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Evaluation of the phytochemical properties and antifungal effects of *Abrus precatorius* and *Morinda lucida* plant parts against clinical isolate of *Candida albicans*

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ABSTRACT

Experimental study was done on the phytochemistry and antifungal effects of *Abrus. precatorius* (seed) and *Morinda lucida* (leaves, root, bark) plant parts on *Candida albicans* in Nnewi, Anambra state, Nigeria. Qualitative phytochemistry was by standard methods, plant materials prepared by air-drying, crude extraction by Soxhlet extraction and extractive index analyzed. Antifungal susceptibility was by standard methods using paper - disc agar - diffusion and punch hole methods, assessing Minimum Inhibitory Concentration (MIC) and Fungicidal Concentrations (MFC). Data was analyzed using SPSS version 23. Results revealed, in 16 plant part, highest percentage extractive

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value was in methanolic extract of *Morinda lucinda* leaf (11.2%) and best solvents for extraction yield for each plant were, water extract for *Abrus precatorius* seed (8.2%); acetyl - acetate for *Morinda lucinda* root (5.2%) and bark (4.2%); methanolic extract for *Morinda* leaf (11.2%). Phytochemistry showed ten components extractable from 16 plant parts solutions. *Morinda lucida* (root) ethanol, ethyl-acetate, and water extracts (bark and leaf parts) *had* the highest, (9) bio-active compounds. Water extract was the best (9) in 3 out of the 4 herbal plant parts. Zones of inhibition by extracts on *Candida albicans* by disc and Agar - well diffusion method showed highest clearance in methanolic extracts of *Abrus precatorius* seed (17.00±0.00) mm, ($p < 0.02$), ethyl-acetate extracts of *Morinda lucinda root* (16.00±1.41) mm ($p<0.04$) and *Morinda lucinda* bark (16.50±2.12mm) in all using Agar-well diffusion method. For *Morinda lucida* roots and bark, clearance was higher in ethyl - acetate solvent (16.00±1.41) mm ($p=0.04$); (16.50±2.12mm) compared with controls (20.50±2.12mm); (20.50±4.95). There was zero clearance using *Morinda lucinda* leaves and all n-hexane solvent. Performance of agar-well diffusion method was better than disc-diffusion. MIC against *Candida albicans* showed *A. precatorius* methanol seed extract as best MIC (6.25) mg/ml, while methanol extract of *Abrus precatorius* best MFC (25mg/ml). Conclusively, all extracts have anti-candida properties though water extract of *Abrus* seed gave highest percentage yield. All *plants* parts are quite promising as a multipurpose medicinal agent though agar-well diffusion method is recommended as best method for testing the herb's parts. Extensive research will expose their therapeutic potentials.

Keywords*: phytochemical properties, antifungal effects, Abrus precatorius ,Morinda lucida, Candida albicans*

INTRODUCTION

The use of different parts of several medicinal plants to cure specific ailments/diseases has been in vogue from ancient times. Extracts of many plants are highly efficient against some pathogenic

fungal, as well as other microbial infections. It is estimated that around 70,000 plant species, have been used at one time to other for medicinal purposes (Purohit and Vyas, 2004). The judicious use of medicinal herbs can cure deadly diseases that have subdued the abusive usage of some synthetic drugs, Bhattacharjee (2001), thereby reducing drug resistance.

Medicinal plants are the plants whose parts (leaves, seeds, stem, roots, fruits, foliage etc.), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals (Sofowara *et al.*, 2013). The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although some synthetic antifungal drugs brought about a revolution in controlling different fungal diseases, but these synthetic drugs are out of reach to millions of people. Plants have been used in traditional herbal medicine for many years (Didone *et al*., 2011). In some parts of the world, plants and herbs are still the primary source of remedies used in treating diseases (Humber,2002).

Several plant extracts have been reported to have anti-*Candida* activities including *Allium sativum* (*Garlic*), Bishop *et al*. (2010); Ebrahimy *et al*. (2015); Berberine-containing herbs Varadaraian *et al*., (2015); Dhamgave *et al*. (2014), *Cinnamomum verum* (*Cinnamon*) and *Origanum vulgare* (*Oregano*), Liu *et al*. (2011); Soares *et al*., 2015 and (Haba *et al*., 2014). Moreover, none of these plant extracts have been approved by regulatory agency for human use either because of lack of information regarding their efficacy/toxicity and/or lack of defined chemical structures. Many low molecular mass proteins or peptides with a higher antifungal activity have been extracted in recent years from various plants like *Abrus precatorius*, Osborn *et al.* (1995); Huynh *et al*. (1996;2009) and are believed to be involved in a defense mechanism against fungi.

Candida albicans is the most common fungal pathogen to humans and infects mostly individuals with weak immune defense, producing severe infections. *C. albicans* can form highly ordered biofilms, which are microbial colonies that are enclosed by extracellular matrix and they are attached to a solid surface (Chandra *et al.*, 2001). Biofilms may act as a reservoir for the pathogenic cells which are resistant to host immune system and drugs that may lead to invasive systemic infections of tissue,

organ and infect almost all inner organs. This causes fungemia and life-threatening septicemia (Karkowska-kuleta et *al.*, 2009). *C. albicans* biofilms is found on implanted medical device, causing problems to medical practitioners (Uppuluri and Ribot, 2017).

During the past 10-15 years, interest in the antimicrobial nature has expanded due to the increased resistance of fungal pathogens to some antimicrobial drugs being currently used, and the toxicity or adverse host reactions of other anti-infectives. Many plants contain metabolites with active components against fungi. Those active plant components do not only have antifungal properties, but they also help in healing process and some supposed incurable diseases. The antimicrobial peptides are very effective against microbes, and they show their activities by lysis, by binding to, and disruption of the outer membrane of microbes. (Haung *et al*., 2000). Terpenoids, saponins, phenolics and their compounds, coumarins (simple and furanocoumarins), alkaloids, essential oils, antifungal peptides, proteins and terpenes all possess antifungal properties (Matejuk et *al.*, 2010).

Ever since threats of new diseases in the past few decades, the problem of antimicrobial resistance has emerged (like non-albicans species), due to the evolution/adaptation of microbes and the re-emergence of old diseases due to the development of fugal resistance (Jones *et al.,* 2008). Antifungal resistance has become a global concern and the clinical efficacy of many existing antifungal is being threatened by the emergence of multidrug resistant pathogens (Bandow *et al;* 2003).

Although, most of this traditional knowledge of medicinal plants is in the form of oral knowledge and the active ingredients of herbal remedies are unknown to the traditional healers. The development and knowledge of antifungal activities from plant extracts have the potential for applications in antifungal therapy (Elgharbawy *et al.,* 2020).

Nevertheless, in the current investigation carried out, this research work was designed to study the extractive values, phytochemical constituents and antimicrobial potentials of *Abrus precatorius* (seed) locally called *ojuologbo (Yourba), Damar Zaya (Hausa) and Anya Mumu (Igbo) and Morinda*

lucida (leaves, stem, root) against some clinical isolates of *Candida albicans* in order to discover a possible pharmacologically active compounds or agents against *Candida albicans*. Since, many of the antifungal and other synthetic drugs show sensitization reactions and other undesirable side effects, and there is a possibility that herbal drugs if well processed and monitored are relatively safer than others of multifarious nature (Huynh *et al*., 2020).

Aim of the study

The aim of this study was to determine the phytochemical properties and antifungal effect of *Abrus. Precatorius* (seed) and *Morinda lucida* (leaves, root, stembark) plant parts against some clinical isolates of *Candida albicans.*

MATERIALS AND METHOD

Study design

This is an experimental study to be find out the phytochemistry and antifungal capacity of the selected plant leaves, seeds, stem, and root against *Candida albicans*.

Study Area

The herbal plants were obtained locally from Nnewi town in Nnewi north Government area of Anambra State in Nigeria.

Inclusion and exclusion criteria

- **Inclusion Criteria:** All fresh seeds, leaves, roots and barks of both plants collected from Umudim, Nnewi Anambra state.

- **Exclusion Criteria:** All rotten seeds, leaves, roots and barks of both plants collected outside Umudim, Nnewi Anambra state.

Methodology

Plant collection and identification: The plants and its parts used in this study consists of *Abrus precatorius(seed) and Morinda lucida (leaves, stem, root),* which majorly grows in temperate regions. Fresh quantities of these plants were also obtained from different areas around Umudim, Anambra state. All collected plant parts, were identified and verified by a botanist at Department of Botany Sciences, Faculty of Natural sciences, Nnamdi Azikiwe University, Awka.

Phytochemical Studies: Phytochemical test for the presence of Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, Tannins, Protein, Carbohydrates, Cardia glycosides and Resins as described by (Evans, 1989).

Preparation of plant materials: *Morinda lucida* leaves, stem, root and *Abrus precatorius* seed were air-dried in a cool sun-free area for 7 days as described by (Dilika *et al.*,1996; Bans et *al.*,2013). It was grounded with a sterile grinding machine (Thomas Wiley Laboratory Mill) and pulverized. The stems were washed with tap water to remove debris and stem bark of the plants chopped into smaller pieces using a sharp knife. The washed stems were dried in the shade for two weeks and then crushed mechanically using a grinding machine and sieved using a sieve. The crushed and ground plant parts were weighed with a digital weighing balance (Ohio, England) and then stored in sterile closed polythene containers till further needed in the extraction process.

