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Chemo-profiling of Secondary Metabolites from Endophytic Fungi of *Jatropha tanjorensis*

Omotoso Abayomi E[1]* and Igbinaduwa Patrick[2]

[1]*Department of Pharmaceutical & Medicinal Chemistry, University of Port Harcourt, Port Harcourt, Nigeria*

[2]*Department of Pharmaceutical Chemistry, University of Benin, Benin City, Nigeria*

***Corresponding author e-mail: abayomi.omotoso@uniport.edu.ng**

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ABSTRACT

Jatropha tanjorensis belongs to family Euphorbiaceae and like other medicinal herbs have been used in ethnomedicine for treatment of various ailments such as diabetes, hypertension, inflammation and anemia. This is believed to be due to the presence of bioactive compounds they contain. This study was carried out to isolate the endophytic fungi resident in the midribs and leaf blades of *J. tanjorensis* and consequently to identify the bioactive compounds produced by the fermentation of these fungi using Gas Chromatography- Mass Spectrometry analysis. Leaves of *J. tanjorensis* were subculture into Sabouraud agar from which single colony forming units were isolated and identified microscopically and morphologically. The isolated fungal strains were subsequently fermented in rice media. The secondary metabolites were extracted using ethyl acetate and subsequently concentrated for GC-MS analysis.

Two microorganisms were isolated: *Cryptococcus neoformans* and *Microsporum canis.* Bioactive compounds identified were mainly fatty acids such as n-Hexadecanoic acid and Octadecanoic acid. Many of the compounds identified possess an antioxidant, hypolipidemic, anti-inflammatory, anti-cancer, and pesticides, anti-microbial and anti-androgenic property which justifies the folklore use of the leaves in traditional system to cure various ailments. The results suggest that the medicinal properties of *J. tanjorensis* can be attributed to endophytes (endophytic fungi) present in the leaves.

Keywords: *Jatropha tanjorensis*, Endophytic fungi, GC-MS, Bioactive compounds.

INTRODUTION

Microorganisms are ubiquitous in nature hence their existence on virtually all surfaces of both living things and fomites (ectophytes), within the stem and tissues of plants (endophytes) and also on the surface of smooth muscles and organs of human where they are termed normal flora. This thus explains the colonization of the organs and tissues of both stems and leaves of plants by endophytes. Endophytes are microorganisms which are found within the cells, tissues and organs of plants.

Endophytes are ubiquitous and have been found in all species of plants in which they exhibit variable life history strategies of the symbiosis, ranging from facultative, saprophytic to parasitic to exploitive and to mutualistic [1-5].

Endophytes may be transmitted either vertically (directly from parent to offspring) or horizontally (among individuals); vertically transmitted fungal endophytes

are typically considered clonal and transmit via fungal hyphen penetrating the embryo within the hosts' seeds, while reproduction of the fungi through asexual conidia or sexual spores leads to horizontal transmission, where endophytes may spread between plants in a population or community. Endophytes are a reliable and abundant source of bioactive and chemically novel compounds, with great potential for exploitation in a wide variety of medical, agricultural, and industrial arenas

Jatropha tanjorensis J. L Ellis & Saroja (Euphorbiaceae) a shrub with thick stout stem and sparse branching. Leaves alternate, palmate five lobed, light to dark green with no pigmentation except on very young leaves, margins distantly serrate, long petiole with dense pigmentation, has 8 stamens and does not produce fruits. *J. tanjorensis* is used ethno-medicinally in the management of several disorders and pathological conditions in the body and as such possesses antidiabetic, antimicrobial, antioxidant, antianaemic, antimalarial, anticancer and anti-HIV activities. The medicinal properties of this plant have been attributed to the secondary metabolites which have been isolated from this plant. It is believed that these secondary metabolites are actually produced by the endophytic fungi present in this plant. With the isolation of a number of secondary metabolites from endophytic fungi from other medicinal plants, hence the need to evaluate the endophytic fungi in *Jatropha* and hence the secondary metabolites [6-10].

