

LEAF SURFACE MYCOFLORA OF SOME MEDICINAL PLANTS IN BENIN CITY, NIGERIA: A PRELIMINARY STUDY

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ABSTRACT

The leaf mycoflora of some common orally administered medicinal plants was studied to determine the presence of fungi that might pose health risks. Leaf-washings of *Terminalia catappa*, *Spondias mombin*, *Chromolaena odorata*, *Caesalpinia pulcherrima*, *Sida acuta*, *Azadirachta indica*, *Talinum triangulare*, *Dialium guineense*, *Vernonia amygdalina* and *Carica papaya* were cultured on Potato Dextrose Agar (PDA) and Actidione-chloramphenicol Sabouraud agar (Sabouraud Glucose Agar + cycloheximide + chloramphenicol). Leaf surface fungi were isolated after incubating at 27±2°C for 3 -7 days. Fungal viable count was recorded as colony forming units (cfu/ml). Five fungal species (*Aspergillus niger*, *A. flavus*, *Penicillium roqueforti*, *Trichophyton rubrum* and *Microsporum canis*) were isolated. *Aspergillus niger* occurred on all the eight plants (= 80% occurrence) while *T. rubrum* had 20% occurrence. *Aspergillus niger* also had the highest fungal count (2.7x10⁶ cfu/ml) and *T. rubrum*, the lowest (1.1x10⁶). Two of the fungi (*A. flavus* and *A. niger*) are potential toxin-producing fungi which could cause harm. The presence of *M. canis* and *T. rubrum* (dermatophytic fungi) has health implications for people who collect the plants and for those who take them.

Keywords: *Dermatomycosis, fungi, leaf surface, Nigeria, toxins, tradomedicine.*

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INTRODUCTION

The use of plants for treating various ailments (a practice known as herbal medicine, herbology or phytotherapy) has been on for several centuries. Report has shown that many compounds used in orthodox medicine were derived from ethnomedicinal plants and 80% of these compounds were used in the same or related manner as the traditional ethnomedical use (Fabricant and Farnsworth 2001). Even in the present technological era, traditional medicine still remains the predominant means in the third world, for the preservation of health of the rural majority who constitute over 70% of the total population. According to Gbile and Adesina (1986), the Nigerian flora has made and would continue to make great contributions to the health care of Nigerians. Infact the indigenous medicinal plants form an important component of the natural wealth and culture of Nigerians.

Some of these plants (especially their leaves) are used orally in tradomedicine (Kubmarawa *et al.* (2013). Examples of such plants are *Citrus aurantifolia* (Family Rutaceae), *Gossypium hirsutum* (Malvaceae) and *Vernonia amygdalina* (Asteraceae). Fungi causing diseases in plants in plants and plant parts include *Curvularia* sp. (leaf spot of *Jatropha*), *Aspergillus* and *Penicillium* spp (soft rot of leafy vegetables) and *Xylaria* sp. (dry rot of *Raphia* fruit). Most exposed materials and surfaces, including plants, are prone to fungal contamination. This is because of the dispersal of fungal spores in the air, water and by human and animal activities. Fungi have been isolated from the air, water and various other substances (Woodcock *et al.*, 2006; Horner, 2006; Okungbowa and Dede, 2010; Edema and Okungbowa, 2011 and Ismael, 2011). In order for the efficacy of these plants not to be reduced by the presence of microorganisms growing on their surfaces, steps have to be taken to counter this effect. However, such organisms have to be identified first, since this is the first step in the control/elimination of offending organisms.

This study was carried out to determine and document the mycoflora of some orally administered medicinal plants and ascertain the health implications of the presence of these fungi.

MATERIALS AND METHODS

Collection of samples and confirmation of tradomedical uses

Fresh samples of ten predetermined medicinal plants were collected from home gardens, uncultivated farmlands and rain forests within the University of Benin and its environs. The plants were *Talinum triangulare*, *Azadiracta indica*, *Vernonia amygdalina*, *Carica papaya*, *Chromolaena odorata*, *Sida acuta*, *Terminalia catappa*, *Spondias mombin*, *Caesalpinia pulcherrima* and *Dialium guineense*. Selection of plants was based on the availability of plants as well as availability of documented information on their tradomedical uses (Achaya, 2008; Idu *et al.*, 2008; Kubmarawa *et al.*, 2013). A confirmation of tradomedical uses of the plant samples was done by oral interview. Three herbal medicine practitioners in Benin City were consulted. Where at least two similar independent answers were given concerning oral use of plant, such was considered as confirmatory. Plant branches (with 12-20 leaves, depending on size of leaf) were collected aseptically with the aid of a pair of sterile scissors and gloves, after which they were placed in sterile polythene bags. Each plant was collected from two different sites located 1- 3km apart. The bags containing leaves were immediately sealed and labelled and taken to the laboratory for study.

