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Exploring antifungal activities of *Rhazya stricta* and *Azadirachta indica* leaves against human black fungus

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Abstract

Mucormycosis or black fungus infection originates from Mucor which belong to Mucorales order of fungi. At present mucormycosis spread vastly attacks human beings. Immunosuppressive glucocorticoid drugs are used widely in SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). These article aimed to discover antifungal therapies using medicinal plants has increased for the following reasons: 1- The richness of plant flora in Saudi Arabia and the low knowledge in the use of medicinal plants, 2- the 2030 vision of Saudi Arabia's mutual interest in medicinal plants using *Rhazya stricta* and *Azadirachta indica,* were collected during January 2021 from saline soil, Plant Extraction using; methanol (70%), and distilled water, GC-MS analysis, then antifungal activity were examined, and observe the extraction effect on the fungus cell by Electron microscope. Results declared that the aqueous and methanolic leaf extracts of *R. stricta* and *A. indica* possess efficient antimicrobial effects against the test bacterial strains, particularly the methanolic extracts.

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Introduction

Mucormycosis is the third aggressive fungal disease after the candidiasis and aspergillosis (Reid and Lynch, 2020). Mucormycosis or black fungus infection originates from Mucor which belong to Mucorales order of fungi (Riley *et al.*, 2016). Mucorales order has 150 genera and 250 species. They are saprophytic species which grow on dead organicmaterial and few species are parasitic in nature. These types of fungus are also known as black molds because ofthe presence of black spores. The mycelium is profusely branched aseptate. At present mucormycosis spreadvastly attacks human beings. Widespread uses of immunosuppressive drugglucocorticoids in SARS–CoV–2 (severe acute respiratory syndrome coronavirus–2) treatment causedsecondary bacterial and fungus infections leads to higher fatality among the COVID–19 (coronavirus disease–2019) patients (Ibrahim *et al.*, 2012; Skiada *et al.*, 2020 and Hernández *et al.*, 2021). SARS–CoV–2 infection damages the lungs and other vital organs (Chakrabarti and Dhaliwal, 2013; Ruhnke *et al.*, 2015 ; Prakash *et al.*, 2019). Additionally, the immune system of the host may be suppressed due to use of glucocorticoids, an immunosuppressive drug (Roden *et al.*, 2005; Saegeman *et al.*, 2010). To combat the situation of current pandemic COVID–19, the Governments taken strict action by posing the lockdown in different phases, which was a challenge for the society, education, industry, and economy as well (Mehta and Pandey, 2020; Sharma *et al.*, 2021).

Presence of black fungus mostly infects sinuses and nose although it can be evident in the lungs. After the commonsite the spores spread to eyes and brain causing blindness and headache respectively. Black fungus enters the body through skin injury mediated by burnt, cut and scrape. Rhinocerebral (brain & sinus), disseminated mucormycosis, lung mucormycosis, gastrointestinal mucormycosis and cutaneous mucormycosis are the types of mucormycosis (Choudhary, *et al.*, 2021 and Vasudevan *et al.*, 2021).

In the background of the COVID-19 pandemic, an invasive fungus, mucormycosis has also set its foot in, worsening the scenario. Mucormycosis is an opportunistic angio-fungal infection predominantly occurring in subjects with low immunity, which can turn fatal. It is a severe but uncommon systemic mycosis caused by Mucor or Rhizopus species of Mucoraceae family. This disease, which is most prevalent in the tropical regions, is more pronounced in Asia, leading among them. The sudden rise in cases in the background of COVID-19 due to multitudinous reasons and the high mortality rate, despite appropriate therapies, has created panic among the public as well as the health-care system (Kasimadom, 2021).

Many indoor and outdoor biotic environments, with almost food items, offering all the growth element of Mucorales (Richardson *et al.*, 2020). Outdoor habitat such as soil considered the Mucorales major habitat. *Rhizopus* spp. and *Mucorales* spp. are hydrophilic which required a high moisture for growth (Caetano *et al.*, 2018). A variety of *Mucorales* spp. (Syncephalastrum, Lichtheimia, and Rhizopus) has also been found in food items including; Mucorales-contaminated food items which mainly affected immunocompromised individuals (Richardson, 2009; Mousavi *et al.*, 2018; Chandley *et al.*, 2022).