This study seeks to isolate endophytic fungi present in the mid ribs and leaf blades of the study plant - *Jatropha tanjorensis* and identify the products of secondary metabolites produced by the isolates.

MATERIALS AND METHODS

Materials used

The materials used in this experiment were: *Jatropha tanjorensis* leaves, Petri-dishes, Universal bottles, Bijou bottles, fermentation bottles, sterile 0.5 ml syringes, sterile rubber pipette, sterile stirrer, sterile surgical blades, sterile wire loop, sterile microscopic slips and slides, Compound microscope, Centrifuge, Autoclave, Sabouraud agar (Potato dextrose agar), Absolute ethanol, Ethyl acetate, Separating flask, Distilled water, Double-distilled water (sterile distilled water), Sodium hypochlorite 3.5% w/v, Beaker, Measuring cylinder, Sulphone blue, Aluminium foil, coloured marker pen, disinfectant jar, nose mask, scarf, sterile surgical gloves, Bunsen burner, Retort stand, Tripod stand, Wire gauze.

Plant collection

The leaves of *Jatropha tanjorensis* were freshly collected from Pharmacognosy garden, Department of Pharmacognosy, University of Benin, Benin City, Edo State, Nigeria on the 15th of March, 2017. The fresh leaves were identified by Pharm. Uwumarongie O.H. of the Department of Pharmacognosy, University of Benin. The leaves were stored in a polythene bag to prevent moisture loss prior to commencement of the experiment. The plant materials were washed thoroughly under a running tap water before surface sterilization.

Preparation of the leaves

The surface of the leaves were sterilized by immersion into 70% ethanol and washed for 2 minutes, afterwards, the leaves were transferred to 2% Sodium Hypochlorite and washed for 3 minutes and finally to double-distilled water for 3 minutes. The process was repeated for each of the 40 leaves.

The process can be illustrated using the Figure 1 below;

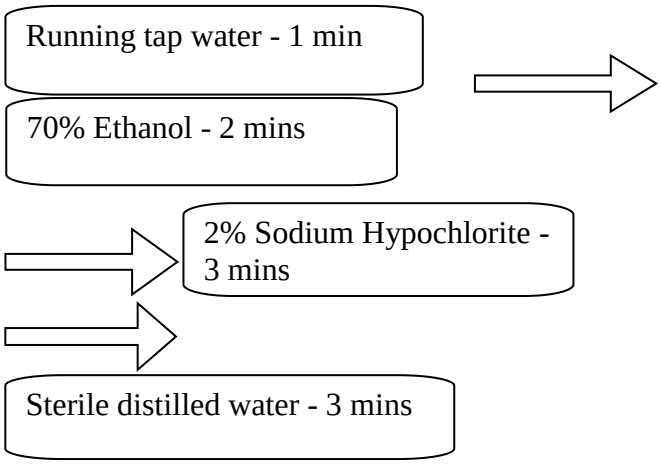

Figure 1: Procedure for surface sterilization of leaves.

Preparation of media

According to manufacturer's specification, Sabouraud (in this case Sabouraud broth) was prepared and this involved weighing the calculated amount of the Sabouraud in a sterile beaker followed by the addition of calculated quantity of distilled water

using a measuring cylinder. The amount of Sabouraud Dextrose Agar (SDA) used is calculated thus; according to manufacturer's specification; 65 g of SDA is dissolved in 1000 ml of water; therefore, 10 ml of Sabouraud broth for each of the 40 universal bottles will require 400 ml of water. Conversely, 400 ml of water will require; 400 x 65/ 1000 = 26 g of SDA. The solution was stirred properly using a sterile stirrer, the beaker was covered with aluminum foil and then transferred to the autoclave for sterilization and timed for 30 minutes. After 30 minutes of sterilization, the Sabouraud broth is transferred into each of the (10 ml-marked) 40 sterile universal bottles using a sterile pressure pipe with each corked immediately. All were performed within a sterile zone. A colored marker pen was used to label each bottle according to number.

