

Antifungal Property and Phytochemical Screening of the Crude Extracts of *Funtumia Elastica* and *Mallotus Oppositifolius*

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ABSTRACT

The antifungal properties of aqueous and ethanol extracts of *Funtumia elastica* and *Mallotus oppositifolius* were carried out using the disc diffusion agar assay. The crude extracts exhibited definite significant antifungal activity on most of the fungi. The zone of inhibition varied for the fungi, which were: *Aspergillus flavus*, *Candida albicans*, *Microsporium audouinii*, *Penicillium sp.*, *Trichophyton mentagrophytes*, *Trichoderma sp.* and *Trichosporon cutaneum* with respect to the type of plant extract. The aqueous extracts of *Mallotus oppositifolius* had the highest zone of inhibition of 24.75 ± 0.86 mm on *Penicillium sp.* The fulcin antibiotic had zone of inhibition of 11.94 ± 0.43 mm on *Microsporium audouinii*, being its highest inhibition on any of the fungi tested. Preliminary phytochemical studies of *F. elastica* and *M. oppositifolius* extracts revealed that they contain anthocyanins, butacyanin, flavonoids, steroids and tannins. Phytobutanin was absent in the extracts. Heavy metal analysis of plant materials showed absence of cadmium, zinc, lead, chromium and nickel, while the presence of copper, iron and manganese was less than 0.95%.

Propiedades Antifúngicas y Pesquizado Fitoquímico de los Extractos Crudos de *Funtumia Elastica* y *Mallotus Oppositifolius*

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RESUMEN

Las propiedades antifúngicas de los extractos acuosos y etanólicos de *Funtumia elastica* y *Mallotus oppositifolius* fueron probadas mediante ensayo de difusión con discos en agar. Los extractos crudos mostraron poseer una actividad antifúngica definitivamente significativa sobre la mayoría de los hongos. La zona de inhibición varió en correspondencia con los hongos, a saber, *Aspergillus flavus*, *Candida albicans*, *Microsporium audouinii*, *Penicillium sp.*, *Trichophyton mentagrophytes*, *Trichoderma sp.* y *Trichosporon cutaneum*, con respecto al tipo de extracto de planta. Los extractos acuosos de *Mallotus oppositifolius* presentaron la zona de inhibición más alta, a saber, 24.75 ± 0.86 mm, al aplicarse sobre *Penicillium sp.* El antibiótico fulcin presentó una zona de inhibición de 11.94 ± 0.43 mm al aplicarse sobre *Microsporium audouinii*, resultando ser ésta la inhibición más alta en relación con todos los hongos sometidos a prueba. Los estudios fitoquímicos de los extractos de *F. elastica* y *M. oppositifolius* revelaron contenidos de antocianinas, butacianinas, flavonoides, esteroides, y taninas. La fitobutanina estuvo ausente en todos los extractos. El análisis de metales pesados en las muestras de plantas mostró ausencia de cadmio, zinc, plomo, cromo y níquel, en tanto que la presencia de cobre, hierro y manganeso estuvo por debajo de 0.95%.

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INTRODUCTION

The recorded use of plants in the treatment of ailments dates back to antiquity (1). *Mallotus oppositifolius* (Geisel) and *Funtumia elastica* (Preuss) are among the plants listed by

Nigerians as treatment for skin diseases (2). *M oppositifolius* is an erect branching perennial shrub up to 3.6 m high when fully matured. The plant is commonly found in drier types of forest and secondary growth throughout the West Africa region (3). Economically, *M oppositifolius* twig is used as chewing sticks for cleaning the teeth, the stem is used as yam stakes. It has also been recorded that in Nigeria the leaves are taken by the Hausas in cold infusion to expel tapeworm, while the decoction is a vermifuge in Ivory Coast. In Ghana, the crushed leaves are applied to inflammation of the eye during an attack of small pox (3). Rottlerin has also been found in its bark and leaves (4).

Funtumia elastica belongs to the family Apocynaceae, commonly called Lagos silk rubber and 'Ire' in Yoruba. The genus *Funtumia* consists of two species namely: *F elastica* (female) and *F africana* (male). The leaves of both species are very similar, glabrous, leathery, elongated, elliptic more or less acuninate, cuneate at the base with short stalks (5). *F elastica* is distinguished through the flowers and fruits which are both shorter than those of *F africana*. *F elastica* is about 30 metres high and 2.50 metres in girth (3) *F elastica* is used for carving spoons and bowls in Sierra Leone and found to be suitable for match manufacture in Nigeria. The decoction of the leaf is used as a cure for mouth and venereal diseases (6).