The medicinal plants discovered new methods of treatment, which have higher economic value and fewer side effects, has become particularly important worldwide (Aware *et al.*, 2022; Jităreanu *et al.*, 2023; Tnah *et al.*, 2019). These valuable biological resources to discover antifungal therapies using medicinal plants has increased for the following reasons: 1- The richness of plant flora in Saudi Arabia and the low knowledge in the use of medicinal plants, 2- the 2030 vision of Saudi Arabia's mutual interest in medicinal plants (Alharbi, 2017).

Rhazya stricta is one of Apocynaceae family and the Rauwolfioideae subfamily. This species is distributed and has been used as a drug to many diseases in; Qatar, India, Pakistan, Afghanistan, Iran, Iraq, and Saudi Arabia (Khan *et al.*, 2016; Fazeli-Nasab *et al.*,

2021). *R. stricta* (known in Saudi Arabia as harmful) is a poisonous, erect shrub small, evergreen, with hairless leaves (Ribeiro-Santos *et al.*, 2018; Shehzad *et al.*, 2018).

R. stricta and *A. indica* metabolites' containing many effective compounds which can be used as treatment a lot of dangerous diseases like; hypertension, syphilis, cancer, rheumatism, skin diseases, sore throat, and fever. Many studies have declared that different parts of *R. stricta* had several phytochemical elements such as flavonoids, terpenes, and alkaloids. Research on *R. stricta*, a plant native to Asia, has shown that it has a number of healing properties. Antimicrobial activity of different concentrations of five solvent extracts, i.e. alkaloids, aqueous, non-aqueous alkaloids, organic alkaloids, organic non-alkaloids and complete aqueous extracts (Mohamed *et al.*, 2023). Raw ethanolic extract of *R. stricta* fruit has antimicrobial activity. Methanolic and chloroform root extracts of *R. stricta* plant have antifungal and antimicrobial activity (Gao *et al.*, 2019; Wu and Shaw, 2022).

From many years ago, neem beneficial properties have been determined which includes its medicinal effects inaddition to its agricultural pest control and in traditional medicine. *A. indica* initially sparked interest across the globe because of its potential to be used in farming as a non-toxic infection-control agent. In fact, azadirachtin, one of the most prevalent substances in neem plants, is a biopesticide that is becoming more and more popular (Chaudhary *et al.*, 2017; Pasquoto-Stigliani *et al.*, 2017; Kilani-Morakchi *et al.*, 2021; Wylie *et al.*, 2022).

However, different parts of the neem tree have been used for thousands of years in traditional Indian medicine due to their supposed benefits for various conditions, including dental, antifungal, anticancer, antidermatitic, antipyretic, antacid, antiparasitic, antibacterial, antiviral, antidiabetic, contraceptive, and antidermatitic (Alzohairy, 2016).

Nearly all of *A. indica's* parts, including the gum, fruits, bark, gum, stem, roots, leaves, flowers, seeds, and have been utilized as common home cures for ailments in people. Additionally, millions of individuals utilize neem twig chewing sticks for oral hygiene throughout the world (Gupta *et al.*, 2017; Aumeeruddy and Mahomoodally, 2021; Iman *et al.*, 2021; Patil *et al.*, 2021; Singh *et al.*, 2021; Yarmohammadi *et al.*, 2021).

While there are many methods for preventing fungal infections, chemical control is still one of the most important ones because it can reduce the incidence of fungal infections without having to worry about secondary side effects or the development of microbial resistance to the chemicals. To safeguard Corona patients, researchers are looking for naturally occurring antifungal medicines that may be employed. Present article goal to determine the antifungal efficacy of *R. stricta* and *A. indica* leaf extracts against pathogenic fungi that cause the "black fungus" disease *Mucormycosis*.