Preparation of leaf-washing

With the aid of a pair of sterile forceps 4-12 leaves from a plant were introduced into a flask containing 20ml of sterile distilled water and agitated in a shaker for 1min (Osono, 2008) and left to stand for about 5-10 minutes. Ten-fold serial dilution of leaf-washing was done by transferring 1 ml of leaf-washing into 9 ml sterile distilled water. Further ten-fold (1:10) serial dilutions were done three times.

Inoculation of media with leaf-washing

Inoculation of media to determine fungi present in leaf-washing was done following the pour plate method (Okungbowa, 2012). One milliliter of 40g/ml gentamycin (Gentalek) was aseptically introduced into each sterile Petri dish with the aid of a syringe. Then 1ml of the appropriate dilution of each leaf - washing was transferred to each Petri dish. About 18ml of Potato Dextrose Agar, PDA (Oxoid, England) was poured into each Petri dish, making 20ml load per plate. Another set of plates containing Actidione-chloramphenicol Sabouraud agar (Sabouraud Glucose Agar + cycloheximide + chloramphenicol) instead of PDA, were prepared for isolation of dermatophytes (Ismael, 2011). The Petri dishes were swirled to allow proper mixing and left to solidify. The plates were incubated at room temperature ($27 \pm 2^{\circ}\text{C}$) for 7 days. Since each plant had two samples from a site, and two locations were assigned to each plant, there were a total of four samples per plant. Two plates in which sterile distilled water was substituted for leaf-washing were used as control and were also incubated for 3 days (for other fungi) and 7 days (for dermatophytes).

Fungal Viable Count

After incubation, fungi present in each plant leaf (each leaf - washing) were estimated by counting the number of colonies of each fungus on each culture plate (Nwafor and Ikenebomeh, 2009) and calculating the number of colonies in the total volume of undiluted leaf-washing. The fungal count was recorded as colony forming units/ml (cfu/ml) leaf-washing. Fungal count was determined twice and the mean calculated.

Isolation and Identification of Fungi

At the end of incubation, fungal colonies were subcultured onto fresh PDA plates for purification. Fungal colonies observed were identified by comparison with the descriptions and illustrations of Barnett and Hunter (1998), Klich (2000) and Larone (2000).

Statistical Analyses

Data were analyzed by comparing means using the student t-test. Significance was determined at a probability of 0.05.

RESULTS

Table 1 shows study plants, their medicinal uses and modes of administration as confirmed from oral interview. Five fungal species were isolated from the leaf surfaces. They were *Aspergillus niger*, *A. flavus*, *Penicillium roqueforti*, *Microsporum canis* and *Trichophyton rubrum*. The leaves of all ten plants had fungi isolated from them. The control plates had no fungal growth.

Aspergillus niger was isolated from eight plants, and therefore, had the highest fungal occurrence (80%) while *T. rubrum* had the lowest (20%) as shown in Table 2. The plants from which *T. rubrum* was isolated were among the ones from which *M. canis* was isolated.

Table 1: Study plants, their traditional uses and modes of administration

S/N	Scientific name	Common name	Traditional use	Mode of administration
1	<i>Talinum triangulare</i>	Water leaf	Scabies, fresh wounds	Leaf extract/oral
2	<i>Azadirachta indica</i>	Neem	Malaria	Decoction/oral
3	<i>Vernonia amygdalina</i>	Bitter leaf	Heart disease	Leaf extract/oral
4	<i>Carica papaya</i>	Papaya	Asthma	Smoke from burnt leaf inhalation
5	<i>Chromolaena odorata</i>	Siam weed	Diarrhoea	Decoction/oral
6	<i>Sida acuta</i>	Wire weed	Gonorrhoea	Leaf extract/oral
7	<i>Terminalia catappa</i>	Indian almond	Diabetes	Decoction/oral
8	<i>Spondias mombin</i>	Hogplum	Allergies	Leaves chewed
9	<i>Caesalpinia pulcherima</i>	Red bird of paradise	Purgative	Decoction/oral
10	<i>Dialium guineense</i>	Velvet tamarind	Hiccups	Leaves chewed

