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Phytochemical analysis and *in vitro* screening of antifungal activity of *Jatropha multifida, Euphorbia hirta, Occimum gratissimum* and *Mitracarpus scaber* leaves extract

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Abstract

The research study was to understudy the qualitative and quantitative phytochemical compounds and antifungal activity of methanolic extracts of *Jatropha multifida*, *Euphorbia hirta*, *Occimum gratissimum* and *Mitracarpus scaber* leaves against 12 species of fungi.

The 8 phytochemical compounds tested were saponin, phenol, tannin, flavonoids, steroids, terpenoid, alkaloid and glycoside. The antifungi sensitivity test was analysed by Kirby-bauer disc diffusion method. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was analysed by broth tube dilution method followed by subculturing the relative samples. The plant extracts were prepared in the concentration range of 6.25, 12.5, 25, 50,100 and 200 mg/ml. The antifungal sensitivity test showed that *Jatropha multifida* (200mg/ml) was weakly effective against *Fusarium solani* and resistant against others. *Occimum gratissimum, Euphorbia hirta* and *Mitracarpus scaber* at (200mg/ml) respectively was highly effective on most of the fungi species tested with zone of inhibition of 10-20mm. *Jatropha multifida* had MIC and MFC (>200mg/ml), *Occimum gratissimum* had MIC and MFC (100 and >100mg/ml), *Euphorbia hirta* (25-200 and 50 - >200mg/ml) and *Mitracarpus scaber* had (25 - 100 and 50-200mg/ml) on most test isolates respectively. Investigations showed, *Mitracarpus scaber* were highly effective, followed by *Euphorbia hirta*, *Occimum gratissimum* and *Jatropha multifida* on the test organisms and could be attributed to the presence of phytochemical compounds and as such can be harnessed and used as a source of alternative medicine especially in the tropics like the South-eastern Nigeria.

Keywords: Phytochemicals; *Jatropha multifida; Euphorbia hirta; Occimum gratissimum* and *Mitracarpus scaber*.

1. Introduction

The use of medicinal plants in the treatment of diseases are of immense importance in the health of people. It is closely linked with conventional medicine as it is being used now in curing both emerging and re-emerging infections [1]. Herbs derived from different parts (leaves, roots, seeds, berries, bark or flowers) of plant extracts have gone a long way in treating a wide range of clinical diseases [2]. These plant parts possess certain chemicals known as primary and secondary metabolites.The primary metabolites includes amino acids, sugar, purines and primidines while the secondary metabolites incudes alkaloids, glycosides, phenols, terpenoids. The distribution and concentration of these chemicals differ from one plant to another and from one plant part to another. Chemical compounds such as alkaloids

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and glycosides are known to be poisonous if present in large quantities but can be useful and harmless if used in smaller quantity [3]. About three quarter of the world today relie on plants and their extracts for their healthcare [4].These extracts are now known as a potentially source for the development of chemotherapeutic agents [5].

1.1. Mitracarpus scaber

This plant belongs to the family Rubiaceae and it is popularly known as the madder family. It belongs to the Gentianales order which is now called Rubiales order. This family consists of approximately 500 genera and about 6,000 species which can be found all over the world. Some of them can be seen as tropical trees and shrubs (erect, struggling or twining) while some are found as herbs (erect or decumbent). *Mitracarpus Scaber* is a herb that is about 30cm in height or less and possess rough leaves. In Nigeria, different tribes have different names for it. The Igbos' call it Obuobwa, Hausas' call it Gududal while the Yorubas' call it Irawo [6]. Its leaves extract can be used in treating a lot of diseases traditionally like headache, toothaches, hepatic diseases, veneral diseases as well as leprosy. It is also believed to possess both antibacterial and antifungal activities [7]. In Nigeria, its leaf extract have been used in treatment of some skin diseases like, eczema, ringworm, lice, craw-craw, lice. It can be used in dressing cuts, ulcers and wounds [6]. Due to the immense importance of this plant a lot of scientific studies have been carried out to determine the antibiotic effectiveness of its leaf extracts.

In a study by [7], syrup were formulated from crude extracts of *Mitracapus scaber* "zucc". The antimicrobial activity of the syrup was analysed using agar plates containing these extracts at different concentrations ranging from 25mg/ml and 300mg/ml. This preparation was used to determine the minimum inhibitory concentration against a fungal organism namely *Candida albicans* at 37°C. At the end of the experiment results showed that *Candida albicans* were inhibited by the formulation at a minimum inhibitory concentration of 75mg/ml. [8] also evaluated the antimycotic activity of crude methanolic extracts of *Mitracarpus scaber* on *Candida albicans* and *Trichophyton mentagrophytes.* In his work, 20mg/ml solution of methanol crude extract was prepared as against standardized solution of Nystatin and Griseofulvin prepared from conventional products. 0.05ml of each preparations were added to sterilized sabouraud dextrose agar containing chloramphenicol, after which the test organisms were inoculated onto the agar slants and then incubated at 37°C. Results showed that *Mitracarpus scaber* inhibited the growth of both organism at 4mg/ml while Nystatin did not inhibit the growth of *Candida albicans* but inhibited the growth of *T.mentagrophytes* at 7mg/ml. [9], surveyed the efficacy of two commonly used antifungal herbs in Nigeria against clinical isolates of fungi. In their work *Mitracarpus scaber* and *Occimum gratissmum* were used to test its antifungal activities against fungal isolates (moulds and yeast) recovered from subjects in a community. The test isolates were from skin/scalp scrapings, *Candida albicans,* sputum, urine, endocervical swab, groin, mouth thrush and palm. *In-vitro* antifungal activity of the ethanol extract of *Mitracarpus scaber* (50μgml^{-1}) showed that the clinical isolates were sensitive to the herbal extracts but were more sensitive to *O. gratissium* oil extract (MIC range of 0.8 – 1.25 μgml^{-1}) than to ketoconazole (MIC range of 0.31-5.00 μgml^{-1}). [10] worked on the action of miscamates soap in the treatment of ringworm. In their research a soap was produced by combining plant oils for the treatment of skin infections (*Mitracarpus scaber, Cassia alata* and *Mareya micrantha).* This soap was subjected to an *in-vitro* test on *Trichophyton mentagrophytes* and clinical trials on infected patients. Studies from this work showed that the soap is active *in-vitro* on *T. mentagrophytes* and its application provides total cure for ringworm.