Inoculation and incubation

Using a sterile surgical blade, the outer tissues are removed from the leaf and the inner tissues of the mid rib and leaf blade carefully excised and placed inside the Sabouraud broth. This procedure was repeated for each of the leaves and then, all were incubated at 25-28°C (Room temperature) for 4-7 days. After 24 hours, each of the broth samples were shaken to activate the growth process of the endophytes [11-14].

Subculture into Petri-dishes

5 days from the day the broth samples were shaken, the hyphal tips of the endophytic fungi emerging from each of the broth were subculture into new petri dishes containing freshly prepared Sabouraud Dextrose Agar (SDA) according to their labeled numbers, using a sterile inoculating loop within a sterile zone and incubated at 25°C for 7 days.

The Sabouraud Agar plates were prepared using the manufacturer's specifications as explained above. The preparation was heated with continuous stirring for at least 5 minutes until the Agar dissolves completely in water. The solution was then covered with an aluminium foil and placed in the autoclave for sterilization and timed for 30 minutes. After 30 minutes of sterilization, the Sabouraud medium was transferred into each of the petri dishes by slightly tilting the lid with the left hand and then holding the sterile pressure pipe with the right hand, each plate being covered immediately and allowed to set. The volume of SDA required to fill each of the 42 plates (2 plates used as control) was calculated to be 20 ml per plate. Thus using manufacturer's specification of 65 g of SDA in 1000 ml of water for dissolution, conversely, the 42 plates will require 54.60 g of SDA.

Isolation of microbial colonies

The whole process outlined above was repeated for another 42 plates, here, the colonies formed from the previous Agar plates were isolated by picking a loop full of a colony forming unit (C.F.U) and carefully transferring into already prepared Agar plates within a sterile zone. They were incubated at 25°C, this time, for 3 days.

Identification of the endophytic fungi

Following the isolation of the fungal colonies from each plate; they were identified morphologically and microscopically. Microscopic identification was carried out on a microscopic slide by placing a loop full of the organism and addition of 1 drop of Sulphones Blue for clear vision under microscope. For morphological identification; the endophytic fungal strains were mainly identified based on the morphological characteristics of reproductive structures with the aid of several relative monographs and original descriptions of species.

Fermentation process

After identification of the fungal strains, they were subculture each into two different freshly prepared rice media and incubated at 25°C for 5 days. After which the subculture process above was repeated using 15 ml of freshly prepared rice media for each organism, though this time, it was transferred into a fermentation bottle and incubated at 25°C for 10 days.

Centrifugation of the broth samples

Sequel to the fermentation process, the rice media were centrifuge (1000 g) for a spin time of 4 minutes. After which the apparent sedimentation of the primary metabolites were observed revealing a clear supernatant (Secondary metabolites). The supernatant of the 4 broth samples were then transferred carefully to 4 empty sterile universal bottles using sterile 0.5 ml syringe for each sample, caution was taken not to disrupt the sediment fraction of the samples [15-17].

Chemical extraction of the supernatant

After the collection of the supernatant from each sample, chemical extractions of the secondary metabolites were performed using 30 ml of Ethyl acetate. Each of the samples were shaken rigorously with 10 ml of Ethyl acetate (thrice) and poured into the separating flask which was supported firmly on a retort stand. The mixtures were separated using separating funnel into separate containers and labeled accordingly.

Concentration of the Ethyl acetate sample extracts

The Ethyl acetate extract for each sample were then heated over a water bath using a crucible in order to concentrate the secondary metabolites to ensure accurate analysis. Finally, the concentrates for each sample were labeled appropriately and a standard analysis using Gas Chromatography-Mass Spectrometry (GC/MS) was performed.