Material and methods

Plant collection

Leaves of *Rhazya stricta and Azadirachta indica* respectively about (1 kilo gram from each plant) were collected during January 2021 from saline soil of Taiba region (latitude: 21.7874726, longitude: 39.1459682) about 17 kilometers from the Red sea. The plant was gathered, labeled, and delivered to the University of Jeddah's College of Science laboratories. There, the leaves were dried, rinsed, and ground using a blender before being sieved through a 1 mm aluminum sieve and kept in labeled, airtight bottles until needed (Mukne *et al.*, 2022).

Plant extraction techniques

Plant Extraction techniques including using solvents: methanol (70%), and water that has been distilled. In a nutshell, 100g of dry powder was extracted using 1000ml and 500ml of solvents through maceration at room temperature for 48 hours for each solvent. Next, each mixture was filtered twice using N°1 whatman paper and filter paper with a porosity of 0.45 µm. Separate drying of the obtained filtrates was done at 50°C in a Laborota 4000 rotary evaporator. Aqueous and aqueous methanol crude extract powder

were utilized to screen for antioxidants, determine the total phenol content, and study phytochemical components (El-Shora and Abd El-Gawad, 2014).

Test fungal organisms

The test fungal organisms in the present study Mucormycosis "black fungus" (Meyen ex Hansen) were collected from Department of microbiology, collage of science, University of Jeddah.

GC-MS analysis

GC-MS analysis About 3 minutes hold period was used with a 35°C initial column temperature. The temperature was programmed to rise by 8°C every minute, with a maximum temperature of 280°C. After injecting 1µl of the sample into the port, it vaporized instantly and down the column. After the sample was inserted into the port during the operation, a 1 ml/min flow of helium was utilized as the carrier gas to move it down the column. The MS spectrum was recorded at 70 eV. After the separation of the columns, FID identified the components and performed further analysis on them. The names, molecular weights, and structures of the compounds were ascertained by matching their spectra with those of recognized compounds in the NIST MS 2.0 structural database.

Antifungal activity examination

Each extract was given a stock solution containing 0.2 g/mL of dimethyl sulfoxide (DMSO) for the antifungal activity testing. The stock was kept at 4C until it was needed, and the extracts were kept at 20C. The agar well diffusion method was utilized to evaluate the fungicidal activity of the extracts using Sabouraud's Dextrose Agar. The extracts were incubated at 28±2˚C/7d and the fungal growth was monitored on a regular basis to estimate the minimum inhibitory concentration (MIC) in millimeters (mm). For every clinical isolate, the antifungal activity was replicated twice using two replicates of each plant extract at every test concentration (Zain *et al.*, 2012). The test extracts of desired concentrations (500 ppm, 1000 ppm) in Aqueous and methanollic solvents, each inoculated with an inoculum of 5 mm diameter of each extract and incubated for seven days (Zohra *et al.*, 2013).

Electron microscope

Samples were processed through an automated tissue processor (Leica EM TP, Leica Microsystems: Austria) and fixed with gluteraldhyed 2.5% and dehydrated with ethanol while being stirred. A CO, critical point drier (Model: Audosamdri-815, Tousimis; Rockville, Maryland, USA) is then used to dry the material. Gold sputter coater (SPI-Module, USA) applied to the sample. Using scanning electron microscopy (model: JSM-5500LV; JEOL Ltd – Japan), the sample was examined.

Statistical analysis

11.0 for Windows was used for the analysis, and the results were presented as means ± standard error (SE). To determine whether there were any significant differences between the means, multiple comparisons and one-way analysis of variance (ANOVA) were used. At the 5% confidence level, differences in means were regarded as significant. In SPSS 11.0 for Windows, correlations between variables were calculated using the regression model.

Results

Gas chromatography of Rhazya stricta and Azadirachta indica leaf extracts

Utilizing gas chromatography and mass spectroscopy, the bioactive components of Rhazya stricta's methanolic extract were examined.