1.2. Jatropha multifida

The genus name of this plant Jatropha is a greek word *jatros* (doctor) and trophie (food) which means medicinal uses [11]. This plant belongs to the family Euphorbiaceae. It can be seen as a shrub or a tree that is drought resistant. It can be found in the wild or semi-cultivated areas in central and South America, Africa, India and South East Asia [12]. Different parts of the Jatropha (seeds, leaves, bark etc) are known to be of great importance in traditional medicine and for veterinary purposes [13]. On the other hand *Jatropha* is a non edible oil seed plant, whole extracts from different parts is known to be toxic. This toxic effect of the extracts is as a result of the presence of a toxic ingregient called phorbol ester which is known to contain moluscicidal, Piscicidal, insecticidal, rodenticidal, anti-microbial and cytotoxic properties. Its adverse effects on animals including rats, poultry and ruminants are also well established [14]. A study by [15] showed that *Jatropha multifida* latex (whole plant) can be used to cure wounds but toxic when they surveyed the ethanomedical of plants used by local society in Poncokusumo district Malang, East Java Province, Indonesia.Wongsatit [16], observed that the watery sap of *Jatropha multifida* obtained by decoction can be used to treat stomach pain when he surveyed the utilization of medicinal plants used by herbalists in Khok Pho District, Pattani Province (Thailand). [5] in their work screened the antibacterial and antifungal activities of *Jatropha multifida* (Ogege) sap against some pathogens which include *Pseudomona aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Candida* sp. usuing agar well diffusion and broth dilution methods to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at concentration

of 1050mg/ml to 2mg/ml (by two fold dilution using sterile distilled water, results showed that all the organisms were susceptible to the sap. [17] studied the antimicrobial activity of *Jatropha multifida* extracts and chromatographic fractions against microorganisms responsible for sexually transmitted infections. In their research, hexane, ethyl acetate and methanol extracts of the plant and chromatographic fractions were screened against seven pathogenic organisms comprising gram positive and gram negative bacteria and fungi. The results showed potent antimicrobial activity against the isolates.

1.3. Occimum gratissimum

This is a shrub that belongs to the family Lamiaceae. It is popularly known as scent leaf or dove basil. It is usually found in tropical countries. Although Africa and Asia are the two continents where most variants of the plant exists [18]. In Nigeria, different tribes have different names for it. In Igbo it is called Ncho-anwu, Ahuji, in Yoruba it is called Efinrin, in Hausa it is called Daidoya while Edo people calls it Aramogbo [19]. *Occimum gratissimum* has been known to possess an antibacterial and antifungal properties and as a result has been of great importance to the medical field [20]. It has been proven that *O. gratissimum* is very useful in the medication for people living with Human Immunodeficiency virus (HIV) and Acquired Immuno Deficiency Syndrome Virus (AIDS) [21]. It is also useful in the treatment of gonorrheal infection, vaginitis, vaginal douches for metritis and mental sickness [21].

Occimum gratissimum have also been known to be active against some pathogenic bacteria like *Escherichia coli, klebsiella pneumonia, Pseudomonas aerugnosa, Proteus vulgaris, Streptococcus vivadians* [19] and some fungi like *Trichophyton rubrum, Crytococcus neoformans, T. mentagrophytes,Candida albicans* [20]. Traditionally it is used in the treatment of ailments like urinary tract, wound, skin and gastrointestinal infections [22]. The plant is an erect small plumb with many barnacles usually not more than 1m high [22]. Phytochemical evaluation of *Occimum gratissimum* reveals that it is rich in alkaloid, tannis, phytates, flavonoids and Oligosaccharides [23]. Around the coastal area of Nigeria, this plant is used in the treatment of epilepsy [24].