Preparation of plant extracts and extraction of the crude extracts for extractive index: The resulting dried powders of the plant parts were weighed, 20g collected and extracted using water, methanol, n-hexane and ethyl-acetate as described by Eloff (1998) after soaking in 100 ml solvents at room temperature for 72 hours, shaking regularly. Soxhlet extraction method was used for extraction according to (Evans, 1997; Harbone,1998). Extraction was done as for the leaves, seed,

stem bark and roots respectively and extracts filtered using Whatman filter paper No 1. The filtrates were evaporated to dryness at 35°C using evaporating dish and percentage yield calculated. Extracts obtained were stored at 2-4^0 C until needed. Extractive values of the aqueous extracts were obtained after triplicate analysis was made.

Determination of extractive value: Concentration of the aqueous extracts were detected by evaporating 0.1ml of each extract in an evaporating dish of known weight in an oven (Gallemkemp, United Kingdom) to dryness. The dish with the residue was allowed to cool, weighed and the weight of the residue obtained by subtracting the weight of the empty dish from the weight of the dish plus residue. This was repeated in triplicate in each case all as described by (Trease and Evans,1983).

Qualitative determination of phytochemical constituents

Grounded plant parts were used for phytochemical screening of the plants using method describe proteins and cardiac glycosides by Harbone (1998) and (Trease and Evans,1983). Phytochemical screening tests for active compounds such as tannins, saponins, terpenoids, glycosides, steroids, flavonoids, carbohydrates, and alkaloids were carried out on the different plant parts of *Morinda lucida* and *Abrus precatorius*. Possible presence of color changes of the solutions after addition of the testing reagents was used to check for the presence or absence of these secondary metabolites. Resultant color changes were read and indicated as positive (+), which shows the presence of the phytochemicals and negative (–), which indicates the absence of phytochemicals and degree of the content's grades in (+).

Antimicrobial sensitivity test

Preparation of yeast strains for antifungal screening/ susceptibility screening

Fungal isolates: Clinical isolates confirmed to be *Candida albicans* were obtained from the Medical Microbiology Department of Nnamdi Azikiwe University Teaching Hospital Nnewi Anambra State, Nigeria. They were collected in slants in McCartney bottles containing sterile Nutrient-glycerol broths and appropriately stored until needed. Isolates were re-activated by re-culturing on Candida

chrome agar (Liophilchem, Italy) and then incubated at 37ºC for 24 hours. Germ-tube test was used to confirm aquamarine green colonies of *Candida albicans* from other yeast as described by ((Reynolds and Braude, 1956).

Preparation of McFarlane's standard

McFarland standard (0.5) concentration was prepared to give an approximate cell density (1.5×10^8 CFU/ml) giving optical density (absorbance) of 0.132 at 530nm wavelength. The standard was stored into screw cap tubes which was tightly sealed to prevent loss by evaporation and stored protected from light at room temperature.

Preparation of standard control drug concentration

The drug used as control drug was Nystatin (µg/ml) (1000IU) (Biomedicine, India) prepared as recommended by CLSI (Clinical and laboratory Standards Institute, 2020).

Preparation of plant extract's standard concentrations

One (1) gram each of plant solvent pre-prepared extract (each separately) was taken and dissolved in 5ml of Dimethylsuphoxide (DMSO). Thus 200mg/ml of stock was obtained as a standard concentration of the extracts.

$$1g = 1000mg$$

1000mg dissolves in 5ml of DMSO

 X mg dissolves in 1ml of DMSO

$$X = \frac{1000 \times 1}{5}$$

$$X = 200mg/ml$$

Preparation of fungal yeast (inoculum)

Three (3) fungal yeast colonies were picked from overnight growth (48 hours) and suspended in Sabouraud dextrose broth using a sterile loop and incubated for about 2-6 hours. The turbidity standard was shaken vigorously before use and used to make a visual comparison with the density of the suspension against a white background with black lines. Density of the suspension was adjusted to 1.5 McFarland standard.

Antimicrobial susceptibility studies

The paper disc agar - diffusion method and punch -hole methods were used to compare the diameter of zones of clearance for antifungal susceptibility of each herb and their parts as described by (Alastruey-Izquierdo *et al.,* 2015; Baeur *et al.,* 1966; Drago *et al.,* 2004). Standard aseptic microbiological technique was observed. With regards to paper disc-diffusion method, sterile paper discs which had been impregnated with the different plant extracts (1ml each at a concentration of 200mg/ml) was impregnated by press-applying on the *Candida albicans*-inoculated Sabouraud Dextrose Agar (SDA) using sterile forceps. Prior to that, pre-prepared Sabouraud Dextrose Agar (SDA agar plate was overturned in an incubator in order to dry. A sterile cotton swab was dipped into the standardized yeast inoculum already adjusted to the standard opacity and excess fluid was drained by pressing the swab on the walls of the tube. The inoculum was evenly spread over the whole dried surface of a 90mm Mueller-Hinton agar plates twice during which the agar plate was rotated about 60 degrees each time to ensure that the inoculum is spread throughout the entire agar surface. Inoculated plates were allowed to dry for ten minutes.

Each of the test plates comprised of six discs which was placed at equidistance to each other to avoid the overlapping of inhibition zone. The plates were kept at room temperature for 10 minutes (pre-diffusion time). They were incubated for 24 hours at 25ºC. The diameter of inhibition zone either around the treated discs or around the control discs was measured and results recorded in millimeter (mm) for the antifungal activity assessment to the nearest whole millimeter with a pair of transparent rulers. The tests were carried out in duplicates to ensure the reliability and the mean average for each zone of inhibition calculated in millimeters.

In the punch-hole agar - well diffusion method as described by Balouiri *et al.,* (2016), a cork borer of 6mm diameter well and 4mm in depth was used to make strategic points on the already inoculated Sabouraud Dextrose agar plates. Each well was labelled appropriately for each extract and pipetted with 30µl of the standard concentration of the extracts using an automatic pipette. The plates were left on the bench for about 30 minutes for diffusion of extracts to take place before incubating at 37^0 c for 24hrs after which the zone of inhibition was read with a micrometer screw gauge and recorded in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) was determined for each plant extract showing antimicrobial activity against the tested *Candida albicans* yeast. Modified micro broth - dilution method was followed for determination of MIC values as described by Abu-Shanab *et al.* (2006); Abou - Elkhair *et al.* (2010) and (Radojevic *et al.*, 2012). This test was performed in sterile 96-well micro plates prepared by dispensing 50µl of Sabouraud broth into each well. A 50µl aliquot from the stock solution of tested extracts containing concentration of 200mg/ml was added into the first row of the plate. Then two- fold serial dilutions were performed by using a micropipette. The obtained concentrated range is expected to be from 100 to 0.0004 mg/ml and then 10µl of inoculum was added to each well except in the positive control (plant extract with media) while inoculum with media used as a negative control.

Inoculum was adjusted to contain approximately 1.5 x 10^8 CFU/ml). The test plates were incubated at 25ºC for 24hours. After 24hours, 50µl of 0.01% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) was added to the wells and the plate incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active fungi, the inhibition of growth can be detected when the solution in the well remained clear after incubation with TTC.

Determination of Minimum Fungicidal Concentration (MFC)

From the tubes in MIC showing no visible sign of growth/turbidity, 0.1ml of the sample was inoculated onto sterile Sabouraud Dextrose Agar by streak - plate method. The plates were incubated at 25ºC for 48 hours. The plate with the lowest concentration that results in killing 99.9% fungi indicated no cultural growth was noted and recorded.

Statistical analysis

The data collected was analyzed using Statistical Package for Social Science (SPSS) version 23. Values obtained were presented as mean and standard deviation in millimeters diameters. Then p-value was considered to be significant when ($p< 0.05$) at 95% confidence interval (CI). Analysis of variance (ANOVA), frequency, distribution and students t-test were performed to determine statistically significant differences amongst both plant type used, plant extracts and the methods used.

RESULTS

The percentage extractive values

All the plant parts dissolved and were extractable with all the solvents used but at different extractive values. The plant part with the highest extractive value among all the plant parts analyzed approximated to nearest whole number was methanolic extract of *Morinda lucinda* leaf (11.2%) > water extract of *Abrus precatorius seed* (8.2%) > acetyl -acetate extract of *Morinda lucinda* root (5.2%) and > methanol extract of *Morinda lucinda* bark (3.3%). So methanolic extract of *Morinda lucinda* leaf showed the highest percentage bio-active phytochemical yield and its methanolic bark extract the least.

In relation to solvent used for each plant part, the solvent that had the highest percentage extractive value yield in each plant were; Water extract in *Abrus precatorius* seed (8.2%); acetyl -acetate extract of *Morinda lucinda* root (5.2%); acetyl -acetate extract of *Morinda lucinda* bark (4.2%); and methanolic extract leaf for *Morinda lucinda* leaf (11.2%) respectively in table 1.

Table 1: Extractive values of the various plant parts

Name of Plant	part	solvent	Percentage extractive values (%)
Abrus precatorius	seed	water	8.13831
		methanol	7.662268
		ethyl acetate	4.171214
		n-hexane	5.736049
Morinda lucida	root	water	4.706996
		methanol	4.173598
		ethyl acetate	5.184121
		n-hexane	4.328014
Morinda lucida	bark	water	3.656658

		methanol	3.277264
		ethyl acetate	4.158688
		n-hexane	4.026565
Morinda lucida	leaf	water	8.13831
		methanol	11.16494
		ethyl acetate	10.30321
		n-hexane	10.08223

Phytochemical constituents of the various plant parts extracts

A total of ten (10) phytochemical components were extractable from 16 plant parts solutions in all (Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, Tannins, Proteins, Cardiac glycosides, Carbohydrates and Resins) at various quantitative degrees represented qualitatively. In order, herbal parts with highest number of phytochemical bioactive components and the solvents with the highest number of active components were;

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Morinda lucida (root) ethanol extract and ethyl-acetate extracts (9 active components); *Morinda lucida* (bark and leaf parts) water extracts (9); *Abrus precatorius* (seed) water extract, methanolic and ethyl acetate extracts, *Morinda lucida* (leaf) ethyl-acetate extract (8); *Morinda lucida* (root) water extract and *Morinda lucida* (bark) methanolic extracts (7); *Morinda lucida* (leaf) methanolic extract (6); *Morinda lucida* (root) n-hexane and *Morinda lucida* (bark)ethyl-acetate extracts (5); *Abrus precatorius* (seed) n-hexane and *Morinda lucida* (bark) n-hexane extract (4) and *Morinda lucida* (leaf) n-hexane extract (3) respectively.