Fungal related diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosuppressed persons (7). The treatment given by the traditional doctors often includes the administration of entire plants, or extracts of roots, stems, bark, leaves, fruits, seeds or juice of the plant. The treatment might be wrong sometimes, hence the need to scientifically analyze the medicinal plants for their efficacy. Antifungal activity of several Nigerian plants has been investigated and documented. These include that of *Mitracarpus villosus* (8), *Ritchiea capparoides* (9) and *Khaya ivorensis* and *Tetracera potatoria* (10). Research on bioactive substances from plant sources has great scope and could lead to the development of antifungal agents that can combat fungal resistance. The antifungal activity of the bark of *Funtumia elastica* and the leaf of *Mallotus oppositifolius* has not been reported in the literature.

As a continuation of studies in this laboratory into the scientific basis for the use of Nigerian plants for medicinal purposes, two medicinal plants, the bark of *F elastica* and the leaf of *M oppositifolius*, used locally in the treatment of various skin diseases were examined for antifungal activity. The phytochemical properties and heavy metal constituents were screened in the plant materials used and are also reported in this paper.

MATERIALS AND METHODS

Source of plant materials

The plant materials used were collected from the Olokemeji forest in Ogun state of Nigeria (lat 5.2°N, Long 14.8°E). The bark of *Funtumia elastica* and the leaf of *Mallotus*

oppositifolius were collected and shade – dried at room temperature (28–30°C) for 14 days. Samples of the plants were authenticated by the Department of Botany and Microbiology, University of Lagos, Nigeria, and also by using the text in vernacular names of medicinal plants by Gbile (11). Voucher samples (LUH 200102 for *Funtumia elastica* and LUH 200103 for *Mallotus oppositifolius*) have been deposited at the Department of Botany and Microbiology, University of Lagos, Nigeria.

Source of micro-organisms

The fungi used were obtained from scrapes from an infected finger of a patient at the National Primary Health Centre, Yaba, Lagos. The fungi were *Candida albicans*, *Microsporium audouinii*, *Aspergillus flavus*, *Trichophyton mentagrophytes*, *Penicillium* sp, *Trichosporon cutaneum* and *Trichoderma* sp. The fungi were identified by a mycologist at the Department of Botany and Microbiology, University of Lagos, Nigeria and by comparing the morphology of the fungi with the ones in standard mycology texts such as Alexopoulos (12), Talbot (13), Deacon (14) and Bryce (7). These fungi were stored on sabouraud dextrose agar (Oxoid) slants in the refrigerator at 4°C prior to use.

Preparation of soluble extract

The dried bark of *F elastica* and dried leaves of *M oppositifolius* were ground into fine powder with an electric blender. A weight of 1.8 kg was obtained for each of the powdered plant parts. Two, 600g portions of the powdered leaves were soaked separately in 1.8 litres of 70% aqueous ethanol and 1.8 litres of distilled water for 24 hours. Thereafter, each extract was filtered through Whatman filter paper No 1832 and concentrated by evaporating in a rotatory evaporator at 40°C, producing the ethanol and water extracts of each plant part. The two extracts of each plant were stored in the refrigerator at 4°C prior to use.

Antifungal activity assay

The antifungal testing was carried out using the disc diffusion agar method of Irobi and Daramola (9). Spore or conidia suspension of 10^5 – 10^7 cells/ml, counted with haemocytometer, was made. About 10 ml of the Sabouraud dextrose agar (SDA) was poured into Petri dishes and allowed to solidify. A micropipette was used to introduce 0.1 ml of the spore or conidia suspension onto the agar plate before spreading with a glass spreading rod under sterile conditions. Sterilized discs (6 mm) Whatman No AA2017006, were soaked in each of the crude extracts being assayed, for 6 hours. Four of these soaked discs were placed on a fungal spore or conidia seeded plate with the help of sterile forceps. There were three controls, one contained the fungal inoculum but with discs that were soaked in sterilized distilled water, another had sterilized discs without soaking in water or extract. The third control contained the fungal inoculum but with discs soaked in orthodox antibiotic fulcin 100 mg/ml. Three repli-

cates were produced for each fungus. All the plates containing the discs were then incubated at 28–31°C. Zone of inhibition was measured after 48 hours of incubation. The assay was repeated once. The antifungal activity test results were statistically analyzed as described by Parker (16).