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

GC/MS analysis of the partitioned extracts (Ethyl acetate) was carried out on GC-MS-QP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer (GC/MS) instrument employing the following conditions: column VF-5MS fused silica capillary column (30.0 m x 0.25 mm x 0.25 µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70eV; helium (99.999%) was as carrier gas at a constant flow of 1.5 ml/min and an injection volume of 5 µl was employed for *Candida stellatiodea* extract (split ratio of 50:1) while 2 µl was employed for *Candida albican*, *Cryptococcus neoformans* and *Microsporium canis* extracts (split ratio of 20:1), injector temperature was 240°C; ion-source temperature was 200°C. The oven temperature was programmed from 70°C (isothermal for 3 min), with an increase of 10°C/min, to 240°C, then 5°C/min to 300°C, ending with a 9 min isothermal at 300°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 440 Da.

Identification of Phytoconstituents

Interpretation on mass spectrum of GC/MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with spectrum of known component stored in NIST library. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC/MS. The names, molecular weight, retention time and peak area percentage of the test materials were ascertained [18,19].

RESULTS

Microbiological and Microscopic Results

Sequel to the isolation of colonies and their identification, four microorganisms (Endophytic fungi) were discovered. They include: *Cryptococcus neoformans, Microsporum canis, Candida albican* and *Candida stellatoidea.*

Gas Chromatography - Mass Spectrometry (GC/MS) Analytical Results

The results from GC/MS analysis performed on the Ethyl acetate extracts of each above listed organisms, revealed several organic compounds which are illustrated in Tables 1-4 below;

DISCUSSION

In present studies, bioactive compounds have been identified in the ethanol extract of the leaves of *Jatropha tanjorensis* using Gas Chromatography/Mass Spectrometry analysis (GC/MS); this revealed compounds such as Squalene which possesses antioxidant, antitumor activities, it also has emollient properties which makes it a potential useful compound in Cosmetic Dermatology. Amyrin, Phytol and alpha-Sitosterol were also identified and were shown to possess antimicrobial and anticancer properties. Finally, Octadecatrienoic acid was also identified from the ethanol extract and was proven to have anti-inflammatory and hepatoprotective properties.

In this study, ethyl acetate was used to extract the secondary metabolites of endophytic fungi isolated from the leaves of *Jatropha tanjorensis* which were then subjected to GC/MS analysis. From the GC/MS analysis, the Ethyl acetate extract for each sample showed several peaks on the chromatogram indicating several compounds. *Cryptococcus neoformans* extract showed 23 peaks, *Microsporum canis* extract showed 13 peaks, *Candida albican* extract showed 11 peaks and *Candida stellatoidea* extract showed 8 peaks.

From Table 1; 13 bioactive compounds were identified which

are classified thus: a dicarboxylic acid (3-Propylglutaric acid), aromatic dicarboxylic acid (Isophthalic acid), saturated fatty acids (Dodecanoic acid, Eicosanoic acid, Tetradecanoic acid, n-Hexadecanoic acid, 15-Hydroxypentadecanoic acid, 18,19-Secoyohimban-19-oic acid), Methyl ester (Cyclopropane Octanoic acid), Peroxide (Lauryl peroxide), Acyclic hydrocarbon (2-Methyltetracosane), Ketones (Pyrrolo [1,2-a]pyrazine-1,4-dione, 9,9-Dimethoxy bicyclo [3,3,1] nona- 2, 4-dione) and a Diol (9,10-Secocholesta-5,7,10[19]-tiene-1,3-diol).

Table 1: Bioactive compounds identified in the Ethyl acetate extract of *Cryptococcus neoformans.*

Name of compound	Chemical formula	Molecular weight (g/mol)	Peak Area %	Retention Time (mins)
3-Propylglutaric acid	$C_8H_{14}O_4$	174	0.47	3.574
Dodecanoic acid	$C_{12}H_{24}O_2$	200	9.17	6.173
Eicosanoic acid	$C_{20}H_{40}O_2$	312	2.19	6.394
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.63	7.985
Pyrrolo[1,2-a]pyrazine-1,4-dione	$C_7H_{10}N_2O_2$	154	0.83	8.39
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.88	9.708
9,9-Dimethoxybicyclo[3,3,1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	9	10.993
15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	5.22	11.927
Lauroyl peroxide	$C_{24}H_{46}O_4$	398	2.55	12.553
Cyclopropane Octanoic acid	$C_{22}H_{38}O_2$	334	17.34	12.931
2-Methyltetracosane	$C_{25}H_{52}$	352	8.48	13.092
18,19-Secoyohimban-19-oic acid	$C_{21}H_{24}N_2O_3$	352	2.46	13.345
9,10-Secocholesta-5,7,10(19)-triene-1,3-diol	$C_{30}H_{52}O_3Si$	488	4.47	13.959
Isophthalic acid	$C_{27}H_{44}O_4$	432	7.21	14.035