Solvents showing the highest promise as a dissolving agent for bio-active constituents quantitatively represented (+) for each plant part in descending order include; for *Abrus precatorius* (seed); water, methanol, ethyl-acetate extracts (8) active components, n-hexane extract (4). *Morinda lucida* (root); methanolic, ethyl- acetate extracts (9), water (7) and n-hexane (4); *Morinda lucida* (bark); water (9), ethanolic (7), ethyl-acetate (5) and n-hexane extracts (4); *Morinda lucida* (leaf); water (9), ethyl- acetate extract (8), methanolic (6) and n-hexane (3).

In summary, *Morinda lucida* (root) ethanol extract and ethyl-acetate extracts (9 active components); *Morinda lucida* (bark and leaf parts) water extracts (9) extracted the highest number of bio-active compounds, while *Morinda lucida* (leaf) n-hexane extract (3) the least. Water extract was the best in 3 out of the 4 herbal plant parts studied (*Abrus precatorius* (seed), Morinda *lucida* (bark) and *Morinda lucida* (leaf) (9), while the least was in n-hexane extracts for each of the 4 plant parts (4,4,4,3(*Morinda lucida* leaf) in table 2.

Table 2: Phytochemical constituents of various plant parts extracts

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Name of plant	part	solvent	AK	FL	SP	ST	TER	TAN	PRO	CARB	CARD	RS
Abrus precatorius	seed	water	+	+++	-	++	+++	+++	+	++	+	-
		methanol	++	++	-	++	++	+++	+	++	+	-
		ethyl acetate	+	-	+	+	+	+	+	-	+	+ n-
		hexane	-	-	+	++	++	-	-	-	-	+
Morinda lucida	root	water	+	+	+	++	++	-	+	+	-	-
		methanol	++	+	+	++	++	++	+	++	-	+
		ethyl acetate	++	++	-	+	+	+	+	+	+	+
		n-hexane	-	+++	+	++	++	-	-	-	+	-
Morinda lucida	bark	water	+	++	+	++	++	+	+	+	+	-
		Methanol	+++	+++	+	++	-	+	+	+	-	-
		Ethyl acetate	-	-	-	++	++	-	+	++	-	++ n-
		hexane	-	-	+	+	++	-	-	-	-	+
Morinda lucida	leaf	water	+	+	+	++	++	+	+	+	+	-
		methanol	++	+++	-	-	+	-	+	++		- ethyl
		acetate +	+++	++	++	+	+	+		-	-	+
		n-hexane	-	-	-	++	+	-	-	-	-	+

Keys: for phytochemical tables

AK = Alkaloid	TAN= Tannin	+	=	Present
FL = Flavonoid	PRO=Protein	-	=	Absent
SP = Saponin	CARB=Carbohydrate	++	=	Moderately

present

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ST = Steroid CARD=Cardiac glycoside +++ = Highly Present

TER=Terpenoids

Table 3 Zones of inhibition (in mm) of various plant parts on *Candida albicans* by disc and Agar- well diffusion method

Methanolic extracts of *Abrus precatorius* seed showed the highest mean ±SD clearance 17.00±0.00mm using Agar-well diffusion method and 19.00±5.65mm in Nystatin control drug, differing significantly with control (p=0.02), but 14.5±0.71mm with Disc-diffusion method with methanol extract, and 18.00±1.41mm in control with significant difference (p=0.02;0.04)

Ethyl-acetate extracts of root of *Morinda lucinda* showed the highest mean ±SD clearance 16.00±1.41mm using Agar-well diffusion method and 20.50±2.12mm in Nystatin control drug, differing significantly with control (p=0.40), but with Disc-diffusion method, no clearance zone was observed in all extracts except in control drug (20.00±4.24)>0.05.

Ethyl-acetate extracts of bark of *Morinda lucinda* showed the highest mean ±SD clearance using Agar-well diffusion method (16.50±2.12mm) and 20.50±4.95mm in Nystatin control drug, but with Disc-diffusion method, no clearance zone was observed in all extracts except in control drug (19.50±9.19)>0.05.

Generally, intermediate clearance zones were observed in all plant herbs analyzed, higher in methanolic (17.00±00mm) and ethyl acetate extracts (15.50±0.00mm) in agar- well diffusion and (14.5±0.71) in disc-diffusion method with *Abrus precatorius* seed compared with break -point diameter of control drug (Nystatin µg/ml) for intermediate susceptibility (14-17mm) and susceptible diameter of clearance (>18mm) (p=0.02).

For *Morinda lucida* roots, clearance was higher in ethyl acetate solvent (16.00±1.41) mm (p=0.04), than methanol (12.00±1.41) mm, (p>0.05) when compared with control (20.50±2.12mm). In *Morinda lucida* (bark), clearance was higher in ethyl acetate extract (16.50±2.12mm) than in methanolic (13.00±1.41)>0.05, compared with control (20.50±4.95).

There was no zone of clearance in all extracts with *Morinda lucinda* leaves(0.0±0.00mm) in both methods, in n-hexane method in both methods, and in root and bark extracts of the plant using disc-diffusion method, though clearance zone was observed with control drug in agar-well and disc-diffusion methods (19.50±2.12 ;18.50±4.95mm), (20.50±2.12; 20.00±4.24mm) and (20.50±4.95; 19.50±9.19) for the plant parts respectively, p>0.05. In general, the performance of Agar-well diffusion method was better than disc-diffusion for susceptibility testing of all the herb's parts.

Table 3 Comparative study of zones of inhibition (in mm) of various plant parts on *Candida albicans* by disc and Agar- well diffusion method

			Agar-well diffusion		Disc-agar Diffusion	
Name of plant	**part**	**solvent**	**Mean ±SD mm**	**P Value**	**Mean ±SD**	**P Value**

Key: for control drug Resistant = ≤13 Intermediate =14 - 17 Sensitive = ≥18
NYT= Nystatin

4 The Minimum Inhibitory Concentrations (MIC) of Plant extracts against *Candida albicans*

Among all the plant parts and their solvent extracts, *A. precatorius s*eed extract had the best/least MIC, (6.25) mg/ml < ethyl-acetate extract of *Morinda lucida* root (25) mg/ml<methanol extract of *Morinda lucida* root and ethyl-acetate extract of Abrus *precatorius seed* (100mg/ml), < methanol and ethyl acetate extracts of *Morinda lucida* bark respectively (200mg/ml).

Abrus precatorius	seed	water	0±0.00		0±0.00	
		methanol	17.00±0.00		14.5±0.71	0.02*
		Ethyl acetate	15.50±0.71	0.02*	13.5±2.12	0.07
		n-hexane	0±0.00		0±0.00	
		control (NYT)	19.00±5.65	0.05	18.00±1.41	0.04*
Morinda lucida	root	water	0±0.00		0±0.00	
		methanol	12.00±1.41	0.05	0±0.00	
		Ethyl acetate	16.00±1.41	0.04*	0±0.00	
		n-hexane	0±0.00		0±0.00	
		control (NYT)	20.50±2.12	0.05	20.00±4.24	0.10
Morinda lucida	bark	water	0±0.00		0±0.00	
		methanol	13.00±1.41	0.05	0±0.00	
		Ethyl acetate	16.50±2.12	0.06	0±0.00	
		n-hexane	0±0.00		0±0.00	
		control (NYT)	20.50±4.95	0.11	19.50±9.19	0.21
Morinda lucida	leaf	water	0±0.00		0±0.00	
		methanol	0±0.00		0±0.00	
		Ethyl acetate	0±0.00		0±0.00	
		n-hexane	0±0.00		0±0.00	
		control (NYT)	19.50±2.12	0.05	18.50±4.95	0.12

Table 4 The MIC of plant extracts against *Candida albicans*

Plant	**part**	**solvent**	**Fungi**	**MIC (mg/ml)**

Ochiabuto, O.M.T.B., Unaeze, B.C., Iyindu, M.E. and Obeagu, E.I. (2022). Evaluation of the phytochemical properties and antifungal effects of *Abrus precatorius* and *Morinda lucida* plant parts against clinical isolate of *Candida albicans. Madonna University Journal of Medicine and Health Sciences.* **2** (1): 249-291

Abrus precatorius	seed	methanol	*Candida albicans*	6.25
		Ethyl acetate		100
Morinda lucida	root	methanol	*Candida albicans*	100
		Ethyl acetate		25
Morinda lucida	bark	methanol	*Candida albicans*	200
		Ethyl acetate		200

Table 5 The Minimum fungicidal concentration (MFC) of plant extracts against *Candida albicans*

The MFC of methanol and Ethyl- acetate extracts of *Abrus precatorius* against *Candida albicans* were 25mg/ml and 100mg/ml respectively.

Table 5 The MFC of plant extracts against *Candida albicans*.