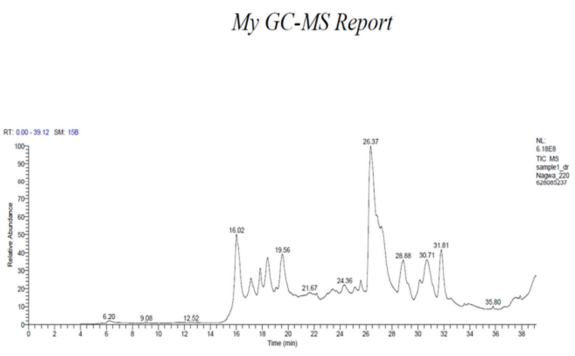

Fig. 1. GC analysis peaks of *Rhazya stricta* leaves methanol extraction (cm)

Table 1. Phytochemical constituents identified within the leaf methanol extract of *Rhazya stricta* by GC-MS analysis

Peak no.	RT	Compound name	Area%	MF	Molecular formula	Molecular weight
1	16.03	Spathulenol	12.80	920	$C_{19}H_{30}O_2$	290
2	17.13	Octadecadiynoic acid, methyl ester	1.53	838	$C_{12}H_{26}OSi$	214
3	17.85	1h-3a,7-methanoazulene, Octahydro-3,8,8-trimethyl-6-methylene	2.34	828	$C_{15}H_{24}$	204
4	18.42	Caryophyllene oxide	4.59	866	$C_{15}H_{24}O$	220
5	24.29	Isoaromadendrene epoxide	0.89	852	$C_{15}H_{24}O$	220
6	24.36	Heptatriacotanol	0.74	807	$C_{37}H_{76}O$	536
7	25.60	Dodecanoic acid,	1.19	763	$C_{19}H_{34}O_6$	358
8	26.34	Heptadecyn	31.71	682	$C_{17}H_{32}o$	252
9	26.89	Hexadecenoic acid	0.72	717	$C_{17}H_{32}O_2$	268
10	27.32	Pentadecanoic acid	3.22	763	$C_{15}H_{30}O_2$	242
11	28.87	Octadecatrienoic Acid, 2,3-dihydroxypropyl Ester	10.3	821	$C_{21}H_{36}O_4$	352
12	30.12	Docosatetraenoic Acid, methyl ester	1.56	800	$C_{23}H_{38}O_2$	346
13	30.71	Octadecadienoyl chloride	9.11	835	$C_{18}H_{31}CIO$	298
14	31.80	Aspidospermidine	9.30	849	$C_{19}H_{24}N_2$	280
15	37.89	Cyclic butylboronate	0.38	687	$C_{25}H_{34}O_7$	446

Table 2. Phytochemical constituents identified within the leaf methanol extract of *Azadirachta indica* by GC-MS analysis

Peak no.	RT	Compound name	Area%	MF	Molecular formula	Molecular weight
1	15.76	Heptadienal, 2-ethylidene-6-methyl	0.41	713	$C_{10}H_{14}O$	150
2	16.06	Heptadienal	1.16	715	$C_{10}H_{14}O$	150
3	16.33	Carbonitrile	1.28	715	$C_{20}H_{27}NO_2$	313
4	16.69	Tetradecadiynoate	1.50	755	$C_{15}H_{22}O_2$	234
5	17.53	Heptadecynyloxy	1.91	718	$C_{22}H_{40}O_2$	336
6	18.77	Octadecadiynoic acid, methyl ester	6.46	782	$C_{19}H_{30}O_2$	290
7	19.30	Cyclopropaneoctanoic acid	0.62	749	$C_{22}H_{38}O_2$	334
8	19.39	Picrotoxin	0.66	756	C15H1606	292
9	19.69	Octadecatrienoic acid	1.43	765	$C_{27}H_{52}O_4Si_2$	496
10	19.95	Pentacosadiynoic acid	0.71	792	$C_{25}H_{42}O_2$	374
11	20.03	Caryophyllene oxide	0.71	778	$C_{15}H_{24}O$	220
12	20.23	Epiglobulol	1.75	786	$C_{15}H_{26}O$	222
13	20.68	Octadecanal	0.46	720	$C_{18}H_{34}D_2O$	270
14	20.97	Retinal	2.04	835	$C_{20}H_{28}O$	284
15	21.10	Eicosapentaenoic acid	1.09	758	$C_{20}H_{30}O_2$	302
16	23.46	Octadecenoic acid	2.14	793	$C_{18}H_{34}O_2$	282
17	23.90	Hydroxyand rostane	0.23	757	$C_{19}H_{24}O_3$	300
18	23.96	Heptatriacotanol	0.43	780	$C_{37}H_{76}O$	536
19	24.31	Aspidospermidine	1.81	769	$C_{21}H_{26}N_2O_2$	338
20	25.29	Oxiraneundecanoic acid	3.75	748	$C_{19}H_{36}O_3$	312
21	25.64	Isochiapin	0.81	710	$C_{19}H_{22}O_6$	346
22	26.56	Hexadecanoic acid	4.65	827	$C_{18}H_{36}O_2$	284
23	27.08	Isopropyl palmitate	14.32	824	$C_{19}H_{38}O_2$	298
24	28.56	Cholestan	9.32	826	$C_{28}H_{48}O$	400
25	28.98	Eicosenoic acid	0.59	739	$C_{20}H_{38}O_2$	310
26	29.75	Cyclopropaneoctanoic acid	11.73	834	$C_{22}H_{38}O_2$	334
27	30.74	Octanoic acid	0.14	845	$C_{21}H_{38}O_2$	322
28	30.90	Cyclopropaneoctanoic acid	0.70	803	$C_{22}H_{38}O_2$	334
29	32.50	Docosatetraenoic acid, methyl ester	0.19	787	$C_{23}H_{38}O_2$	346
30	32.60	Eicosatetraenoic acid, methyl ester	0.50	817	$C_{21}H_{34}O_2$	318
31	35.42	Heptadecen	0.64	760	$C_{18}H_{30}O_2$	278
32	36.31	Benzopyran	1.53	717	$C_{27}H_{30}O_{16}$	610