Preliminary phytochemical studies

Preliminary phytochemical studies were carried out using methods described by Fadeyi *et al* (16). The extracts of *F elastica* and *M oppositifolius* were screened for the presence of anthocyanins, butacyanins, flavonoids, phytobutanins, saponins, steroids and tannins.

Heavy metal analysis

The heavy metal analysis of the plant materials was carried out at the Federal Institute of Industrial Research (FIRO), Oshodi, Nigeria. One gram of each of the ground plant material was weighed into an already weighed crucible. The crucible was placed on the flame of a burner to burn off the carbon. This was then put in a Eurotherm furnace at 550°C for one hour to ash the plant material. The ash was allowed to cool and was then weighed to obtain the percentage ash content. The ash was dissolved in 1ml distilled water placed into a 100 ml volumetric flask and 1ml of hydrochloric acid (HCl) was added and gently shaken for proper homogenization. The volume of the solution was made up to 100 ml of the flask using distilled water. This was then placed in an Atom Absorption Spectrometer (AAS) to aspirate for the presence of heavy metals. Aspiration was done by using Hollow cathode lamps for each metal and the percentage concentration of each heavy metal was measured against a standardized grade (17).

RESULTS

The powdered *F elastica* produced 20g of ethanol extract and 63g of water extract. The powdered leaf of *M oppositifolius* produced 27g of ethanol extract and 59g of aqueous extract. The results on Table 1 show that all the crude extracts had definite significant antifungal activity on most of the fungi. The zone of inhibition varied for the fungi with respect to the type of plant extract. The ethanol and aqueous extracts of *F elastica* did not inhibit the growth of *Microsporium audouinii*, while only the aqueous extracts of *F elastica* did not inhibit the growth of *Trichosporon cutaneum*. The ethanol extracts of both plants used did not inhibit the growth of *T cutaneum*. Overall, the aqueous extracts of *Mallotus oppositifolius* had the highest zone of inhibition of 24.75 ± 0.86 mm. The zone of inhibition of the aqueous extracts of *F elastica* on *Penicillium* sp and *Trichophyton mentagrophytes*, and the ethanol extracts of *M oppositifolius* on *Trichophyton mentagrophytes* were less than 10 mm. Generally, the crude extracts were more active against fungus than fulcin antibiotic (Table 1).

The crude extracts (ethanol and aqueous) of the two plants contained anthocyanins, butacyanin, flavonoids, steroids and tannins. Saponin was absent only in the ethanol extract of *F elastica* while phytobutanins were absent in all the plant extracts (Table 2).

Heavy metal analysis of the bark of *F elastica* and the leaves of *M oppositifolius* is shown on Table 3. The result showed that the plant materials did not contain heavy metals such as cadmium, zinc, lead, chromium, and nickel. The presence of copper, iron and manganese was less than 0.95% individually which is of significance. The ash content was 8.80% for *F elastica* and 7.65% for *M oppositifolius*.

Table 1: Antifungal activity of ethanol and water extracts of *Funtumia elastica* and *Mallotus oppositifolius*

Extracts or solution	Zone of Inhibition (mean \pm SE mm) produced by extracts FUNGI						
	<i>Aspergillus flavus</i>	<i>Candida albicans</i>	<i>Microsporium audouinii</i>	<i>Penicillium sp</i>	<i>Trichoderma mentagrophytes</i>	<i>Trichoderma sp</i>	<i>Trichosporon cutaneum</i>
Control	0.00 \pm 0.00 a*	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
Fulcin	11.13 \pm 0.29 b	10.03 \pm 0.49 b	11.94 \pm 0.43 b	10.88 \pm 0.32 b	9.68 \pm 0.26 g	10.69 \pm 0.36 b	10.65 \pm 0.75 b
<i>Funtumia elastica</i> (bark) ethanol extracts	11.89 \pm 0.45 b	10.75 \pm 0.56 b	0.00 \pm 0.00 a	13.31 \pm 0.38 ef	10.69 \pm 0.36 b	14.19 \pm 0.32 e	0.00 \pm 0.00 a
<i>Funtumia elastica</i> (bark) aqueous extracts	11.38 \pm 0.37 b	11.27 \pm 0.37 b	0.00 \pm 0.00 a	4.87 \pm 0.54 h	9.25 \pm 0.17 g	0.00 \pm 0.00 a	17.00 \pm 0.41 n
<i>Mallotus oppositifolius</i> (leaves) ethanol extracts	17.69 \pm 0.23 n	12.75 \pm 0.54 f	13.25 \pm 0.76 ef	14.06 \pm 0.40 e	5.87 \pm 0.25 h	17.12 \pm 0.63 n	0.00 \pm 0.00 a
<i>Mallotus oppositifolius</i> (leaves) aqueous extracts	13.01 \pm 0.45 ef	11.16 \pm 0.53 b	16.00 \pm 0.81 n	24.75 \pm 0.86 c	11.75 \pm 0.60 b	21.62 \pm 0.76 d	21.50 \pm 0.66 d