Plant	**part**	**solvent**	**Fungi**	**MFC(mg/ml)**
Abrus precatorius	seed	methanol	*Candida albicans*	25
		Ethyl acetate		100

Ochiabuto, O.M.T.B., Unaeze, B.C., Iyindu, M.E. and Obeagu, E.I. (2022). Evaluation of the phytochemical properties and antifungal effects of *Abrus precatorius* and *Morinda lucida* plant parts against clinical isolate of *Candida albicans. Madonna University Journal of Medicine and Health Sciences.* **2** (1): 249-291

Discussion

The prevalence of fungal infections as well as drug resistance to them is growing at an alarming pace. Recurrence of these fungal diseases is often due to their evolutionary avoidance of antifungal resistance. The development of suitable novel antimicrobial agents for fungal diseases continues to be a major problem despite much efforts for a search for new antifungal agents. Hence, it is urgently necessary to develop new surrogate agents that are more effective than conventional drugs available. Among the remarkable innovations from earlier investigations on natural-drugs, *Morinda lucida* and *Abrus precatorius* are a group of plant-derived substances capable of promoting many valuable effects on humans. *Morinda lucida* (Brimstone) is a tropical plant found in most African countries including some parts of Nigeria like Anambra State. It belongs to the family of rubiacea. The leaves can also be used in the treatment of fever, malaria and jaundice. The plant Abrus precatorius Linn a medicinal plant belongs to the genus Abrus (Family: Fabaceae), commonly called "Indian liquorice, black-eyed rosary seed, Jequirity, Crab eye" in English and in vernacular "Kundumani" (Elumalai *et al.*, 2009). The present study was carried out to test the antifungal efficacy of the seeds extract of *Abrus precatorius Linn* and *Morinda lucida* roots, bark and leaves with reference to fungal *Candida albicans* spp.

The phytochemical analysis of both plant parts revealed the presence of some phytochemical constituents that are of pharmacological benefits. The research showed that all herbs' parts used in the research contained the presence of extractable active secondary metabolite contents with antifungal properties, though at varying qualities. Ten (10) bioactive phytochemical components were identified from the 16 plant parts solutions (Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, Tannins, Proteins, Cardiac glycosides, Carbohydrates and Resins) in table 1 from both *Abrus precatorius* (seed) and Morinda *lucinda* leaves, barks and roots. The active metabolites in the seed of Abrus precatorius Linn has been found by Rajaram and Janardhanam (1992) to contain abrin, abrus agglutinin, glycyrrhizin gallic acid, trigonelline, precatorine and lipolytic enzymes, Glucine, Coumestrans, resin, asparagines, sapoins, alkaloids and sterols. Ojo *et al*.(2016) also in a qualitative

study found that tannin, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols were present in seed, leaves and roots of all of the plant parts. In a comprehensive review by Narendra and Atul (2014) several active metabolites were stated to be its constituents including several essential amino acids, flavonoids and triterpenoids, steroids, saponins, flavones, flavonol glycosides, reducing sugars, phenolic compounds glycosides Shatish *et al.* (2010) to mention a few. Morinda *lucinda* leaves, barks and roots analyzed by Dahunsi *et al*. (2020) in Zaria, Northern Nigeria show the presence of tanins, alkanoids, flavinoids, terpanoids, saponins, Steroid, phenols and cardiac glycosides in various concentrations all in line with findings in this research.

Various studies have shown that many plants are rich source of antioxidants. For instance, vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignins, found in plants, all act as antioxidants (Suffredini *et al.,* 2004). Plant extracts have shown strong antioxidant capacity both in vitro and in vivo, and the extracts can be considered a good source of natural antioxidants and antimicrobials, Altemimi *et al*. (2017) as well as antifungals (Elgharbawy *et al.,* 2020). Jabubawski *et al*. (1997) have shown that many antioxidant compounds can be found in fruits and vegetables including phenolics, carotenoids, anthocyanins, and tocopherols. Elgharbawy *et al.* (2020) in an extensive review of several plant-based researches with antifungal activities pointed out that most plants with antifungal properties contain terpenoids, saponins, coumarins, phenolic compounds, essential oils, proteins, alkaloids and peptides, which agrees with findings in this research. Okwuosa *et al.* (2012) confirmed in their study that *Uvaria. chamea* leaves and roots, *Spondias mombin* leaves and bark and *Combretum racemosum* leaves possessed phytochemical metabolites with antifungal activities.

The different solvents used extracted bioactive components in the order showing that plant part with the highest extractive value among the 16 solvent parts analyzed was methanolic extract of *Morinda lucinda* leaf (11.2%) > water extract of *Abrus precatorius seed* (8.2%) > acetyl -acetate extract of *Morinda lucinda* root (5.2%) and > methanolic extract of *Morinda lucinda* bark (3.3%) in table 1,

maybe as a result of their differing affinities for extraction and difference in internal and external factors as well as effects of soil types, organic contents, heavy metals in the soil and minerals.

It has been reported by many scientists that different solvents such as methanol, hexane, and ethyl alcohol, used for the purpose of antioxidant extraction from various plants parts, such as leaves and seeds produces different impacts. Wong and Kitts (2006) suggested the use of various solvents of differing polarities in order to extract different phenolic compounds from plants with a high degree of accuracy. This is supported and confirmed by findings in this research. Variations in the content of bioactive compounds according to Çirak and Radusiene (2019) may be due to many internal and external factors, including plant organs, phenological stage, genetic profile, environmental abiotic and biotic factors, like growing site, light, temperature, radiation, soil drought and salinity, pathogens, and herbivores attack. Moreover, scientists have discovered that highly polar solvents, such as methanol, have a high effectiveness as antioxidants.

In this study, four different solvents were used as solvent extract and each produced varying degrees of bioactive components with different percentage yield probably as a result of good separation due to suitability of polarity of solvents on the chemical constituents. Plant materials have been known to have high amounts of complex phytochemicals, which make a good separation difficult, therefore, it has been suggested that it is very useful to use increasing polarity with multiple mobile phases for achievement of a highly valued separations (Zhang *et al.,* 2005). Soil contamination affects medicinal plants and other agricultural products grown in the soil (Haruna *et al.,* 2021). Gao *et al.* (2010) noted the resistance developed by some microorganisms and bacteria against drugs due to the presence of toxic heavy metals in the soil like cadmium, mercury, lead, chromium, arsenic, nickel, copper and zinc.

In this study in table 1, the result indicated the highest presence of percentage extractive yield value in water extract of *Abrus precatorius* seed (8.2%); acetyl -acetate extract for *Morinda lucinda* root (5.2%); acetyl -acetate extract for *Morinda lucinda* bark (4.2%); and methanolic extract leaf for

Morinda lucinda leaf (11.2%) respectively. Reasons could be attributed to the fact that water solvent is the most polar solvent, and also that the solvents used matched that of the polarity of the solute of interest in the specific plant part and hence water matched the polarity of the bioactive substances in seed of *Abrus precatorius*, while acetyl -acetate matched that *Morinda lucinda* root, bark and leaf part well enough to achieve the desired results. This could also be due to different parts (tissues) in a plant, many of which will produce different compounds, in addition to the diverse chemical structures and physicochemical properties of the bioactive phytochemicals as reported by (Sarajlija *et al.*, 2012).

Altemimi *et al.* (2017) stated that solvents used for the extraction of biomolecules from plants parts are chosen based on the polarity of the solute of interest and hence a solvent of similar polarity to the solute will properly dissolve the solute. They also noted that the polarity, from least polar to most polar, of a few common solvents is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water respectively. Therefore, findings from this research confirms this statement as aqueous extract produced more yield.

In table 2, ten (10) phytochemical constituents were derived from all plant part from the two herbs in all. (Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, Tannins, Proteins, Cardiac glycosides, Carbohydrates and Resins). These active substances are the constituents that confer antimycotic properties to these herbs and their parts. Their presence in the extracts show they have anti-fungal properties, though varying at different levels. The presence of these chemical could possibly explain the scientific basis of the plants in the medicinal utilization by the traditional healers in treatment and prevention of various diseases across West African Countries.

Terpenoids have been proven to have antifungal activity, Thoppil and Bishayee (2011), main activity determined by thymol and chavchrol etc. act by forming hyphae, reducing gene expression, destroying mitochondrion activity dysfunction, Haque *et al*. (2016), destroying fungal cell walls Onishin *et al*. (2000); depolarization of membranes and calcium ion stress (Rao *et al.*, 2010). Saponins kill fungi by damaging cell membranes, cause the content to leach out leading to fungal

death (Mshvildadze, 2000). Alkaloid's mode of action in fungi in different herbs are usually pleiotropic, inhibiting protein synthesis, intercalating DNA or creating and boosting development of fungi inhibitors (Elgharbawy *et al.*, 2020). Alkaloids are suggested to cause malfunction of mitochondria, cause direct impact on fungal cellular growth, respiratory and enzyme activity to achieve cellular imbalance (Dhamgaye, 2014).

Flavonoids in form of favanols, flavanones, Isoflavanones, flavones, favans and Anthocyanidins act to destroy fungi by several ways including inhibiting efflux pumps, cell division, RNA /DNA synthesis, cell wall formation, disrupt plasma membrane and cause mitochondrial disrupting (Aboody and Mickymaray, 2020). Zhu *et a*l. (2019) proposed that the mechanism of action of Tannins is that they disrupt cell wall and plasma membrane in fungal pathogens.