In Fig. 1 and Table 1, the active compounds are displayed together with their molecular weight, retention period, and beak area. The plant extract revealed the existence of several phytochemical substances with antibacterial properties, including; Spathulenol, Octadecadiynoic, Caryophyllene oxide, Isoaromadendrene epoxide, Heptatriacotanol, Dodecanoic acid, Heptadecyn, Hexadecenoic acid,

Pentadecanoic acid, Octadecatrienoic, Docosatetraenoic Acid, Octadecadienoyl chloride, Aspidospermidine, Cyclic butylboronate. Although the active compounds of *Azadirachta indica* are shown with their retention time, molecular formula, molecular weight and beak area in Fig. 2 and Table 2 as follows; Heptadienal, Carbonitrile, Tetradecadiynoate, Heptadecynyloxy, Octadecadiynoic acid, Cyclopropaneoctanoic acid, Picrotoxin, Octadecatrienoic acid, Pentacosadiynoic acid, Caryophyllene oxide, Epiglobulol, Octadecanal, Retinal, Eicosapentaenoic acid, Octadecenoic acid, Hydroxyand rostane, Heptatriacotanol, Aspidospermidine, Oxiraneundecanoic acid, Isochiapin, Hexadecanoic acid, Isopropyl palmitate, Cholestan, Eicosenoic acid, Cyclopropaneoctanoic acid, Octanoic acid, Docosatetraenoic acid, Eicosatetraenoic acid, Heptadecen, Benzopyran.

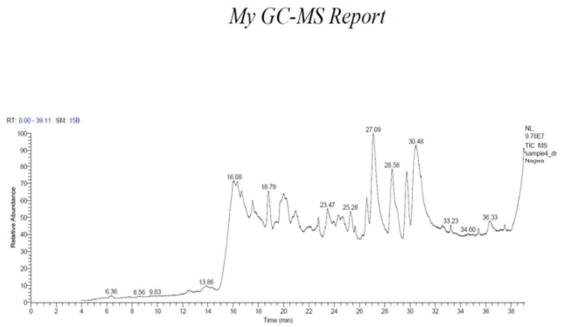

Fig. 2. GC analysis peaks of *Azadirachta indica* leaves methanol extraction (cm)

Table 3. Antifungal assay of *Rhazya stricta* and *Azadirachta indica* extracts against Mucormycosis "black fungus" extract in different solvents (per millimeter)