Tables 2 and 3 have 7 bioactive compounds each comprising majorly Fatty acids (Dodecanoic acid, n-Hexadecanoic acid, 15-Hydroxypentadecanoic acid), Methyl ester (Cyclopropane Octanoic acid), Carboxylic acid (Chloroacetic acid), Aldehyde(7-Hexadecenal), Ketones (9,9-Dimethoxybicyclo[3,3,1]nona-2,4-dione, Spiro[androst-5-ene-17,1'-cyclo-butan]-2'-one) and a Diol (E,E,Z-1,3,12-Nona decatriene-5,14-diol).

Table 2: Bioactive compounds identified in the Ethyl acetate extract of *Microsporum canis.*

Name of compound	Chemical formula	Molecular weight (g/mol)	Peak Area %	Retention Time (mins)
Chloroacetic acid	$C_{17}H_{33}ClO_2$	304	0.60	4.778
Dodecanoic acid	$C_{12}H_{24}O_2$	200	9.21	6.173
9,9-Dimethoxy bicyclo [3,3,1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	1.77	6.396
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.49	9.699
15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	13.78	11.926
Cyclopropane Octanoic acid	$C_{22}H_{38}O_2$	334	29.69	12.931
Spiro[androst-5-ene-17,1'-cyclo-butan]-2'-one	$C_{22}H_{32}O2$	328	5.33	13.833

Table 3: Bioactive compounds identified in the Ethyl acetate extract of *Candida albican.*

Name of compound	Chemical formula	Molecular weight (g/mol)	Peak Area %	Retention Time (mins)
Chloroacetic acid	$C_{17}H_{33}ClO_2$	304	0.80	4.779
Dodecanoic acid	$C_{12}H_{24}O_2$	200	10.80	6.176
9,9- Dimethoxybi cyclo [3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	3.22	6.395
15- Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	8.34	11.926
Cyclopropane Octanoic acid	$C_{22}H_{38}O_2$	334	29.17	12.932
7- Hexadecenal	$C_{16}H_{30}O$	238	9.76	13.886
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	7.75	13.958

Table 4 has 8 bioactive compounds comprising Aromatic carboxylic acid (3- Furan acetic acid), Fatty acids (n-Hexadecanoic acid, Octadecanoic acid, 8, 11, 14-Eicosatrienoic acid), Acyclic hydrocarbon (Eicosane, Tetracosane), Epoxide (Cyclododecane epoxide) and an Alkyne (1-Pentadecyne).

Table 4: Bioactive compounds identified in the Ethyl acetate extract of *Candida stellatoidea.*

Name of compound	Chemical formula	Molecular weight (g/mol)	Peak Area %	Retention Time (mins)
3-Furanacetic acid	$C_{12}H_{16}O_5$	240	0.81	16.178
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	68.56	22.163
1-Pentadecyne	$C_{15}H_{28}$	208	3.21	23.969
Cyclododecane epoxide	$C_{12}H_{22}O$	182	9.67	24.021
Octadecanoic acid	$C_{18}H_{36}O_2$	284	10.14	24.237
8,11,14-Eicosatrienoic acid	$C_{21}H_{36}O_2$	320	1.47	24.900
Eicosane	$C_{21}H_{44}$	296	1.57	27.340
Tetracosane	$C_{24}H_{50}$	338	4.56	29.186

The compounds: n-Hexadecanoic, Octadecanoic acid and 15-Hydroxyl pentadecanoic acid are saturated fatty acids which possess hypolipidemic properties by reducing significantly LDL/HDL ratio in cardiovascular risk patients. This apparently justifies the claims that *Jatropha tanjorensis* can be used to treat various cardiovascular diseases such as Coronary heart disease (CHD) and Ischemic stroke. 7-Hexadecenal is an aldehyde which possesses antidiabetic properties and is used for treating patients with diabetes, by increasing the secretion of insulin in the body. Thus, the presence of this compound justifies its use tradomedicinally in the treatment of diabetes.