Plant antifungal proteins comprises of chitinases and chitinase-like proteins, chitin-binding proteins, cyclophilin-like proteins, defensins and defensin-like proteins, deoxyribonucleases, embryo-abundant protein-like proteins, glucanases, lectins, lipid transfer proteins, peroxidases, protease inhibitors, ribonucleases, ribosome-inactivating proteins, storage 2S albumins, and thaumatin-like proteins differing in amino-acid sequences, (Wong *et al.*, 2010). Despite the diversity, they exhibit similar biological activities probably due to having a common intracellular effector. Proteins display antifungal activity by inhibiting fungal growth (Wong *et al.*, 2010). To achieve this, Chitinases hydrolyze chitin, (Graham and Sticklen 1994). Some thaumatin-like proteins evoke a mitogen-activated protein kinase signal translation mechanism that results in changes in the fungal wall and enhanced toxicity, Yun *et al.* (1998) while other thaumatin-like proteins are capable of hydrolyzing β-1,3-glucans (Grenier *et al.*, 1999).

Cardiac glycosides in plant parts exhibit anti-fungal effects by inhibiting Na/K-ATPase causing changes in intracellular calcium concentration (Aronson., 2008). Carbohydrates according to (Wink and Wyk, 2010; Van and Wink, 2015), such as glucose, galactose or fructose are used to form glycosides with SM and are thus participants of both primary and secondary metabolism and some complex Ca^{2+} and Mg^{2+} ions and functions as an antinutritive substance. Polysaccharides can interact with proteins and cell surfaced by forming several hydrogen bonds (Wink, 2015). Resins in plants

attribute their antimicrobial effects to the presence in the molecules of functional groups such as the hydroxyl, aldehyde, and ketone or to their cis or trans configurations. They act by morphological alterations which is consistent with an unspecific mode of action causing inhibition of the fungal growth or damaging the fungal cells in parallel with a mechanism of resistance based on the retention of the compound by the lipid accumulation (Savluchinske-Feio *et al.,* 2006). Presence of the secondary metabolites in the herbal parts of *Morinda lucida* and *Abrus precatorius* in this research proves they possess antifungal activities, agreeing with other researches.

Interaction between the root, leaf and stem-bark extracts of *Morinda lucida* was investigated by Nweze *et al*. (2005) for their antifungal activities of Morinda lucida against *Candida albicans* based on disk diffusion. Additive interactions were observed in each pair of the combinations showed neither synergistic nor antagonistic interactions were recorded. Phytochemical analysis suggested the presence of similar phytochemicals in all the extracts. In a study by Elumalai *et al.* (2009), the aqueous and methanol extractions of seed of *Abrus precatorius* Linn were evaluated for the antifungal activity against selected fungal spps viz. *C.albicans, C.tropicalis, C.krusei, C.kefyr. C.glabrata, C.guilliermondi, Aspergillus niger, Aspergillus fumigates* and *Aspergillus flavus* using agar well diffusion method. Methanol extract of the seeds of Abrus precatorius Linn revealed higher antifungal activity against *C.albicans, C. tropicalis, C. krusei , Aspergillus fumigates and Aspergillus flavus* whereas intermediate activity was recorded against *C. kefyr, C.glabarata , C. guilliermondi, and A.niger*. On the other hand aqueous seeds of the above plant recorded higher antifungal activity against *C.krusei,C.guillermondi* and *A.fumigates* and an intermediate antifungal effect on *C. albicans, C. tropicalis, C. kefyr, C.glabrata, Aspergillus niger* and *Aspergillus flavus*, after which they suggesting that the methanol and aqueous extract of the seeds of *Abrus precatorius* Linn could be used to develop a novel broad spectrum of antifungal herbal formulation, agreeing with this research findings.

Morinda *lucida* (root) ethanol and ethyl-acetate extracts (9 active components) and *Morinda lucida* (bark and leaf parts) water extracts (9) produced the highest number of bio-active contents, while the

n-hexane extracts the least, leading with *Morinda lucida* (leaf) n-hexane extract (3) in the study. This could be because ethanol and ethyl acetate are highly polar solvents and extracted active phytochemical constituents that had antifungal activity. A study by Oshuntokun (2015) observed that the ethanol extracts of leaf and bark of *Morinda lucida* had better antimicrobial activity against the clinical isolates of *Escherichia coli* (ATCC 0157), *Escherichia coli* (ATCC 25922), *Salmonella typhi, Candida albicans* (ATCC 90029), *Klebsiella pneumonia* (ATCC 35657), *Mycobacterium fortuitum* (ATCC 6841), *Mycobacterium smegmatis* (ATCC 19420), *Mycobacterium abscessus* (ATCC 19977), *Mycobacterium phlei* (ATCC 19240), *Staphylococcus aureus* (ATCC 29213) compared to the ethyl acetate stem using Agar-well diffusion method.

In each leaf part, water extract extracted the highest number of secondary metabolites types out of all studied; (*Abrus* precatorius seed, water and methanol extracts (8); Morinda lucida leaf and bark water extracts (9); but Morinda lucida root methanol and ethyl acetate (9) in 3 out of the 4 herbal plant parts studied, while the least was in n-hexane extracts in each of the 4 plant parts in table 2. Results showed that different solvents resulted in various extraction yields. A higher extraction yield was observed in aqueous water solvent. This indicates that the extraction efficiency favored distilled water and because the plant material (seed, bark, root, leaves) contains high levels of polar compounds that are soluble in solvents with high polarity such as water. Also, there is a probability that factors enhancing the diffusivity and solubility in the process was present to facilitate the extraction. Li *et al*. (2018) pointed out that properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration affects the extraction efficiency. Extraction solvents have an effect on the extraction yield and the content of bioactive compounds, thus significantly affecting the biological activity of the extract (Ngo *et al.,* 2017). This result is consistent with the extraction yield of aqueous seeds of the above plant by Elumalai *et al.* (2009) which recorded higher antifungal activity against *C.krusei, C.guillermondi and A.fumigates* and an intermediate antifungal effect on *C. albicans, C. tropicalis, C. kefyr, C.glabrata, Aspergillus niger* and *Aspergillus flavus but* the methanolic extract of the seeds of Abrus precatorius Linn revealed

higher antifungal activity against *C.albicans, C. tropicalis, C. krusei , Aspergillus fumigates and Aspergillus flavus.*

Comparing the zones of inhibition using Agar- well diffusion and disc-diffusion methods among the plant parts in table 4, the performance of agar-well diffusion method was better than disc-diffusion for susceptibility testing of all the herb's parts. Reasons for this variation could be associated with the use of different non-standardized approaches inoculum preparation techniques, inoculum size, growth medium, incubation conditions and endpoints determination.

Moreover, more advantage of direct-contact with media is created in agar-well diffusion because the antimicrobial extract solution gradually diffuses through the agar medium in which the test substance was added and inhibits the growth of the species faster, unlike the disc-diffusion in which paper on which the extract was added could even absorb a reasonable amount of substance contributing to more inhibitions as well as limitations like, the type of media used, agar percentage, thickness of the media , solubility, etc. could lead to lots of ambiguity, hence no activity seen in some of the experiment. This was also noted by (Balouiri *et al.,* 2016).

Variations in diameters of clearance of tested organism using different plant parts and different solvents in table 4 could be because of effects of solvent of varying polarities, as well as to their intrinsic properties that are related to the permeability of their cell surface to the extracts. Thavamoney *et al.,* (2018) also noted variations produced by properties in fruit parts of *Dacryodes rostrata* influenced by different solvents. In the present work, agar-well diffusion method was better (17.00±0.00) mm than disc diffusion (14.5±0.71) mm for methanolic extracts of *Abrus precatorius* seed and was significantly comparable with control (NYT) in both methods (19.00±5.65mm; 18.00±1.41mm) ($p<0.02;0.04$). This result was higher than that obtained by Elumalai *et al.* (2009) with methanolic seed extract of the same herb on *Candida albicans*. Ethyl-acetate extracts of root of *Morinda lucinda* and bark of *Morinda lucinda* were better and cleared only with agar diffusion (16.00±1.41; 16.50±2.12) mm and significantly compared with control (20.50±2.12 mm) ($p=0.04$) respectively for both methods.

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Methanol and ethyl acetate extracts are polar solvents, which makes them good extractors of secondary metabolites. () Ethyl-acetate solvent produced the highest percentage extractive value yields in *Morinda lucinda* root (5.2%) and *Morinda lucinda* bark (4.2%), while methanolic extract of *Abrus precatorius* (seed) had (7.7%) yield in each plant part compared with other solvents used in the present research. Ethyl acetate extract of *Morinda lucida* produced 9 phytochemical constituents' secondary metabolites, on top list, while the bark extract produced 5 and methanolic extract of *Abrus precatorius* (seed) (8) in the research. Explanation to their antifungal activities comparable significantly to controls is because of the phytochemical constituent present in the parts. The contents despite the quantitative yield and number of phytochemicals present must have been active enough to produce desired results. Variation in activity despite not being the best yields or solvent extractors in this work can be explained by effects several factors as reported by Altemimi *et al.* (2017) like different parts (tissues) in a plant, many of which will produce quite different compounds and diverse chemical structures and physicochemical properties of the bioactive phytochemicals (Saraljlija *et al.*, 2012).

Abrus precatorius has been noted in previous researches as a potential medicinal plant and reviews revealed chemical constituents of leaf, root and seeds of *Abrus precatorius* contains principally flavonoids, triterpene glycosides, abrin and alkaloids which have antimicrobial activities (Narendra and Atul, 2014). The seed is well documented to have hundreds of essential amino acids, growth inhibitors, Alkaloids and nitrogen compounds, acids and so many rich compounds which makes a "jequirity" used in various ayurvedic formulations used to treat various diseases and is very valuable medicinal plant for its chemical constituents(Narendra and Atul, 2014).It is a potential of good insecticide and with antimicrobial activity, Adelowotan *et al.* (2008); Bobbarala *et al.* (2009), and an anti -yeast (Atal *et al*., 2010;Jahan *et al.*, 2009 and Sirsi , 1963).