Also, [20] studied the effects of *Occimum gratissimum* leaves on common dermatophytes and *Malassezia furfur* in Rivers state, Nigeria using well-in-agar diffusion technique with different concentrations of ethanolic extracts. Its isolates were gotten from scalp, skin, toes and feet. Results showed a significant inhibitory effect of *Occimum gratissimum* at five different concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml used. [25] surveyed the antifungal activity of *Occimum gratissium* towards dermatophytes caused by *Microsporum canis, M.gypseum, Trichophyton rubrum* and *T. mentagrophytes. Occimum gratissimum* extracts (hexane, chloroform fractions, the essential oil and eugenol) were investigated using agar dilution method against their dematophytes. Results showed that hexane and eugenol were the most active although hexane inhibited the growth of all isolates 100% at the concentration of $125\mu ml^{-1}$. [22] justified the use of *Occimum gratissium* as a herbal medicine when they evaluated its interaction with disc antibiotics. This was achieved by testing the antibacterial and antifungal properties of the ethanolic extract of the leaves of *Occimum gratissimum* as against four clinical isolates namely *Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.* Also a typed bacterium of *Escherichia Coli* (ATCC 11775) and another typed fungal strain of *Candida albicans* (ATCC 90028) were included. The research also went further to verify if the concomitant administration of conventional antibiotics with *Occimum gratissimum* will affect its activity negatively since it is normally eaten as food (spice). This experiment were carried out using agar diffusion method to test the *in-vitro* activity of this plant extract and some of the conventional disc antibiotics used are Ciprofloxacin, Septrin, Streptomycin, Ampicillin, Nysatin and Ketoconazole. Results were positive although there were varying *in-vitro* activities against all the isolates used. [24] in his work, studied the antibacterial properties of *Occimum gratissimum* against some selected entero-bacteriacea. Water and ethanol were used for the extraction of the active constituents of the plant and results revealed that water extracts of the plant was not as active as the ethanolic extracts against the tested organisms.

1.4. *Euphorbia hirta* (Leaves)

Euphorbia hirta (linn) belongs to the family Euphorbiaceae. It is a small annual plant commonly found in tropical countries. Its height is usually about 40cm. Its stem is slender and often reddish in colour. Its young ones are covered with a yellowish bristly hairs. Its leaves are oppositely arranged and lanceolate [26]. When plucked its stem and leaves produces whitish or milky juice [26]. Its leaves are known to treat dysentery, cough, asthma, worms and vomiting. The white latex is used as eye drops to cure conjunctivitis, it is applied on swellings, piles and boils [27]. Its main components are flavonoids, terpenoids and phenols [28]. So many antimicrobial works have been carried out using extracts from different parts of *Euphobia hirta* plant. A work by [29] showed the antibacterial activity of *Euphorbia hirta* extracts against some clinically important bacterial species. Ethanol and petroleum ether extracts of *Euphorbia hirta* were prepared at different concentrations (25μg/ml, 50 μg/ml, 75μg/ml and 100μg/ml) and its antibacterial activity was tested using cup-plate method. Results showed that the ethanol extracts have potentially deleterious effects on the

microorganisms. [30] in his work, assessed extracts of *Euphorbia hirta* L. Leaf, flower, stem and root for their antibacterial and antifungal activity and brine shrimp lethality against some medically important bacteria and yeasts using the agar disc diffusion method. Results showed that leaves extract inhibited the growth of all tested organisms including the yeast (*Candida albicans)* with large zone of inhibition.

[31] in their work extracted and tested the antimicrobial activity of alkaloids of *Euphorbia hirta* against four bacteria (*Escherichia Coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus*) and four fungi (*Aspergillus flavus, Aspergillus niger, Candida albicans* and *Trichophyton mentagrophytes).* This was achieved by using the disc diffusion assay. Results showed that all micro organisms were sensitive against all the tested extracts from different parts (leaf, stem, root and fruits) of *Euphorbia hirta.* [32] carried out a review on plants used to treat skin diseases, amongst *Euphorbia* species, of all the species *Euphorbia hirta* showed to have the highest antioxidant activity.

2. Material and methods

2.1. Test isolates

The fungal strains were isolated from cattle skin samples using cultural methods on sabouraud dextrose agar plates and then identified by DNA sequencing using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The fungal strains were *Penicillum citrinum* (MH990629), *Aspergillus fumigatus* (MK4610, *Aspergillus welwitschiae* (MG576117), *Aspergillus aculeatus* (MK461093), *Aspergillus flavus* (Mk 299130), *Fusarium succisae* (Mk 418691), *Curvularia kusanol* (MG975624), *Cladosporium tenuissimum* (MK 357638), *Pestalotiopsis microspora* (MK 224482), *Fusarium solani* (MH517359), *Fusarium lichenicola* (KH921661) and *Absidia* spp.

2.2. Collection and identification of plant materials

Fresh leaves of *Mitracarpus scaber, Jatropha multifida*, *Occimum gratissimum* and *Euphorbia hirta* plants were collected from the Federal research institute Ibadan Nigeria.They were identified and authenticated at the department of Botany Igbenedion University Okada, Edo State by a taxonomist. To prepare the plant material (leaves) for analysis, the leaves were washed and rinsed with distilled water, dried initially at room temperature and finally in a thermostatically controlled hot air oven at 40 ℃ until constant weight is maintained [33].

The leaves were ground into fine powder in an electric blender.The powdered materials were stored in screw capped bottles and kept in a refrigerator at 4 ℃ until required for use [34].