Similarly, the presence of Tetracosane, 1-Pentadecyne, Cyclododecane epoxide, 2-Methyltetracosane, Eicosane, n-Hexadecanoic acid, Tetradecanoic acid, 15- Hydroxyl pentadecanoic acid and Pyrrolo [1, 2-a] pyrazine-1, 4- dione, which all possess antioxidant properties, proves that *Jatropha tanjorensis* leaves possess antioxidant properties due to its ability to prevent onset of degenerative diseases by knocking off free radicals thus protecting vital organs from oxidative stress.

Jatropha tanjorensis has been known to possess antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. This is justified by the presence of Dodecanoic acid, 2-Methyltetracosane, Cyclododecane epoxide, 7-Hexadecenal, Spiro [androst-5-ene-17, 1'-cyclo-butan] -2'-one and E, E, Z-1,3,12-Nona decatriene-5,14-diol, which all possess wide range of antimicrobial properties. Tetradecanoic acid and 1-Pentadecyne particularly possess antifungal activities, thus, the ethyl acetate leaf extract of *Jatropha tanjorensis* can be used in treating various infectious diseases.

8,11,14-Eicosatrienoic acid, 2-Methyltetracosane and Tetradecanoic acid all possess anticancer properties, this in turn justifies the fact that leaf extract of *Jatropha tanjorensis* exhibits anticancer activity by scavenging free radicals, inhibiting peroxidation of lipids, inhibiting Ehrlich Ascites Carcinoma cells and CaCo-2 cells.

The compounds such as Octadecanoic acid, Eicosanoic acid, n-Hexadecanoic acid, 8, 11, 14-Eicosatrienoic acid and 18, 19-Secoyohimban-19-oic acid, possess anti-inflammatory properties. Thus, the leaf extract of *Jatropha tanjorensis* may serve as a potent analgesic in treatment of pain and various inflammatory episodes.

From the afore-mentioned bioactivities of the various identified compounds, the therapeutic properties of *Jatropha tanjorensis* can thus be attributed to the presence of endophytes (in this case endophytic fungi) in the leaves which produces secondary metabolites of medicinal value. In this study, four endophytic fungi were isolated from the leaves of *Jatropha tanjorensis* and identified thus; *Cryptococcus neoformans*, *Microsporum canis*, *Candida albican* and *Candida stellatoidea*. *Cryptococcus neoformans* is a basidiomycetous yeast-like fungus which causes respiratory and neurological disease in humans and animals. The different varieties of *C. neoformans* are easily differentiated by their biochemical properties and antigenic composition of capsular polysaccharides.

Microsporum canis is a pathogenic organism, a zoophilic dermatophyte, which typically colonizes the outer surface of animal's body. Hence, animals, cats and dogs are believed to be the major hosts of this fungus, while humans are occasional

hosts, in which the fungus can induce secondary infections. Microsporum canis has been identified as a causal agent of a ringworm infection in pets, tinea capitis and tinea corporis in humans, and children in particular. Microsporum canis generally invades hair and skin; however, some nail infections have been reported. When hair shafts are infected, *M. canis* causes an ectothrix-type infection where the fungus envelopes the exterior of the hair shaft without the formation of internal spores. This colonization of the hair shaft causes it to become unsheathed, resulting in characteristic round or oval non-inflammatory lesions that develop on the scalp. Infection triggers an acute leukocytic reaction in subcutaneous tissues, which gradually becomes highly inflammatory and leads to hair loss, in the case of tinea capitis.