Documented evidences by Adewole *et al.* (2021) reported that hundreds of secondary metabolites compounds have been isolated from *M. lucida* including alkaloids, tannins, anthraquinones, sterols, saponins, polyphenols, terpenoids, phenols and cardiac glycosides. Though range of mass of extract obtained by Nweze *et al.* (2005) was (3-200mg/dl), that obtained in this research was (3-11%) from the

plant parts. Zones of inhibition diameters of clearance zones obtained from both works were similar for yeast infection.

There was no zone of clearance in all n-hexane extracts of all parts and in extracts of *Morinda lucinda* leaves in both methods, as well as in disc-diffusion method of root and bark extracts of the plant, though clearance was observed with control drug in both methods could be explained to be caused by compounding factors earlier noted. N-hexane solvent is the least polar out of all the solvents and hence extracted the least yield in this research. This explains why it produced no clearance. However, intermediate clearance zones were observed in all plant herbs analyzed, higher in methanolic (17.00±00mm) and ethyl acetate extracts (15.50±0.00mm) in agar- well diffusion and (14.5±0.71) in disc-diffusion method with *Abrus precatorius* seed significantly compared with break -point of control for intermediate susceptibility (14-17mm) and susceptible clearance (>18mm) ($p=0.02$).

Among all Minimum Inhibitory Concentration (MIC) tested against the plant parts, *Abrus precatorius* methanolic extract had the lowest and best MIC at 6.25mg/ml, lower than methanol extracts of *Morinda lucida* root (100mg/ml) and bark extracts (200mg/ml). Ethyl acetate extract of Morinda root produced the lowest MIC (25mg/ml) than *M. lucida* bark (200mg/ml) and *A. precatorius* seed (100mg/ml). The Minimum Fungicidal Concentration (MFC) of methanol and ethyl- acetate extracts of *Abrus precatorius* against *Candida albicans* differed and were 25mg/ml and 100mg/ml respectively due to differing antifungal strength.

These differences were as a result of variations in the quantitative compositions in their phytochemicals, types of constituents, dissolution power of solvent, and concentration of active metabolites in different plant parts. Method of assay and other physiological factors may have influenced the result also. In a study using serial dilution, Paranto *et al*. (2007) had a lower Minimum Inhibitory Concentration (MIC) of saga leaf extract (*Abrus precatorius L*) towards *Candida albicans* at 2 mg/ml. Their conclusion that the concentration of 2 mg/ml or more of (*Abrus precatorius L*) saga leaves extract having antifungal effect against *Candida albicans* proves that MIC of methanol extract of *Abrus precatorius* seeds (6.25mg/ml) obtained in the present work had better antifungal activity, agreeing with their findings. *A. precatorius* seed is a unique

source of hundreds of potential phytochemicals which makes this plant very important and versatile for its large number of medicinal properties (Narendra and Atul, 2014). Several plant growth inhibitors Anderson *et al.*, (1972) and antifungal metabolites have been obtained from the seeds (Attal *et al.*, 2010). The seed kernel according to Chakradhari *et al.*, (2019) consisted of stored oil (3.2%), protein (92.0%) and starch (4.8%), total polyphenol and flavonoid contents of 24710 and 2520 mg/kg (dw) with remarkably high content of polyphenols in the seed coat and the seed pod. Phosphorous, Sulphur and (mainly) potassium nutrients were present too. These explains reasons for its higher potency than leaves. Dahunsi *et al.* (2020) also showed the presence of tannins, alkaloids, flavonoids, terpenoids, saponins, Steroid, phenols and cardiac glycosides in various concentrations of *Morinda lucida* leaves, roots and stem parts were significantly different supporting results in this work.

Conclusion

In conclusion, above research results concludes all the extracts have anti-fungal properties. Water extract of *Abrus precatorius* seed had the highest percentage extractive phytochemical yield. *A. precatorius* and *Morinda lucida* leaves, roots and stem parts is quite promising as a multipurpose medicinal agent as they had very high potential pharmacokinetically and pharmacological applications. Agar-well diffusion method is recommended as a better method than disc-diffusion for susceptibility testing of the herb's parts. Hence extensive research should be done to exploit the therapeutic utility to fight against various fungal diseases.

REFERENCES

Aboody, M.S. and Mickymaray, S. (2020). Anti-fungal efficacy and mechanisms of flavonoids. *Review: Antibiotics (Basel*) 26; **9**(2):45.

Abu-Shanabi, B., Adwan, G., Jarrar, N., Abu-Hijeh, A., and Adwan, K. (2006). Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against Methicillin-Resistant *Staphylococcus aureus*. *Turkey Journal of Biology* **30**: 195-198.

Adelowotan, O., Aibinu, I., Adenipekun, E., and Odugbemi, T. (2008). The in-vitro antimicrobial activity of *Abrus precatorius (L) fabaceae* extract on some clinical pathogens. *Nigerian Postgraduate Medical Journal* **15**(1):32-37.

Adewole, K.E., Attah, A.F., and Adebayo, J.O. (2021). *Morinda lucida* Benth (Rubiaceae): A review of its ethnomedicine, phytochemistry and pharmacology. *Journal of Ethnopharmacology* **276**: 114055.

Alastruey-Izquierdo, A., Melhem, M.S.C., Bonfietti, L.X., and Rodriguez-Tudela, J.L. (2015). Susceptibility test for Fungi: clinical and laboratorial correlations in medical Mycology. *Revista Instituto Medicina Tropical Sao Paulo* **57**(Suppl 19): 57–64.

Almandil, N.B., and AbdulAzeez, S. (2021). Emerging status of Multidrug-Resistant bacteria and fungi in the Arabian Peninsula. *Biology (Basel)* **10**(11): 1144.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., and Lightfoot, D.A. (2017). Review Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts *Plants (Basel)* **6**(4): 42.

Elgharbawy, A.A., Samsudin, N., Zuhanis, Y., Hashim, H.Y., and Salleh, H.M., Santhanam, J. and Ben belgacem, F.F. (2020). Phytochemicals with antifungal properties: cure from nature. *Malaysian Journal of Microbiology* **16**(4); xxx-xxx.

Anderson, J.D., Mandava, N., and Gunn, C.R. (1972). Plant growth inhibitor from *Abrus precatorius* seeds. *Plant Physiology **49**:1024*–1026.

Aronson (2016). 17 - Positive inotropic drugs and drugs used in dysrhythmias. *Side Effects of Drugs Annual:* 209-222.

Attal, A. R., Otari, K. V., Shete, R. V., Upasani, C. D., and Nandgude, T. D. (2010). *Abrus precatorius* Linnaeus: a phytopharmacological review. *Journal of Pharmacy Research* **3** (11): 2585-2587.

Balouiri, M., Sadiki, M., and Ibnsouda, S.K. (2016). Methods for invitro evaluating antimicrobial activity: a Review. *Journal of Pharmaceutical analysis* **6**(2); 71-79.

Ban, N.K., Giang, V.H., Linh, T.M., Lien, L.Q., Ngoc, N.T., Thao, D.T., Nam, N.H., Cuong, N.X., Kiem, P.V., and Minh, C.V. (2013). Two new 11-noriridoids from the aerial parts of *Morinda umbellate. Phytochemistry Letters* **6**(2): 267-269.

Bandow, J.E., Brötz, H., Leichert, L.I., Labischinski, H., and Hecker, M. (2003). Proteomic approach to understanding antibiotic action. *Antimicrobial Agents and Chemotherapy* **47**:948–955.

Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* **45**(4):493-496.

Bhattacharjee, S.K. (2001). Handbook of medicinal plants. (3rd Ed.), Pointer publishers Jaipur, India. 377: 1–6.

Bishop F.L., and Lewith G. (2010). Who uses CAM? A narrative review of demographic characteristics and health factors associated with CAM use. *Evidence Based Complement Alternative Medicine* **7(1)**:11–28.

Bobbarala, V., and Vadlapudi, V. (2009). *Abrus precatorius* l. seed extracts antimicrobial properties against clinically important bacteria. *International Journal of Pharm Tech Research* **1**(4):1115–1118.

Borgio, J.F., Rasdan, A.S., Sonbol, B., Alhamid, G., Almandil, N.B., and Azeez, S.A. (2021). Emerging status of multidrug-resistant bacteria and fungi in the Arabian Peninsula. *Biology (Basel)* **10**(11): 1144.

Chakrabarti, A., Sood, P., Rudramurthy, S.M., Chen, S., Kaur, H., Capoor, M., Chhina, D., Rao, R., Eshwara, V.K., Xess, I., Kindo, A.J., Umabala, P., Savio, J., Patel, A., Ray, U., Mohan, S., Iyer, R., Chander, J., Arora, A., Sardana, R., Roy, I., Appalaraju, B., Sharma, A., Shetty, A., Khanna, N., Marak, R., Biswas, S., Das, S., Harish, B.N., Joshi, S., and Deepak M.D. (2015). Incidence, characteristics and outcome of ICU-acquired candidemia in India. *Intensive Care Medicine **41***(2):285–295.

Chakradhari, S., Khageshwar, S., Patel, K.S., Towett, E.K., Martín-Ramos, P., and Grabowski, A. (2019). Chemical composition of *Abrus precatorius* L. seeds. *European Journal of Medicinal Plants* **28**(1): 1-6.

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormick, T. and Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: Development, architecture, and drug resistance. *Journal of Bacteriology* **183**(18): 5385- 5394.