Solvent	Conc.	*Rhazya stricta*		*Azadirachta indica*	
		500 ppm	1000 ppm	500 ppm	1000 ppm
Aqueous	Minimum	00	00	00	00
	Maximum	25	40	12	40
	Mean	15±0.45	20±0.70	8±0.22	18±0.60
Methanol	Minimum	9	12	00	00
	Maximum	28	43	15	25
	Mean	16±0.80	23±0.99	10±0.41	20±0.56

The bioactive analysis of *Rhazya stricta* and *Azadirachta indica* methanolic extracts declared that *Azadirachta indica* contained a lot of active ingredients than *Rhazya stricta* as it was having more than 32 bioactive compounds and their derivatives while *Rhazya stricta* contained about 15 bioactive compounds only. Although both plants extraction bioactive compounds have an antimicrobial effect, but the richness of *Azadirachta indica* was the main cause that increase its effectiveness against different microorganisms.

Antifungal assay of Rhazya stricta and Azadirachta indica extracts against Mucormycosis "black fungus" extract in different solvents (per milimeter)

Table 3 declared the in vitro inhibitory effect of the water and methanolic extractions in comparison with commercial antifungal as follows; the most effective extraction detected by methanolic extraction of *Rhazya stricta* (16±0.8 and 23±0.99) mm at 500 ppm and 1000 ppm respectively, while, aqueous extractions of *Rhazya stricta* has lower inhibition effect as following (15±0.45 and 20±0.7). although methanolic extraction of *Azadirachta indica* recorded wider inhibition zone than water extraction as following; (10±0.41 mm), (20±0.56 mm) at 500 ppm and 1000 ppm respectively, on the otherside it ranged about (8±0.22 and 18±0.6 mm) at 500 ppm and 1000 ppm respectively of *Azadirachta indica* water extraction.

The results showed the better effect of methanolic extracts than aqueous extractions. Although, 1000 ppm has higher effect than 500 ppm. On the otherhands *Azadirachta indica* has lower effect than *Rhazya stricta*.

The effect of Rhazya stricta and Azadirachta indica extracts on the morphology of normal Mucormycosis "black fungus"

Fig. 3 shows how the typical shape of the Mucormycosis "black fungus" was compared to the altered shape caused by the addition of varying amounts of Rhazya stricta and Azadirachta indica extracts using images taken using a scanning electron microscope (SEM) at two different magnifications (X5000, X1000). The ovoid "yeast" cells with budding that were indicative of the Mucormycosis "black

fungus" were depicted in Fig. (A). Even though Fig. (B) depicts the fungus cells' and their colonies' altered shapes, this change began with the cells shrinking to take on their typical oval shape. Additionally, the colony's prior cell stacking was lost when Mucormycosis, also known as the "black fungus," was exposed to extracts from Rhazya stricta and Azadirachta indica at higher concentrations, which caused the cells to deform until their original shape vanished entirely.

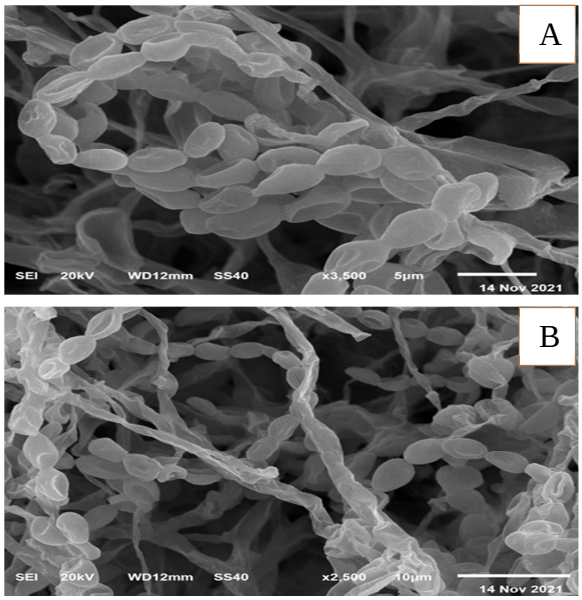

Fig. 3. Scanning electron microscope of antifungal activity of *Rhazya stricta* and *Azadirachta indica* extracts against Mucormycosis “black fungus”; (A) represent the fungal cell at ×5000, (B) represent the fungal cell picture at ×1000