2.3. Extraction of active components from plant materials

The active principle of the plant (leaves) were extracted, using soxhlet extractor and rotary evaporator in a microbiology laboratory of Igbenedion University Okada, Edo State. One kilogram of the powered materials, was extracted in 250ml of 95% methanol to extract the active principle using soxhlet apparatus as described [33 -35]. The crude extract was allowed to evapourate to dryness using rotary evaporator [35].

2.4. Phytochemical screening of the plant leaves

2.4.1. Test for saponins

3g of plant powder was boiled in 10ml of water, warmed and filtered. 2 ml of filtrate was mixed with 5ml of distilled water and then warmed on the water shaken vigorously for a stable persistent froth. The presence of frothing which lasted for 3 – 5 minutes indicated the presence of saponins [36-37].

2.4.2. Test for phenolic compounds

5g of the sample was also boiled with 25ml of methanol, then warmed on water bath and filtered. 2ml of the filtrate was mixed into 2ml of 1% ferric chloride solution. The formation of brownish- green precipitate was taken for the presence of condensed tannin while bluish-black precipitate was taken for the presence of hydrolysable tannin [36-37].

2.4.3. Test for flavonoids

Another 2ml of filtrate obtained from methanol extract of the plant samples was added to 2ml dilute ammonia solution.The appearance of a yellow colour was taken for the presence of flavonoids [36-37].

2.4.4. Test for steriods

2ml of acetic anhydride was added to 0.5g methanol extract of sample in the presence of 2ml H_2SO_4. Colour changes from violet to blue or green indicates the presence of steroids [36-37].

2.4.5. Test for terpenoids

0.5g of methanol extract was mixed in 2ml of chloroform. 1ml of concentrated H_2SO_4 was carefully added to form a lower layer. A reddish brown coloration at interface was taken for the presence of steroids [36-37].

2.4.6. Test for alkaloids

A quantity of 0.1g of the ground samples were boiled with 5ml of 2% hydrochloric acid on a steam bath. This was filtered and 1ml portion of the filtrate reacted with 2 drops of the following reagents [38].

- Wagners reagent (Iodine in potassium iodide solution), and observed for reddish brown precipitate.
- Meyers reagent (potassium mercuric iodide solution), and observed for creamy coloured precipitate.

2.4.7. Test for phlobatannin

0.5g of methanol extract was added 3ml of 1% HCL and water on water bath at 90 ℃ for 15 minutes. The formation of red residue at the base of test-tube was taken for the presence of tannin [36-37].

2.4.8. Test for cardiac glycosides (Keller-Killani)

The presence of cardiac glycoside (five membered lactone, characteristics of cardiac glycoside) was tested by adding of 1ml methanol extract of the plant samples with 1ml of kedde's reagent and 1ml of dilute sodium hydroxide, the formation of violet colour was taken as presence of cardiac glycosides [36-37].

2.5. Quantitative analysis of plant leaves

2.5.1. Alkaloid determination

The plant leave extracts (1mg) was dissolved in dimethyl Sulphoxide (DMSO), 1ml of 2N Hcl was added and filtered. 1ml of the filtrate was mixed with 1ml of bromocresol green solution (BCG), 2ml of chloroform and was later diluted with 6ml of water.

A standard solution was also prepared in the same manner as described earlier. The absorbance of for test and standard solution was determined against the reagent blank at 470nm wavelength using the UV spectrophotometer [39].

2.5.2. Phenol determination

The phenolic content of the plant leaf were determined using Folin-Denis reagent. 10ml of the sample filterate was mixed with 1ml of Folin-Denis reagent and 1ml of sodium carbonate. The solution was kept at room temperature for 5minutes.

For standard solution gallic acid was prepared (5g of gallic acid was dissolved in 100ml of water and mixed with 1ml of sodium carbonate). This was used to measure absorbance for the test at 750nm using spectrophotometer [39].

2.5.3. Glycoside determination

1gram of sample was solubilized in 50ml of water and filtered. 1ml of the filtrate was mixed with 2ml of dinitricsaliculic acid (10g of sodium hydroxide was dissolved in 250ml of water, 60ml of this solution was then mixed with 2grams of saliculic acid) and allowed to stand for some minutes.

For standard solution, glucose solution was prepared and used to determine total glycosidic content for test at 540nm wavelength [39].

2.5.4. Saponin determination

0.5gram of sample was dispensed in 50% methanol in a conical flask. This was heated for 3hours after which the solution was filtered. 1ml of filterate was mixed with 5ml of magnesium carbonate and 1ml of 5% ferric chloride. The absorbance of saponin content was determined using spectrophotometer at wavelength at 380nm [39].

2.5.5. Flavonoid determination

1gram of sample was weighed and dissolved in 50ml of 50% methanol.1ml of extract was mixed with 200 microliter sodium nitrate (100ml,5%) , 200microliter of 10% aluminum chloride and allowed to stand for 5 mins after which 1ml sodium hydroxide was added and allowed to stand for 10minutes. The absorbance was of the test was measured using spectrophotometer at 510nm [40].

2.5.6. Tannin determination

1ml of sample filtrate was mixed with 0.5ml of Follin-Dennis reagent and 5ml of 5% sodium carbonate. This was kept undisturbed for about 30mins.