Table 2: Fungi and the plants from which isolated

S/N	Fungus	Presence/Absence*										
		1	2	3	4	5	6	7	8	9	10	%
1	<i>A. niger</i>	-	+	+	-	+	+	+	+	+	+	80
2	<i>A. flavus</i>	+	+	-	-	+	+	+	+	-	-	60
3	<i>P. roqueforti</i>	-	-	-	-	+	-	+	-	+	-	30
4	<i>M. canis</i>	+	-	+	-	+	+	-	-	+	-	50
5	<i>T. rubrum</i>	-	-	+	-	+	-	-	-	-	-	20

*+ = Present

- = Absent

% = Percentage in total plant sample (10)

Fungal viable count is represented in Table 3. *Aspergillus niger* with a count of 2.7×10^6 cfu/ml had the highest value, followed by *P. roqueforti* (2.0×10^6 cfu/ml) while *T. rubrum* (1.1×10^6 cfu/ml) had the lowest. There were significant differences between some of the fungal counts ($p < 0.05$). The fungal counts of *M. canis* and *T. rubrum* were significantly lower than those of the others ($p < 0.05$).

Table 3: Fungal viable count

S/N	Fungus	Viable count (cfu/ml) $\times 10^6 \pm \text{SD}^*$
1	<i>A. niger</i>	2.7 ± 0.4^a
2	<i>A. flavus</i>	1.8 ± 0.0^b
3	<i>P. roqueforti</i>	$2.0 \pm 0.6^{a, b}$
4	<i>M. canis</i>	$1.4 \pm 0.2^{b, c}$
5	<i>T. rubrum</i>	1.1 ± 0.1^c

* Values are means of two independent experiments. SD = Standard deviations

Values with the same superscript letters along the same column are not significantly different.

DISCUSSION

The leaves of all ten plants were used orally, either in the raw form or dried or boiled, which confirmed literature reports (Idu *et al.*, 2008; Kubmarawa *et al.*, 2013; Olatunji *et al.*, 2014). *Aspergillus* species are found commonly in the air and soil (Patron, 2006). *Aspergillus niger* and *A. flavus* were isolated earlier by Mohamed (2001) from leaf surface of onion plant (which is also a medicinal plant) and from other sources (Okungbowa and Dede, 2010; Zarrin and Haghighi, 2011). Thus, these fungi might have contaminated the leaves through contact with air, soil and man. *Aspergillus flavus* is a known producer of mycotoxins, called aflatoxins, which are carcinogenic and can affect the liver (Mounjouenpou *et al.*, 2008). Two health implications of their presence on the leaves are that they could cause allergic reactions through inhalation of their spores (Horner, 2006; Woodcock *et al.*, 2006) in those who collect the plant leaves for traditional medicine, and toxic reactions (due to toxins produced by *Aspergillus* on the leaves) if the leaves are consumed. Some of the leaves are used in the raw form. *Aspergillus niger* also produces ochratoxins (Mounjouenpou *et al.*, 2008).

Penicillium is a common environmental mould and a spoilage fungus (Patron, 2006; Okungbowa and Dede, 2010).

It is not surprising that *P. roqueforti* was isolated from the leaves since leaves could easily be contaminated through air and soil.

Trychophyton rubrum and *M. canis* are dermatophytes which infect humans and animals (Sharma *et al.*, 2011). *Microsporum gypseum* and *T. mentagrophytes* have been isolated from soil by Zarrin and Haghighi (2011). Species of these dermatophytes were previously isolated from dust by Sugimoto *et al.* (1995). So apart from possible contact of these plants with animals that may harbour them, the dermatophytes isolated here could have reached the plants through contact with soil. *Microsporum canis* is known to cause ring worm mainly in cats and dogs. Cats and dogs were usually seen roaming in the study areas, partly explaining the presence of *T. rubrum* and *M. canis* on study plant leaves. *Trichophyton rubrum* and *M. canis* are not known to cause infection through ingestion. However, they could pose danger to humans whose skin comes in contact with these fungi when they are present on the leaves. Some of the leaves are administered by squeezing leaves with the hand to bring out the sap. By so doing, these dermatophytes could infect the skin.

Some toxins produced by *A. niger* and *A. flavus* are harmful to man while *M. canis* and *T. rubrum* can infect man through contact. Therefore, caution should be observed when handling them. Thorough rinsing and boiling of leaves before use would be a preventive measure.

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