Cirak, C., and Radusiene, J. (2019). Factors affecting the variation of bioactive compounds in *Hypericum species. Biologia Futura* **70**: 198–209.

Clinical and Laboratory Standards Institute (CLSI) (2020). Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi. 2nd ed. CLSI supplement M61. 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.

CLSI (2017). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. In: PA Wayne (Ed.). CLSI document M38. 3rd ed. Clinical and Laboratory Standards Institute, Pennsylvania, 2017.

CLSI (2017). Reference method for broth dilution antifungal susceptibility testing of yeasts. In: PA Wayne (Ed.). CLSI document M27. 4th ed. Clinical and Laboratory Standards Institute, Pennsylvania, 2017.

d'Enfert C. (2009). Hidden killers: Persistence of opportunistic fungal pathogens in the human host. *Current Opinions in Microbiology* **12**(4):358–364.

Dahunsi, O.M., Sodimu, A.I., Olaifa, R. K., and Baba, G.O. (2020). Comparative analysis of phytochemical composition of leaf, bark and root of Brimstone (Morinda lucida. Benth) tree in Igabi Eco region of Kaduna State, Nigeria. *Natural Ayurvedic Medicine* **4**(4): 000280.

Dhamgaye, S., Devaux, F., Vandeputte, P., Khandelwal, N.K., Sanglard, D., Mukhopadhyay, G., and Prasad, R. (2014). Molecular mechanisms of action of herbal antifungal alkaloid berberine, in *Candida albicans*. *PLoS One* **9**(8): e104554.

Didone, L., Oga, D., and Krysan, D.J. (2011). A novel assay of biofilm antifungal activity reveals that amphotericin B and caspofungin lyse *Candida albicans* cells in biofilms. *Yeasts.* **28(8)**:561–568.

Dilika, F., Afolayan, A.J., and Meyer, J.J.M (1996). Comparative antibacterial activity of two Helichrysum species used in male circumcision in South Africa. *South African Journal of Botany* **63**(3): 158-159.

Dilika, F., Bremner, P.D., and Meyer, J.J.M. (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia* 71: 450-452.

Drago, M., Scaltito, M.M., Morace, G., and GISIA-2group. (2004). In vitro activity of variconazole and other antifungal agents against clinical isolates of *Candida glabrata* and *Candida krusei*. *European Journal of Clinical Microbiology and Infectious Diseases* **23**: 619-624.

Ebrahimy, F., Dolatian, M., Moatar, F., and Majd, H.A. (2015). Comparison of the therapeutic effects of Garcin® and fluconazole on Candida vaginitis. *Singapore Medical Journals* **56**(10):567–572.

Elgharbawy, A.A.M., Samsudin, N., Benbelgacem, F.F., Hashim, Y.Z.H., Salleh, H.M., and Santhanam, J. (2020). Phytochemicals with antifungal properties: Cure from nature. *Malaysian Journal of Microbiology* **16**(4): xxx-xxxs.

Elkhair, E.A., Fadda, H., and Mohsen, U.A. (2010). Antibacterial activity and phytochemical analysis of some medicinal plants from Gaza strip-Palestine. *Journal of Al Azhar University–Gaza (ICBA)* **12**: 45-54.

Eloff, J.N. (1998). Which extractant should be used for the screening of antimicrobials components from plants? *Journal of Ethnopharmacology* **60**(1):1-8.

Elumalai, E.K., Sivamani, P., Thirumalai, T., Vinothkumar, P., Sivaraj, A., and David, E. (2009). In vitro antifungal activities of the aqueous and methanol extract of *Abrus precatorius* Linn (Fabaceae) seeds. *Pharmacology online* **2**: 536-543

Evans, W. C. (1997). An index of medicinal plants. A Text book of Pharmacognosy, 14th edn. J & A Churchill, London **7**(5):12–14.

Evans, W. C. (1997). An index of medicinal plants. A Text book of Pharmacognosy, 14th edn. J & A Churchill, London **7**(5):12–14.

Gao, Y., Zhou, P., Mao, L., Ye, Z., and Shi, W.J. (2010). Assessment of effects of heavy metals combined pollution on soil enzyme activities and microbial community structure: modified ecological dose-response model and PCR-RAPD. *Environmental Earth Sciences* **60**(3):603–612.

Graham, L.S., and Sticklen, M.B. (1994) Plant chitinases. *Canadian Journal of Botany* **72**:1057–1083.

Grenier, J., Potvin, C., Trudel, J., and Asselin, A. (1999) Some thaumatin-like proteins hydrolyze polymeric β-1, 3-glucans. *Plant Journals* **19**:473–480.

Haba, E., Bouhdid, S., Torrego-Solana, N., Marqués, A.M., Espuny, M.J., García-Celma, M.J., and Manresa, A. (2014). Rhamnolipids as emulsifying agents for essential oil formulations: Antimicrobial effect against *Candida albicans* and methicillin-resistant *Staphylococcus aureus*. *International Journal Pharmaceutics* **476(1–2)**: 134–141.

Haque, E., Irfan, S., Kamil, M., Sheikh, S., Hasan, A., Ahmad, A., Lakshmi,V., Nazir, A., and Mir, S.S. (2016). Terpenoids with antifungal activity trigger mitochondrial dysfunction in *Saccharomyces cerevisiae*. *Microbiology* **85:** 436–443.

Harborne, J. B. (1998). Phytochemical Methods: A guide to modern techniques of plant analysis. Edition: 3, Springer, Germany.

Haruna, A., and Yahaya, S.M. (2021). Recent Advances in the chemistry of bioactive compounds from plants and soil microbes: a Review. *Chemistry Africa* **4,** 231–248.

Haung, X., W.J., Xie and Z.Z., Gong. (2000). Characterization and antifungal activity of a chitin binding protein from *Ginkgo biloba*. *FEBS* **478**: 123-126.

Huang, W.Y., Cai, Y.Z. and Zhang, Y. (2009). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention *Nutrition and Cancer* **62**(1), 1-20.

Humber, J.M. (2002). The role of complementary and alternative medicine: accommodating pluralism. *Journal of American Medical Association* **288(13)**:1655–1656.

Huynh, D.L., Ngau, T.H., Nguyen, N.H. (2020). Potential therapeutic and pharmacological effects of Wogonin: an updated review. *Molecular Biology Reports* **47:** 9779–9789.

Huynh, K., Borgmeyer, J.R., Smith,C.E., Bell ,L.D., and Shah,D.M.(1996). Isolation and characterization of a 30kDa protein with antifungal activity from leaves of *Engelmannia pinnatifida*. *Biochemistry Journal* **316**:723-727.

Jahan, S., Rasool, S., Khan, M.A. (2009). Antifertility effects of ethanolic seed extract of Abrus precatorius L. on sperm production and DNA integrity in adult male mice. *Journal of Medicinal Plants Research* **3**(10): 809–814.

Jahan, S., Rasool, S., Khan, M.A., Ahemad, M., Zafar, M., Arsahd, M., and Abbasi,A.M. (2009). Antifertility effects of ethanolic seed extract of *Abrus precatorius* L. on sperm production and DNA integrity in adult male mice. *Journal of Medical Plant Research* **3** (10):809–814.

Jakubowski, W., and Bartosz, G. (1997). Estimation of oxidative stress in *Saccharomyces cerevisae* with fluorescent probes. *International Journal of Biochemistry and Cell Biology* **29**(11) :1297–1301.

Jeenkeawpieam, J., Yodkeeree,S., Roytrakul, S., and Pongpom, M.(2021). Antifungal Properties of Protein Extracts from Thai Medicinal Plants to Opportunistic Fungal Pathogens. *Walailak Journal of Science and Technology* **18**(6):9045 1-10.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., and Daszak, P.(2008). Global trends in emerging infectious diseases. *Nature* **451**(7181): 990–993.

Karkowska-kuleta, J., Rapala-kozik, M. and Kozik, A. (2009). Fungi pathogenic to humans: Molecular bases of virulence of *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochemical Polonica* **56**(2): 211-224.

Li, P., Xu, G., Li, S.P., Wang, Y.T., Fan, T.P., Zhao, Q.S., and Zhang, Q.W. (2008). Optimizing ultra-performance liquid chromatographic analysis of 10 diterpenoid compounds in *Salvia miltiorrhiza* using central composite design. *Journal of Agriculture, Food and Chemicals* **56**(4):1164–1171.

Liu X., Han Y., Peng K., Liu Y., Li J., and Liu H. (2011). Effect of traditional Chinese medicinal herbs on Candida spp. From patients with HIV/AIDS. *Advances in Dental Research*. **23**(1): 56–60.

Matejuk, A., Leng, Q., Begum, M. D., Woodle, M. C., Scaria, P., Chou, S. T. and Mixson, A. J. (2010). Peptide-based antifungal therapies against emerging infections. *Drugs of the Future* **35**(3): 197.

Ming, Q., Su,C., Zheng,C., Jia, M., Zhang, Q., Zhang,H., Rahman,K., Han, T., and Qin,L. (2013). Elicitors from the endophytic fungus *Trichoderma atroviride* promote *Salvia miltiorrhiza* hairy root growth and tanshinone biosynthesis. *Journal of Experimental Botany* **64**(18): 5687–5694.

Mshvildadze, V., Favel, A., Delmas, F., Elias, R., Faure, R., Decanosidze, Q., Kemertelidze, E., and Balansard, G. (2000). Antifungal and antiprotozoal activities of saponins from *Hedera colchica. Pharmazie* **55:** 325–326.

Mukherjee, P.K., and Chandra, J. (2004). *Candida* biofilm resistance. *Drug Resistance Update* **7**(4):301–309.