The standard solution of 1mg/ml tannin was prepared by dissolving 100mg stock solution of tannic acid in water.1ml of tannic acid was measured and mixed with 0.5ml of Folin -Denis reagent and 5ml sodium carbonate solution. The tannin content for the test was measured by reading at 760nm against blank reagent using spectrophotometer [41].

2.5.7. Steriod determination

0.5 gram of sample was weighed and mixed with 50ml of 50% methanol which was heated for 30minutes and then filtered. The filterate was mixed with 2ml of 2mole solution of sulphuric acid, 2ml of 10% ferric chloride and 0.5ml of ferric cyanide. The absorbance was read at 750nm wavelength using UV spectrophotometer [39].

2.6. Dillution of plant extracts

Crude extracts of the plant (leaves) were allowed to evapourate to dryness. The solvent used for all plant extracts were dissolve in 99% dimethyl sulfoxide (DMSO). Two grams of the dried extract were dissolved in 10ml 99% dimethlyl sulphoxide (DMSO) to obtain the stock of 200mg/ml. Briefly 5 two-fold extract dilution were prepared in 5ml to obtain the different concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. These were applied on already prepared discs.

2.7. Antifungal susceptibility testing of extracts

Antifungal susceptibility test were carried out on 12 fungal strains using the crude extracts of the plant leaves. This was carried out, using disc diffusion method. In this method, 1ml of the test isolate in sabouraud dextrose broth (SDB) was diluted in 9ml of distilled water. 0.2ml of the dilution was spread evenly on the surface of the solidified sabouraud dextrose agar (SDA) plates, using a sterile bent glass rod spreader (Hockey stick) before introducing the discs.

2.8. Preparation of disc

Sterile paper discs (made of Whatman No.1 filter paper) measuring about 6mm was used to impregnate about 20µl of the different dilutions (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) of the plant extract and then air dried. This discs were transferred onto the solidified sabouraud dextrose agar media evenly spread with the isolate. 20µl of 99% DMSO was also impregnated on a separate disc and delivered as a control and labeled accordingly. This was inoculated on triplicate. The discs were kept on the bench for 40minutes for pre-diffusion of the extract. After diffusion, the discs were aseptically introduced on the surface of the medium with the aid of a sterile forceps and allowed for 10-15 mins, before incubating at 35 ◦ C for 72hours and examined for fungal growth inhibition. The diameter zone of growth inhibition on the different plates was measured in millimeters, using a transparent metric rule.

2.9. Determination of the minimum inhibitory concentration (mic) using tube dilution method

The tube dilution method of broth was used to determine the MIC of the test extract, using two fold serial broth dilution method. The selected plant leaves extract were *Occimum gratissimum*, *Euphorbia hirta, Jatropha multifida* and *Mitracarpus scaber.*

A double strength broth of sabouraud dextrose broth with extract was prepared (5ml of 99% DMSO was used to dissolve 2g of the extract initially and then raised to 10ml using 5ml of double strength sabouraud dextrose broth). This was diluted serially by introducing 5ml of the stock aliquot into 5ml of single strength broth to obtain 100mg/ml. Similarly dilutions up to 10^{-4} was carried out to obtain 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml respectively. The serially diluted test extracts were then inoculated each with 0.2ml of 10^{-2} dilutions of an overnight broth culture of the test isolates. All tubes were incubated for 72 hours and later inoculated onto sterile sabouraud dextrose agar plates and incubated. The least drug concentration that showed least growth was taken as the minimum inhibitory concentration

(MIC) for the particular plant extract while absence of growth indicates a fungicidal concentration; hence the Minimum Fungicidal Concentration (MFC) as described by Clinical Laboratory Standards Institute, CLSI [42].

3. Results and discussion

Table 1 Qualitative phytochemical analysis

Test	**Plants**			
	Occimum gratissium	***Euphorbia hirta***	***Mitracarpus scaber***	***Jatropha multifida***
Saponin	+	+	+	++
Phenol	+	_	_	+
Tannin	_	_	++	+
Flavonoids	+	+	+	+
Steriods	+	++	++	++
Terpenoids	++	++	+++	+
Alkaloids				
Wagner	_	+	++	+
Mayer	+	_	+	_
Glycoside	++	++	+++	+

Key: (+) present, (-) absent and (++) abundance

Table 2 Quantitative phytochemical analysis of the plant leaves.

Test	**Plants (mg/100g)**	**(mg/100g)**	**(mg/100g)**	**(mg/100g)**
	Occimum gratissium	*Euphorbia hirta*	*Mitracarpus scaber*	*Jatropha multifida*
Saponin	27.94	27.94	27.84	27.9
Phenol	0.0085	0.0023	0.0127	0.0023
Tannin	0.0262	0.1606	0.054	0.030
Flavonoids	-2.04	-6.68	-0.310	-2.030
Steriods	35.7	35.86	35.7	35.9
Alkaloids	0.6122	0.3502	0.369	0.383
Glycoside	0.232	0.744	0.660	0.510

Table 3 Antifungal susceptibility pattern of 12 non-dermatophyte molds isolated from this study against the four selected methanolic extracts of the plant leaves.