Discussion

Mucormycosis is a deadly agioinvasive infection caused by fungi of the order Mucorales with an incidence that has grown in the last years (Prakash *et al.*, 2019; Skiada *et al.*, 2020), although this number is likely to be severely underestimated (Skiada *et al.*, 2020; Soare *et al.*, 2020). Traditionally, this infection has received scarce attention because of the low number of cases in comparison with other more frequent fungal infections, but the emerge of the SARS-CoV-2 disease (COVID-19) pandemic has increased the incidence of fungal infections, rising the concern about their risks (Sharma and Goel, 2022). The upsurge in COVID-19-associated mucormycosis with prevalence 50 times higher than the uppermost recorded data (Hussain *et al.*, 2021) has highlighted the unmet need to better understand mucormycosis (Sarkar *et al.*, 2021).

Mucormycosis is a complex fungal infection for several reasons. Despite affecting most frequently individuals with underlying pathologies reducing the immune response, around 19% are immunocompetent patients that have suffered trauma or burn wounds (Roden *et al.*, 2005; Revie *et al.*, 2018). In addition, the clinical presentation is diverse and linked to the underlying pathology with rhino-orbital-cerebral mucormycosis as the most frequent manifestation followed by cutaneous, pulmonary, disseminated, gastrointestinal, and others (Jeong *et al.*, 2019; Muthu *et al.*, 2021).

There are four types of Mucormycosis; Rhinocerebral mucormycosis which considered arare fungal disease which affects the sinuses, nose, and brain (Rapidis, 2009). The sinus's infection spread to the brain.Those persons who have kidney transplantation and uncontrolled diabetes may suffer from rhinocerbral mucormycosis. Some research reaffirms that 9% of rhinocerbral mucormycosis cases found in those patients without any predisposing factors (Spellberg *et al.*, 2005). Symptoms of rhinocerebral mucormycosis are headache and fever. For the treatment of mucormycosis 5mg/ml concentration of Amphotericin B is used (Zhou, *et al.*, 2020). Disseminated mucormycosis is a type of infection commonly affects the brain but when infection spreads through the bloodstream it can affect other parts and organs of the body such as heart, spleen and skin. For the treatment regime is ovuconazole 200 mg/day is utilized to maintain its plasma level above 1000mg/ml (Song *et al.*, 2020). Pulmonary mucormycosis is most common in cancer, organ and stem cell transplant patients. Common traits and symptoms of this disease are high body temperature (>100 °F), chest pain, difficulty in breathing or coughing that produce bloody or dark fluids. For treatment regime liposomal amphotericin B as a

monotherapy is administered in thedoses of (2–4 mg·kg–1·d–1) (Mohindra, *et al*., 2007; Shang *et al*., 2020). Cutaneous mucormycosis is the skin infection when fungi enter the body through damaged skin (due to surgery, severe burn or any typeof skin injury). Symptoms are facial pain, small bubble on the skin filled with serum, skin ulcers, infected skinarea turning black, warmth and excessive reddening, swelling around infection. Amphotericin B is mostactive drug for cutaneous mucormycosis, with a dose of 5–10mg/kg/day (Pak *et al*., 2008). Gastrointestinal mucormycosis is common among young children especially low birth weight and premature infants with age less than 1month, who have had surgery or on medications that lower the body's ability to fight illness. This disease can affect multiple organs and body parts. Discoloration of nose, blurred vision, abnormal breathing, chestpain, and blood in cough are common symptoms which depend upon the sites of infection in the host's body (Bourgonje *et al*., 2020).