Narendra, G., and Atul, B. (2014). Ethno botanical and Phytopharmacological potential of *Abrus precatorius* L.: A review. *Asian Pacific Journal of Tropical Biomedicine* **4**(Suppl 1): S27–S34.

Ngo, T.V., Scarlett, C.J., Bowyer, M.C., Ngo, P.D., and Vuong, Q.V. (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. *Journal of Food Quality* **2017** (9305047):1-8.

Nigussie, D., Davey, G., Tufa, T.B., Brewster, M., Legesse, B.A., Fekadu, A. and Makonnen, E. (2021) Antibacterial and antifungal activities of Ethiopian medicinal plants: a systematic review. *Frontiers in Pharmacology* **12**:633921.

Nweze, E.I., Okafor, J.I., and Njoku, O.U. (2005). Anti-fungal activities of pair combinations of extracts from *Morinda lucid Benth* by decimal additive assay. *Bio-Research* **3**(1): 99-104.

Ochiabuto, O.M.T.B., and Chukwura, I.E. (2020). Impact of Fungal infections and significant count statuses on fertility parameters in commercial motorcyclists and non-cyclists in Nnewi, Nigeria. *European Journal of Pharmaceutical and Medical Research* **7**(3):131-146.

Ojo, J.S., Babatunde, S.K., Ajiboye, A.E., Adedayo, R.M., Ajao, M.A., and Ajuwon, B.I. (2016). Evaluation of phytochemical properties and *in-vitro* antibacterial activity of the aqueous extracts of leaf, seed and root of *Abrus precatorius* Linn. against *Salmonella* and *Shigella. Asian Pacific Journal of Tropical Biomedicine* **6**(9):755-759.

Okwuosa O.M.T.B., E.I., Chukwura, G.O.Chukwuma, C.N., Okwuosa., I.B.,Enweani., N.R., Agbakoba.,C.M.Chukwuma.,P.O.Manafa and C.U. Umedum (2012). Phytochemical and antifungal activities of *Uvaria.chamea* leaves and roots, *Spondias mombin* leaves and bark

and *Combretum racemosum* leaves. *African Journal of Medicine and Medical Science* 41, suppl; 99-103.

Oladele, R., and Denning, D. (2014). Burden of serious fungal infection in Nigeria. *West African Journal of Medicine* **33**(2):107-114.

Onishi, J., Meinz, M., Thompson, J., Curotto, J., Dreikorn, S., Rosenbach, M. et al. (2000). Discovery of Novel Antifungal (1,3)-β-Glucan Synthase Inhibitors. *Antimicrobial Agents and Chemotherapy* **44**(2): 368- 377.

Onishi, J., Meinz, M., Thompson, J., Curotto, J., Dreikorn, S., Rosenbach, M. et al. (2000). Discovery of Novel Antifungal (1,3)-β-Glucan Synthase Inhibitors. *Antimicrobial Agents and Chemotherapy* **44**(2): 368- 377.

Osborn, R.W., De Samblanx, G.W., Thevissen, K., Goderis, I., Torrekens, S., Van Leuven, F., Attenborough, S., Rees, S.B. and Broekaert, W.F. (1995). Isolation and characterization of plant defensins from seeds of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae. *FEBS Letters* 368: 257–262.

Osuntokun, O.T. (2015). Evaluation of antimicrobial activities of crude ethyl acetate and ethanol extracts of Morinda lucida leaf, stem, and bark growing in Adekunle Ajasin University Botanical Garden against selected clinical isolates. *World Journal of Pharmaceutical Research* **1**(11):6-14.

Paranto, M.N.A., Harjadinata, K., and Dewi, W.,(2007). Anti-fungal capacity of Saga leaf (Abrus Precatorius L) towards *Candida Albicans* testing. *Padjadjaran Journal of Dentistry* **19**(2) :97-102.

Purohit, S.S. and Vyas, S.P. (2004). Medicinal plant cultivation: A scientific approach including processing and financial guidelines. Laurier Books Limited, India pp: 624.

Radojević, S.V., and Čomić, L. (2012). Antibacterial activity of naturally occurring compounds from selected plants. In book: Antimicrobial agents chapter: 1 Publisher: *InTech* :1-25.

Rajaram, N., and Janardhanam, K. (1992). The chemical composition and nutritional potential tribal pulse, *Abrus precatorius*. *Plant food and Human Nutrition* **42**(4): 285-290.

Reynolds, R., and Braude, A.I. (1956). The filament-inducing property of blood for *Candida albicans*: its nature and significance. *Clinical Research and Procedures* **4**, 40.

Sarajlija, H., Ckelj, N., Novotni, D., Mrši´c, G., Curi´c, M., Brncic, M., and Curic, D. (2012). Preparation of flaxseed for ´ lignan determination by gas chromatography-mass spectrometry method. *Czech Journal of Food Science* 30: 45–52.

Savluchinske-Feio, S., , Curto, M.J.M., Gigante, B., and Roseiro, J.C (2006). Antimicrobial activity of resin acid derivatives. Review: *Applied Microbiology and Biotechnology* **72**(3): 430-436.

Shatish, M., Balaji, R., Aruna, A., Niraimathi, V., Manikadan, G., and Babu, M.B.V. (2010). Preliminary phytochemical and cytotoxic property on leaves of *Abrus precatorius*. *Journal of Herbal Medicine and Toxicology* ***4***(1):21–24.

Shatish, M., Balaji, R., Aruna, A., Niraimathi, V., Manikadan, G., and Vijayan, P. (2010). Preliminary phytochemical and cytotoxic property on leaves of *Abrus precatorius*. *Journal of Herbal Medicine and Toxicology* **4**(1):21–24.

Sirsi, M. (1963). *In vitro* study of the inhibitory action of some chemotherapeutic agents on a freshly isolated strain of *Cryptococcus neoformans*. *Hindustan Antibiotic Bulletin* **6**(2):39–40.

Lau, S.k., and Lo, G.C., Antifungal Properties of Protein Extracts from Thai Medicinal Plants to Opportunistic Fungal Pathogens.

Soares, I.H., Loreto, É.S., Rossato, L., Mario, D.N., Venturini, T.P., Baldissera, F., Santurio, J.M., and Alves S.H. (2015). In vitro activity of essential oils extracted from condiments against

fluconazole-resistant and -sensitive *Candida glabrata*. *Journal of Medical Mycology* **25(3)**: 213–217.

Sofowora, A., Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary, and Alternative Medicines* **10**(5), 210-229.

Thavamoney, N., Sivanadian,L., Hong Tee, L., Khoo, H.E., and Prasad, K.N., (2018). Extraction and recovery of phytochemical components and antioxidative properties in fruit parts of *Dacryodes rostrata* influenced by different solvents. *Journal of Food Science and Technology -Mysore-* **55**(7): 2523–2532.

Thoppil, R. J. and Bishayee, A. (2011). Terpenoids as potential chemo-preventive and therapeutic agents in liver cancer. *World Journal of Hepatology* **3**(9), 228- 249.

Trease, G.E. and Evans, W.C. (1983) Pharmacognosy (12th ed.) Bailliere Tindal, London.

Trease, G.E., and Evans, C.W. (1985). A Textbook of Pharmacology 13th (ed.) London: ELBS Bailiere Tindall: 181-186.

Uppuluri, P. and Lopez Ribot, J. L. (2017). *Candida albicans* biofilms. *Candida Albicans*: *Cellular and Molecular Biology*: Second Edition **18**(5): 63-75.

Van Wyk, B.E. and Wink, M. (2015). *Phytomedicines, Herbal drugs and Poisons.* Chicago; Royal Botanic Gardens, Kew. The University of Chicago Press: 1-340.

Varadarajan S., Narasimhan M., Malaisamy M., and Duraipandian C. (2015). Invitro anti-mycotic activity of hydro alcoholic extracts of some Indian medicinal plants against fluconazole resistant *Candida albicans*. *Journal of Clinical Diagnosis Research* **9(8)**: 07–10.

Wink, M., (2015). Modes of Action of Herbal Medicines and Plant Secondary Metabolites. *Medicines (Basel)* **2**(3): 251–286.

Wong, J.H., Ng,T.B., Cheung, R.C.F., Ye, X.J., Wang, H.X., Lam, S.K., Lin, P., Chan, Y.S., Fang, E.F., Ngai, P.H.K., Xia, L.X., Ye, X.Y., Jiang, Y., and Liu, F. (2010). Mini Review: Proteins with antifungal properties and other medicinal applications from plants and mushrooms. *Applied Microbiology and Biotechnology* **87**:1221–1235.

Wong, P.Y.Y., and Kitts, D.D. (2006). Studies on the dual antioxidant and antibacterial properties of parsley (*petroselinum crispum*) and *Cilantro (coriandrum sativum*) extracts. *Food Chemistry* 97:505–515.

Yun, D., Ibeas, J.I., Lee, H., Coca,M.A., Narasimhan,M.L., Uesono, Y., Hasegawa,P.M., Pardo, J.M., and Bressan, R.A.(1998). Osmotin, a plant antifungal protein, subverts signal transduction to enhance fungal cell susceptibility. *Molecular Cell* **1:** 807–817.

Zhang, Z., Pang, X., Xuewu, D., Ji, Z., and Jiang, Y. (2005). Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chemistry* **90** (1-2) :47–52.

Zhu, P.C., Lei, M., Andargie, M., Zeng, J., and Li, J. (2019). Antifungal activity and mechanism of action of tannic acid against *Penicillium digitatum. Physiological and Molecular Plant Pathology* **107**: 46-50.