosis, Antibiofilm and Antivirulence activity[25, 26]
5	4(3H)-Pteridinone, 2-amino-7,8-dihydro-8-methyl-6,7-diphenyl-	13.714	331.4	$C_{19}H_{17}N_5O$	1.84		-

6	3-O-Methyl-d-glucose	18.290	194.18	$C_7H_{14}O_6$	67.88		Anti-tumor activity[29]
				Fruit			
1	Undecane	6.169	156.31	$C_{11}H_{24}$	0.95		Mild sex attractant[20]anti-oxidant and anti-inflammatory activities[21]
2	1,2,3-Benzenetriol	12.639	126.1100	$C_6H_6O_3$	63.09		Anti-microbial, Apoptosis, Antibiofilm and Antivirulence activity[25,26]
3	N-[3-[N-Aziridyl]propylidene] hexylamine	17.821	182.31	$C_{11}H_{22}N_2$	0.69		Anti-viral activity[31]
4	Phthalic acid, 5-methylhex-2-yl heptadecyl ester	17.959	502.8	$C_{32}H_{54}O_4$	0.17		Aminoglycoside antibiotic, Neuroprotective[32]
5	3-O-Methyl-d-glucose	18.346	194.18	$C_7H_{14}O_6$	4.03		Anti-tumor activity[29]

The chemicals found in this study were mostly stated to have anti-cancer, antibacterial, anti-virulence, anti-inflammatory, neuro-protecting, and antioxidant effects according to the reported biological activities. 1, 2, 3-Benzenetriol is a phenolic molecule that is most abundantly present in fruit extracts (63.09%), leaf extracts (35.95%) and bark extracts (5.1%). It is an effective anti-microbial and antiviral agent and leads to the death of cancer cells by apoptosis. The highest concentration of 3-O-Methyl-d-Glucose, a glucose derivative with good anti-cancer capabilities, was identified in the bark extract (67.88), followed by the leaf (25.33) and the fruit extract (4.03). Undecane is a hydrocarbon compound that is present in fruit and bark extracts in low amounts and high concentrations in bark extract (7.42).

It possesses anti-inflammatory and antioxidant activities. Decane contains co-carcinogenic and tumor-promoting properties but is reported only in bark extract. 2-Oxo-5-phenyl-4-(4-methoxyphenyl)-1, 2-dihydro-5H-indeno [1, 2-d] pyrimidine reported in leaf extract in high concentration (27.54), although its bioactivities have not been studied. Earlier work by Pratheepa M. *et al.,* 2012 revealed the GC-MS analysis of the plant leaf acetone extract which showed 8 different bioactive compounds from which the novel chemical dioctyl phthalate observed at a peak area of 95.23% which may operate as a very promising medicinal agent for persons suffering from cancer or afflicted with fatal diseases, *etc* [16]. Arulmanickam P. and Chitra T. *et al.,* 2020 studied leaf powder ethanolic extract and revealed 13 different types of bioactive compounds, but only six of them can carry out specific biological functions. These include alpha-methyl mannofuranoside (36.25%) for its antibacterial and antifungal activity, undecanoic acid (1.88%) for its cytotoxic, anticancer, and anti-microbe properties, 5-O-methyl-D-gluconic acid dimethylamide (4.86%) for its antioxidant and anti-microbial properties, Cyclopropanetetradecanoic acid, 2-Octyl-, Methyl ester (3.20) for its anti-microbial activity and Pentanoic acid, 2 (Aminooxy) (3.59%) for its Anti-oxidant and lubricantproperties [17].

Suman *et al.,* 2013 found four bioactive compounds in the leaf's aqueous, hot extracts, with 4-OMethylmannose (70.12%) having antibacterial activity and 1,2,3-Benzenetriol (21.76%) having antioxidant, antibacterial, antiseptic, fungicide, antimutagenic, antidermatitic, pesticide, dye and candidicide properties. In contrast, the methanol extract contained significant amounts of thiophene (antimicrobial action), tetrahydro-2-methyl-myo-inositol (48.54%), 1,2,3-benzenetriol (7.51%) and Vitamin E (d -α-tocopherol) (6.52%). Higher concentrations of 4-O-methylmannose (Secondary messenger) and 5-Methyl-2-trimethylsilyloxy-aceto phenone (7.35%) were found in the ethanol extract. In the ethyl acetate extract, there were 15 compounds detected, the main ones being silane (Coupling Agent) and trimethyl [5-methyl-2-(1-methyl ethyl) phenoxy] anthracene (7.06%) [9].

Parasuraman *et al.*, 2009 reported 17 compounds, of which 14 are from fresh leaves and 5 are from dry leaves. 3-Omethyl-d-glucose (32.88%), Benzenetriol (22.43%), 1.6- Anhydro - â - Dglucopyranose (15.66%), Heptacosane (8.99%), 2-Hydroxy-7methoxy-4,5 diphenyl-5 Hindeno [1.2-d] pyrimidine (7.45%) and Eicosane (3.76%) were found in fresh leaves, whereas 3-O-methyl-d-glucose (41.61%), Benzenetriol (Pyrogallic acid, Pyrogallol) (25.06%), n- Hexadecanoic acid (10.60%), Heptacosane (4.41%) and 1,2-Benzenedicarboxylic acid, diisooctyl ester (3.54%) were present in dried leaves aqueous extracts [18]. Kazi M. and Gude A. *et al.*, 2022 summarized the phytoconstituents and discussed 46 compounds and their biological properties [19]. Only 1,2,3-Benzenetriole (35.95) and 3-O-methyl-d-glucose (25.33) were previously reported of the 9 compounds we found in leaf methanol extract; the other compounds were discovered for the first time. To the best of my knowledge, no studies on the GC-MS analysis of methanol extracts of *C. collinus* fruit and bark have been published yet. In contrast to earlier findings, we present the most recent analysis of the GC-MS profile of the methanolic extracts of leaves, bark and fruits in our investigations.

CONCLUSION: These findings have established a solid scientific foundation for the use of the plant *Cleistanthus collinus* in traditional medicine to treat fungal infections. Compared to chloroform extracts, methanol and ethyl acetate extracts showed high antifungal activity. In particular, *C. albicans* and *S. cerevisiae*, which displayed the strongest antifungal activity, excelled over other fungal strains examined. GC-MS study revealed 15 phytochemical components with antifungal, antibacterial, antioxidant, anti-inflammatory and anti-cancer properties. As far as I am aware, very little work has been done on the antifungal activity of *C. collinus*, as there is very little literature accessible on the antifungal activity of this plant species. We believe that more studies in this area will aid in developing novel antifungal and anticancer drugs.

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