Antifungal activity of *Rhazya stricta* and *Azadirachta indica* extracts against Mucormycosis "black fungus" viewed the inhibitory effect of *Rhazya stricta* in (I) were (A1) Aqueous extraction at (500 ppm) mean measured about 15±0.45 mm., while (A2) Aqueous extraction at (1000 ppm) measured about 20±0.70 mm, on the other hand (B1) methanollic extraction at (500 ppm) were about 16±0.80 mm, (B2) methanollic extraction at (1000 ppm) detected about 23±0.99 mm. The inhibitory effect of *Azadirachta indica* in (I) were (A1) Aqueous extraction at (500 ppm) mean measured about 8±0.22 mm., while (A2) Aqueous extraction at (1000 ppm) measured about 18±0.6 mm, on the other hand (B1) methanollic extraction at (500 ppm) were about 10±0.41 mm, (B2) methanollic extraction at (1000 ppm) detected about 20 ±0.56 mm. nearly similar results reported by Bashir*, et al.,* (1994) *R. stricta* methanolic fractions demonstrated antifungal activities. Another study revealed that fractionated *R. stricta* methanol and chloroform samples showed antifungal activity (Khan and Khan, 2007). The results are in agreement with Jeong et al, (2019b) were found to have inhibitory effect on against fungi. The results of Charmaine *et al*. (2005) stated that *A. indica*'s aqueous extracts showed the strongest inhibition against fungus. Beigomi *et al*. (2021) In *in vitro* or *in vivo* experiments, the fungicidal and bactericidal qualities of neem leaf extracts were demonstrated. Ahmed *et al*. (2015) linked the neem organic extract's ability to block the protease activity of dermatophytes to the antifungal qualities of neem extracts. On the otherside, Gilani *et al*. (2006) conducted an in vitro antifungal investigation by exposing diverse bacteria to methanol-soluble fractions of *Rhazya stricta*.

Recent discoveries about the use of fungal endophytes as a source of antimicrobial compounds have offered alternate strategies for combating pathogen drug resistance, and the ineffectiveness of antifungal activity compounds produced by endophytic fungi has assisted in the prevention of a number of diseases that affect living things (Lamoth *et al*., 2022; Lax *et al*., 2021; Mahalaxmi *et al*., 2021).

Clavatol, chaetomugilin D, guignardic acid, colletotric acid, viridicatol, 7-amino-4-methylcoumarin, altersolanol A, 2-hydroxyl-6 methyl benzoic acid, enfumafungin, xylarenone B, jesterone, fusapyridon A, hypericin, phomopsin A, isopestacin, xylarenic acid, fusaripeptide A, xylarenone A, javanicin, Z-roquefortine C, penitrem A, penijanthine A, fusarithioamide A, pestalone, cryptocandin cryptocin, ecomycins, pseudomycins, pestaloside, and pestalopyrone are a few examples of antifungal compounds produced by endophytic fungi (Martinelli *et al*., 2021; Emad and Gamal., 2013). Numerous antifungal substances produced by endophytes have been documented, including peptides, phenols, terpenoids, steroids, alkaloids, flavonoids, and quinine (Cowen *et al*., 2015).

Conclusion

In conclusion, this study has declared that the aqueous and methanolic leaf extracts of *R. stricta* and *A. indica* possess efficient antimicrobial effects against the test bacterial strains, particularly the methanolic extracts. Furthermore, the results pointed

out that the aqueous extract was more potent than the methanolic one in offering inhibition to the microbial enzymes. The pharmaceutical potential of these plants could be due to the presence of biologically active secondary metabolites in their leaf extracts. These results offer important insights into the management of pathogenic microorganisms and food preservation, particularly with regard *to A. indica*. To attain the best application and utilization of *R. stricta* and *A. indica* extractions, more and more study is required.

Nowadays, there is a lot of interest in and promise for agriculture, pharmacology, and medical research about the microbial world of plants. Scientists have been examining the effectiveness of medicinal plants and their possible pharmacological effects in producing the bioactive chemicals that are naturally found in them. Numerous phytochemical components found in medicinal plants have the potential to treat human illnesses.

Recommendation(s)

The current hunt for novel biologically active metabolites from plant endophytes has become more diverse due to the outlook for medicinal substances and the growing need for benign medications. Despite the discovery of bioactive substances and the application of certain therapeutic plants, little is known about the bioactivities of the fungus that is linked to it. Moreover, investigations into the many pharmacological impacts of bioactive substances are still in their early stages. However, as this review study illustrates, reviewing material on their functions can offer ideas about potential studies.

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