Isolates	***Jatropha multifida***						***Occimum gratissium***						***Euphobia hirta***						***Mitracarpus scaber***					
mg/ml	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**200**	**100**	**50**	**25**	**12.5**	**6.25**
Fusarium solani	15RM	R	R	R	R	R	R	R	R	R	R	R	10	R	R	R	R	R	R	R	R	R	R	R
Fusarium succisae	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R	R	R	R	R	R	R	R	R	R
Fusarium lichenicola	R	R	R	R	R	R	5	R	R	R	R	R	R	R	R	R	R	R	10	R	R	R	R	R
Aspergillus flavus	R	R	R	R	R	R	20	R	R	R	R	R	15	R	R	R	R	R	10	10	R	R	R	R
Aspergillus welwitschiae	R	R	R	R	R	R	10	R	R	R	R	R	16	R	R	R	R	R	15	10	R	R	R	R
Aspergillus fumigatus	R	R	R	R	R	R	15	10	10	10	R	R	10	R	R	R	R	R	15	10	5	R	R	R
Aspergillus aculeatus	R	R	R	R	R	R	15	10	R	R	R	R	R	R	R	R	R	R	12	R	R	R	R	R
Clasdosporium tenuissimum	R	R	R	R	R	R	R	R	R	R	R	R	15	R	R	R	R	R	15	R	R	R	R	R
Curvularia kusanol	R	R	R	R	R	R	20	20	10	10	R	R	10	R	R	R	R	R	10	R	R	R	R	R
Pestaloptiosis microspora	R	R	R	R	R	R	R	R	R	R	R	R	10	9	8	R	R	R	15	10	10	10	10	R
Penicillum citrinum	R	R	R	R	R	R	20	15	R	R	R	R	15	15	10	10	R	R	10	R	R	R	R	R
Absidia Sp.	R	R	R	R	R	R	15	10	10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

KEY: RM – Resistant mutant, R– Resistant. Inhibition Zone (IZ) in Millimetres (mm)

Table 4 Mic's and Mfc's of the four selected plant (leaves) extract against 12 non dermatophyte Molds isolated from the study

Isolate	***Jatropha multifida***		***Occimum gratissium***		***Euphobia hirta***		***Mitracarpus scaber***	
	MIC (Mg/ml)	**MFC (Mg/ml)**	**MIC (Mg/ml)**	**MFC (Mg/ml)**	**MIC (Mg/ml)**	**MFC (Mg/ml)**	**MIC (Mg/ml)**	**MFC (Mg/ml)**
Fusarium solani	>200	>200	100	200	100	200	25	50
Fusarium succisae	200	>200	>200	>200	200	>200	50	100
Fusarium lichenicola	>200	>200	>200	>200	>200	>200	25	50
Aspergillus flavus	>200	>200	200	>200	200	>200	25	50
Aspergillus welwitschiae	>200	>200	200	>200	100	200	25	50
Aspergillus fumigatus	200	>200	100	200	100	200	50	100
Aspergillus aculeatus	>200	>200	100	200	>200	>200	25	50
Clasdosporium tenuissimum	>200	>200	>200	>200	100	200	50	100
Curularia kusanol	>200	>200	100	200	50	100	25	50
Pestaloptiosis microspora	>200	>200	>200	>200	25	50	50	100
Penicillum citrinum	100	200	100	200	25	50	50	100
Absidia Sp.	>200	>200	50	100	200	>200	>200	>200

Key: MIC = Minimum Inhibitory Concentration, MFC =Minimum Fungicidal Concentration

The Methanolic extract of four plant leaves *Euphorbia hirta*, *Occimum grastissium*, *Mitracarpus scaber* and *Jatropha multifida* were subjected to phytochemical analysis. They were tested for eight different compounds. Result reveals presence and absence of these compounds at different concentrations respectively (Table 1). For *Euphorbia hirta*, qualitative analysis reveals abundance of glycosides, terpenoids, steroids and small concentration of flavonoid, alkaloid in wagner reagent and saponin while tannin and phenol were absent. The presence of flavonoids in this study is in agreement with analysis by [43]. Another work by [3] revealed presence of alkaloids, saponin, tannin, flavonoids, steroids and cardiac glycoside while terpeniod was absence when they carried out phytochemical analysis on *Euphorbia hirta*.

Occimum grastissium in this study reveals abundance of glycoside and terpenoid, presence of flavonoid, saponin, phenol, alkaloid with mayer and steroids while terpenoid was absent. This work agrees with other studies [34,44,45,46] while on the contrary, [47] in his study when he screened *Occimum grastissium* leaves flavoniod was absent while all other compounds were present.

Mitracarpus scaber in this study reveals abundance of glycoside, steroids, terpenoid, tannin and presence of flavoniod, saponin and alkaloid with mayer and wagner while phenol was absent. This result is also in agreement with study by [48] and [49] in their study phytochemical analysis of *Mitracarpus scaber* leaves reveals presence of alkaloids, tannin, cardiac glycosides and saponin. In another work by [50], the reverse was the case when he analysed the qualitative analysis of *Mitracarpus scaber* leaves, his result revealed abundance of saponin and flavoniod and presence of tannin, alkaloid, glycosides and steroids.

Jatropha multifida in this study reveals abundance of saponin and steroids, presence of glycosides, terpenoid, tannin, flavonoid, phenol, alkaloid with wagner while alkaloid was absent with mayer reagent. This is in accordance with a study by [51] and [52], in their work *Jatropha multifida* leaves revealed presence of alkaloid, tannin, saponin, flavonoid and phenol.