* Samples with similar letters show no significant differences at $p = 0.01$.
Samples with different letters show significant differences at $p = 0.01$.

Table 2: Phytochemical compounds present in the extracts of the bark of *Funtumia elastica* and the leaves of *Mallotus oppositifolius*

Medicinal plants	Antho-cyanins	Butacyanin	Flavonoids	Phyto-butanins	Saponins	Steroids	Tannins
<i>Funtumia elastica</i> ethanol extracts	+	+	+	-	-	+	+
<i>Funtumia elastica</i> aqueous extracts	+	+	+	-	+	+	+
<i>Mallotus oppositifolius</i> ethanol extracts	+	+	+	-	+	+	+
<i>Mallotus oppositifolius</i> aqueous extracts	+	+	+	-	+	+	+

(+) – Presence of phytochemical compound
 (-) – Absence of phytochemical compound

Table 3: Heavy metal analysis of the barks of *Funtumia elastica* and the leaves of *Mallotus oppositifolius*

Parameter	Percentage content (%)	
	<i>Funtumia elastica</i>	<i>Mallotus oppositifolius</i>
ASH	8.80	7.65
Cadmium (Cd)	N.D*	N.D
Zinc (Zn)	N.D	0.002
Copper (Cu)	N.D	0.002
Lead (Pb)	N.D	N.D
Chromium (Cr)	N.D	N.D
Iron (Fe)	0.0043	0.019
Manganese (Mn)	0.0038	0.018
Nickel (Ni)	N.D	N.D

* ND = Not detected

DISCUSSION

The results obtained on the antifungal activity of *Funtumia elastica* (bark) and *M oppositifolius* (leaf) showed that the plant extracts possess antifungal properties and can be effective antibiotics since they inhibited the growth of fungal casuative agents of skin diseases. This observation is in line with the work of Ajaiyeoba *et al* (9) on *Ritchiea capparoides* var *longipedicallata*. The plant extracts might be host specific in their antifungal activity since zones of inhibition varied for each fungus and there was no inhibition on some fungi. The different rates of inhibition may probably be due to the quantity of the phytochemical compounds present in the extracts.

Hitherto, agreeing with the report of Onolapo and Owonubi (18) that tannin and flavonoids have antimicrobial activities and that at low concentration tannins can inhibit the growth of micro-organisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the

micro-organism. Therefore there is a rationale behind the use of these plants by the local populace.

Traditionally, the aqueous extracts are used orally or topically to cure skin diseases. The report here supports the use of water to produce active fractions. The bioactive constituents of *F elastica* and *M oppositifolius* may be anthocyanins, flavonoids, and tannins, since the presence of these listed phytochemicals in other plants have been reported by Barnabas and Nagarajan (19), and Barapedjo and Bunchoo (20), and implicated to inhibit cell wall formation in fungi leading to the death of the organism. The fact that heavy metals were absent or below the recommended limit of 0.95% or 0.095% (21) in the plants probably confirmed that antifungal activities of the plants were not due to the presence of heavy metal but might be due to the presence of phytochemical compounds.

This current experiment therefore provides some scientific justification for the utilization of extracts from these plants, *F elastica* and *M oppositifolius*, by the Nigerian populace to treat skin disease. However, it is important to point out that crude extracts such as these need to be further purified through antifungal activity guided fractionation to isolate and identify the compounds responsible for biological activity.

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