Candida albican an ascomycete is a dimorphic fungus found worldwide but most commonly in immunocompromised individuals diagnosed with diseases such as HIV and cancer. *Candida* is ranked as one of the most common groups of organisms that cause nosocomial infections. *Candida albicans* infections are ranked high amongst the fungal infections in critically ill or otherwise immunocompromised patients. Parts of the body that are commonly infected include the skin, genitals, throat, mouth, and blood. Distinguishing features of vaginal infection include discharge, and dry and red appearance of vaginal mucosa or skin.

Candida stellatoidea, an emerging yeast pathogen, is closely related to *Candida albicans* and most commonly involved in vulvovaginal candidiasis (VVC) (Table 5).

Table 5: The bioactivities of the different compounds identified in the sample extracts are summarized in the table below.

S/No	Compound name	Bioactivity
1.	Octadecanoic acid	Hypocholesterolemic, antiarthritic, nematicide, 5-alpha reductase inhibitor, anti-acne, hepatoprotective**
2.	3-Propylglutaric acid	No activity reported
3.	Dodecanoic acid	Antimicrobial**
4.	Eicosanoic acid	Anti-inflammatory, muscle growth and repair, neurology**
5.	Tetradecanoic acid	Antifungal, antioxidant, cancer preventive, nematicide, hypercholesterolemia, lubricant [Maruthupandian and Muhan, 2011]
6.	Chloroacetic acid	Herbicide, preservative, bacteriostat**
7.	n-Hexadecanoic acid	Antioxidant, anti-inflammatory, cytotoxic, hypocholesterolemic, nematicide, pesticides, hemolytic, antiandrogen, 5- alpha reductase inhibitor, potent mosquito larvicide (Kumar et al., 2010)
8.	8,11,14-Eicosatrienoic acid	Anti-inflammatory, vasodilatory, antitumor**
9.	15-Hydroxypentadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticides, hemolytic, antiandrogen, 5-alpha reductase inhibitor**
10.	3-Furanacetic acid	Antimicrobial, antiproliferative**

11.	Cyclopropaneoctanoic acid	Potential antidementia drug**
12.	Isophthalic acid	Antimicrobial, anti-fouling**
13.	18,19-Secoyohimban-19-oic acid	Anti-inflammatory**
14.	Lauroyl peroxide	No activity reported
15.	Pyrrolo[1,2-a]pyrazine-1,4-dione	Antifungal, antioxidant**
16.	2-Methyltetracosane	Anticancer, antioxidant, antifungal, antibacterial**
17.	Eicosane	Antioxidant, anticancer, antitumor, cytotoxic, 5 alpha reductase inhibitor**
18.	9,10-Secocholesta-5,7,10[19]-triene -1,3-diol	No activity reported
19.	7-Hexadecenal	Antidiabetic, Antibacterial, Insecticidal**
20.	Tetracosane	Antioxidant, anti-trichomonas, cytotoxic**
21.	1-Pentadecyne	Antifungal, antioxidant**
22.	Cyclododecane epoxide	Antioxidant, antimicrobial**
23.	9,9-Dimethoxybicyclo[3,3,1] nona -2,4-dione	No activity reported
24.	Spiro[androst-5-ene-17,1' cyclobutan-2'-one	Antiarthritic, antimicrobial, anti-inflammatory, antiasthma**
25.	E,E,Z-1,3,12-Nonadecatriene-5,12- diol	Antimicrobial**

** **Dr. Dukes' Phytochemical and Ethnobotanical Databases (Online Database)**

CONCLUSION

In conclusion, *Jatropha tanjorensis* leaves contain endophytic fungi (*Cryptococcus neoformans, Microsporum canis, Candida albican* and *Candida stellatoidea)* to which its medicinal properties can be attributed. Also, the presence of important bioactive compounds identified by GC/MS analysis justifies the use of *J. tanjorensis* leaves by traditional practitioners for treatment of various ailments, hence they might be utilized for development of traditional medicines. This study also warrants further biological and pharmacological investigation in order to elute novel bioactive compounds from *Jatropha tanjorensis* which may create new ways of treating

many incurable diseases.

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