Quantitative phytochemical analysis of the total phenolic, flavonoid, tannin and alkaloid present in the plant leaves were determined by UV spectrophotometric method (Table 2). The total glycosidic compound were found to be in maximum quantity in *Euphorbia hirta* (0.744mg/100g), followed by *Mitracarpus scaber* (0.660 mg/100g), *Jatropha multifida* (0.510mg/100g) and then *Occimum gratissium* (0.232mg/100g).The presence of this chemical compound in ascending order of plants extracts could be attributed to its potentiality of possessing antifungal properties. It is also an implication that *Euphorbia hirta* could possibly be a good producer of glycoside. Our analysis also reveals a high content of saponin (above 27.0mg/100g) present in all the four plant leaves. Saponin have been known to posses antifungal, antiyeast and antibacterial properties. This study also revealed a trace amount of flavonoid (-0.310 to -2.040 mg/100ml) content in all the four plant leaves. This is in disagrement with study by El-Mewafy [53], in their analysis methanolic extract of *Jatropha multifida* leaves contains significant amount of flavonoid (38.22 ±3.26mg/100mg). Alkaloid compound in this study was observed to be maximum in *Occimum gratissium* leaves (0.6122mg/100g) than the other plant leaves extract (≥ 0.35 – 0.40 mg/100g).Tannin content was observed to be in a very high amount in *Euphorbia hirta* (0.1606mg/100g) leaves than in the other plants (0.02 - 0,05mg/100g). Tannin has been known to posses antimicrobial activity. A study carried out by [54], showed that tannin extracted from plant inhibited spore formation of *Aspergillus flavus* and mycelia growth of *Aspergillus niger*. Our study also reveals presence of phenol although not in a very high content, amongst the plant leaves understudied in ascending order *Mitracarpus scaber* (0.0127mg/100g), followed by *Occimum gratissium* (0.0085), *Jatropha multifida* and *Euphorbia hirta* (0.0023mg/100g) respectively. This is in agreement with work by [51], when they analysed phytochemical compounds of *Jatropha* L species in their study phenol had the least concentration (0.18%). This disagrees with [53], in their work methanolic extract of *Jatropha multifida* expressed highly significant content of phenol (17.09 ± 0.010mg/ 100g).

Furthermore, methanolic extract of these four plant leaves (*Jatropha multifida*, *Occimum grastissimum*, *Euphorbia hirta* and *Mitracarpus scaber*) were subjected to antifungal sensitivity test employing the disc diffusion method. Twelve fungal species of the non-dermatophytic molds were tested belonging to *Absidia* spp., *Fusarium solani*, *Fusarium succisae*, *Fusarium lichenicola*, *Aspergillus flavus*, *Aspergillus welwitschiae*, *Aspergillus fumigatus*, *Aspergillus aculeatus*, *Curvulari*a *kusanol*, *Pestalotiopsis microspora*, *Cladosporium tenuissimum* and *Penicillum citrinum* (Table 3 and 4). Results revealed that all isolates were resistant to *Jatropha multifida in-vitro* at all concentrations except for *Fusarium solani* that was found to be weakly sensitive which could be attributed as resistant mutant (RM) at 200mg/ml with an inhibition zone (IZ) of 15mm. To strengthen this, minimum inhibitory concentration(MIC) (Table 4) was carried out using tube dilution method and subculturing of the relative sample and results showed that most isolates had their MIC at concentration > 200mg/ml except *Penicillum citrinum* that expressed its MIC at a concentration of ≥ 100mg/ml and minimum fungicidal concentration (MFC) at 200mg/ml, while *Aspergillus fumigatus* and *Fusarium succisae* showed its MIC at 200mg/ml and MFC > 200mg/ml respectively. This could be attributed to antimicrobial potentials of *Jatropha*

multifida leaves. A study by [55] stated that methanolic extract of *Jatropha multifida* leaves inhibited growth of fungus at an MIC of 25µg/L.

From our results *Occimum grastissimum* leaves extract were sensitive on *Fusarium linchenicola* (200mg/ml), *Aspergillus flavus* (200mg/ml), *Aspergillus welwitschiae* (200mg/ml), *Aspergillus fumigatus* (25-200mg/ml), *Aspergillus aculeatus* (100mg/ml), *Curvularia kusanol* (25-200mg/ml), *Penicillum citrinum* (100mg/ml) and *Absidia* spp. (50-200mg/ml) with inhibition zones (IZ) ranging from 5mm-20mm and was resistance on *Fusarium solani*, *Pestalotiopsis microspora*, *Cladosporium tenuissimum* and *Fusarium succisae* at all concentrations. Afterwards the MIC was also carried out on the isolates and results revealed that *Penicillum citrinum, Aspergillus aculeatus, Aspergillus fumigatus, Curvularia kusanol* and *Absidia* spp. had MIC at 100mg/ml and MFC at ≥ 200mg/ml while *Aspergillus welwitschiae* had a MIC at 200mg/ml and MFC >200mg/ml. This result is an indication that *Occimum grastissimum* possess strong antifungal potentials .This is in agreement with previous works by [34] in their work antimicrobial activities of leaves of *Occimum grastissimum* were observed. Another study by [47] supports that *Occimum grastissium* leaves posseses antifungal properties. In yet another study by [56] revealed that *Occimum grastissium* leaves extract at higher concentration did not support growth of fungi (*Absidia blakslseeana*, *Macrophomina phareolina* and *Fusarium solani*).

Also methanolic extract of *Euphorbia hirta* leaves were tested for antifungal effects on the isolates and results revealed that the extract were sensitive to *Fusarium solani*, *Fusarium succisae*, *Aspergillus flavus*, *Aspergillus weiwitschiae*, *Aspergillus fumigatus*, *Cladosporium tenuissimum*, *Curvularia kusanol*, *Pestalotiopsis microspora* and *Penicillum citrinum* at 200mg/ml with inhibition zones (IZ) ranging from 10mm-20mm and resistance to 3 (*Absidia* spp., *Fusarium lichenicola* and *Aspergillus welwitschiae*). To strengthen this MIC of the extract was carried out and it was observed that the 3 isolates that were resistance had their MIC at concentrations above 200mg/ml others that were sensitive had their MIC at concentration ≥ 200mg/ml except *Pestalotiopsis microspora* that had its MIC at concentration ≥ 50mg/ml and MFC at concentration ≥ 100mg/ml while *Penicillum citrinum* had its MIC at concentration ≥ 25mg/ml and its MFC at concentration ≥ 100mg/ml. This is an indication that methanolic extract of *Euphorbia hirta* leaves posses antifungal quality and this can be attributed to presence of active ingredients present in the leaves extract. This is not in accordance with a work by [57] in their study it was observed that ethanolic extract of *Euphorbia hirta* (leaves) showed no antifungal activity on *Aspergillus fumigatus* and *Aspergillus niger* using dics diffusion method while on the contrary [58] in their study also agrees with our study that leaf extracts of Euhorbiaceae posses growth inhibition against fruit rot fungi.

Methanolic extract of *Mitracarpus scaber* leaves were tested for antifungal sensitivity on the isolates using disc diffusion method and results revealed that the extract were sensitive to *Fusarium linchenicola*, *Aspergillus aculeatus*, *Cladosporium tenuissimum*, *Curvularia kusanol* and *Penicillum citrinum* at 200mg/ml with inhibition zones (IZ) ranging from 10mm-15mm. On *Aspergillus flavus* and *Aspergillus welwitschiae* sensitivity was at 100mg/ml with IZ of 10mm while *Aspergillus fumigatus* showed sensitivity at 50mg/ml with IZ of 5mm and *Pestalotiopsis microspora* had sensitivity at 12.5mg/ml with IZ of 10mm. Three of the isolates (*Absidia* spp., *Fusarium succisae* and *Fusarium solani*) were resistant to this extract. Minimum inhibitory concentration (MIC) on these isolates using tube dilution method and subculturing of the samples revealed *Aspergillus aculeatus*, *Aspergillus welwitschiae*, *Aspergillus flavus*, *Curvularia kusanol* and *Fusarium lichenicola* was at 25mg/ml and minimum fungicidal concentration (MFC) at ≥ 50mg/ml. *Penicillum citrinum*, *Aspergillus fumigatus*, *Fusarium succisae*, *Cladosporium tenuissimum*, *Pestalotiopsis microspora* and *Fusarium solani* had their MIC at 50mg/ml and MFC at > 100mg/ml, while *Absidia* spp. had both MIC and MFC >200mg/ml. This is an indication that *Mitracarpus scaber* posses very strong antifungal potentials which can be attributed to possession of active compounds. This agrees with work by [6] in their study antimicrobial activity of *Mitracarpus scaber* "zucc" were analysed on agar plates and concentration ranging between 25mg/ml and 300mg/ml were used to evaluate minimum inhibitory concentration (MIC). From their results it was observed that fungal isolates such as *Candida albicans* had MIC at 75mg/ml. Another study by [9] analysed the efficacy of ethanolic extract of *Mitracarpus scaber* against fungal isolates (mould and yeast) and results revealed that clinical isolates showed sensitivity at 50µg/ml^{-1}. According to [59] in their work *Mitracarpus scaber* expressed antifungal activity against *Aspergillus flavus*, *Microsporum canis*, *Candida albicans* with MIC and MFC <65µg/ml. This is in accordance with our study.

4. Conclusion

This study has revealed that these plants leaf extracts evaluated contains active compounds that can be used as alternative medicines in the treatment of infections caused by fungal isolates. The extract of *Mitracarpus scaber* showed the highest antifungal activity against the non dermatophytic molds followed by *Euphorbia hirta*, *Occimum grastissum* and *Jatropha multifida*. This is an indication that these our indigenous plants studied could be harnessed and used in the production of antifungal drugs. Further investigation will be to identify the particular active compounds that might be responsible for these antifungal activities.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declared that there is no conflict of interest.

Statement of ethical approval

Based on the compliance of the ethical standard of Imo and Abia State in Nigeria. The Ethical permit No; NAR/Vet/XX of the Ministry of Agriculture and Natural Resources Owerri, Imo state dated 13th March, 2017 and that of the Ministry of Agriculture Umuahia, Abia state Ref. No; DVS/01/RCH/01/18, dated 4th June, 2018, was also issued before the commencement of the screening of the cattles in both States

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