EVALUATION OF ANTIFUNGAL ACTIONS OF STEM-BARK EXTRACTS OF *Entandrophragma cylindricum* AGAINST SOME PLANT PATHOGENIC FUNGI

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ABSTRACT

This study was carried out to investigate the potential of extracts of the stem bark of Entandrophragma cylindricum Sprague for controlling plant pathogenic fungi. The bark of the E. cylindricum was extracted with four different solvents: ethanol, chloroform, petroleum ether and water. The four extracts were tested for the phytochemical constituents and antifungal activities. Five fungal species (Curvularia lunatus Tandam and Bilgrami, Fusarium solani Mart, Aspergillus niger Van Tiegh, Aspergillus flavus Link and Trichoderma longibrachyatum Rafai) were used in this study. These fungi are responsible for various plant diseases. Phytochemical tests showed that the extracts contained alkaloids, tannins, flavonoids and phenols. The antifungal activity was based on inhibition of the growth of the test fungi by the extracts. The inhibition of C. lunatus to all the extracts was significantly ($P < 0.05$) different compared to that of the other fungi. Ethanol extracts exhibited the strongest fungitoxicity against the fungi tested. The results obtained suggest that E. cylindricum could be used in controlling diseases caused by the fungi monitored.

Keywords: *Entandrophragma cylindricum, antifungal, bark extracts, pathogenic fungi*

Introduction

The cost and harmful side effects of synthetic pesticides have prompted investigations on exploiting pesticides of plant origin. Such side effects include residue toxicity on the treated materials, environmental pollution and increasing pesticides resistance (Kumar, 1984; Van Emden and Peakall, 1996). Studies have shown the importance of natural chemicals as a possible source of non-toxic, safe and easily biodegradable alternative pesticides (Singh, 1994; Gorbany and Salary, 2004; Stompor Chrzan, 2004). Furthermore, pesticides of plant origin are cheap and readily available in developing countries such as Nigeria where synthetic fungicides are scarce and expensive for peasant farmers. Numerous natural products of plant origin are pesticidal in action, and have the potentials to control fungal pathogens of crops (Okwu *et al.*, 2007). However Earnsworth (1990) reported that just very few plants; about 10% have been investigated for their anti-microbial activity. Therefore, a large reservoir of potential sources of botanical fungicides such as *Entadrophragma cylindricum* still exists especially in tropical forest awaiting exploitation.

Entandrophragma cylindricum Sprague (sapelli) belongs to the mahogany family meliaceae. Sapelli is one of the species of red wood gown in Nigeria. It is a heliophilous species of the canopy, hermaphrodite and insect pollinated. Its fruits are produced when the trees reach about 50 cm diameter at breast height (dbh) and its seeds are wind dispersed (Garcia *et al.,* 2004). *Entandrophragma cylindricum* and other members of the mahogany family such as

Azadirachtaindica and *Khaya senegalensis* have been variously utilized in a number of folk medicine (Gill, 1992; Shmutterer, 1995). The concoction of the stem bark is claimed by local traditional healers to be used in the treatment of fever, cough, black tongue and diabetes.

Although several workers have reported the toxic effects of plant extracts on plant pathogens (Onifade, 2000; Nwachukwu and Umechuruba, 2001; Wokoma and Anaemene, 2003; Okigbo and Igwe, 2007; Nduagu *et al*, 2008; Okigbo *et al*, 2009; Ijato *et al*, 2011; Gupta and Tipathi, 2011), there is a dearth of information on the use of *Entandrophragma cylindricum* as pesticide of plant origin. This study was designed to determine the antifungal activities of stem bark extracts of *E. cylindricum* against some pathogenic fungi of plants.

Materials and Methods

Collection of plant material

The stem bark of *Entandrophragma cylindricum* was collected from Sapele, Delta State, Nigeria. The identity was confirmed by the Taxonomy Unit of the Department of Forestry and Wildlife, Delta State University, Asaba Campus, Asaba, Nigeria.

Preparation of plant material and extracts

The bark was cut into small bits and air-dried at room temperature for 14 days. The dried bits were pounded to coarse particles using a wooden mortar and pestle. Six hundred and fifty grammes (650g) each of the pounded plant materials was extracted with ethanol, chloroform, petroleum ether and water. The extracts were evaporated to dryness on a water bath. The extract yield of each solvent was weighed and calculated with the formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

Phytochemical screening of crude extracts

Phytochemical screening was carried out on parts of the dry extracts to reveal the presence of secondary metabolites in them according to the methods of Trease and Evans (1989) and Poongothai *et al.* (2011).

Preparation of the test solution

The test solution of each extract was prepared by dissolving 5g of dry extract separately in 50 ml sterile distilled water in a 250 ml Erlenmeyer flask in a water bath. Extracts were subsequently filtered through four folds of cheese cloth.

Source of fungi

Curvularia lunatus (Tandam and Bilgrami), *Fusarium solani* (Mart), *Aspergillusniger* (Van Tiegh), *Aspergillus flavus* (Link) and *Trichoderma longibrachyatum* **(**Rafai) used in this study were obtained from Department of Microbiology, Delta State University, Abraka, Nigeria. The

fungi were maintained on Potato Dextrose Agar (PDA) slant throughout the period of the experiment. They were revived twice on fresh PDA each time before use.

Screening for antifungal activity

Food poisoning technique described by Okigbo *et al.* (2009) was used to test for antifungal activity of the plant extracts. One millilitre of each extract was pipette aseptically into a series of sterile glass Petri dishes. Ten millilitre of cooled PDA medium was poured into each plate and gently swirled on the laboratory bench to ensure even dispersion of extract and then allowed to solidify. Three replicate plates for each extract were then inoculated at the center with 2mm diameter mycelial discs of each fungus and incubated at 28°C. Radial growth was measured daily for 5 days. Radial growth was taken as the mean growth along two pre-drawn perpendicular lines on the reverse side of each plate.

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where:

dc = average diameter of fungal colony in the control plates

dt = average diameter of fungal colony in the treated plates

Method of data analysis

All the data collected from the experiments were subjected to analysis of variance (ANOVA) using SAS 2002 and significant means were separated with the new Duncan's Multiple Range Tests in the SAS 2002 computer software.

Results and Discussion

The result of the percentage yield of the plant extracts using different solvents is shown in Table 1. Ethanol extract was found to have the highest yield of 4.51% while water extract has the lowest yield of 1.60%. This indicates that *Entandrophragma cylindricum* had more non polar constituents than polar ones.

The result of the phytochemical analysis with respect to each solvent is shown in Table 2. All the extracts had alkaloids and flavonoids. In addition, ethanol extract contained tannins and phenols. Chloroform contained phenols. Petroleum ether contained tannins. The components, anthraquinones, reducing sugar, cardiac glycosides saponins, steroids and terpenoids where not detected in the extracts of the plant tested. Other investigators (Gill, 1992; Ahmad *et al.* 1998; Shariff, 2001) have reported the presence of these components in members of the family Meliaceae to which the plant used in the present study belongs. The presence of these secondary metabolites could be responsible for the antifungal activities of the extracts of the plant. Scientists have shown that these metabolites play defensive roles in the plants producing them. For example, Haralampidis *et al,* (2001) reported that secondary metabolites have been implicated as chemical defense against attack by soil fungi. In the same paper, they further reported that many plants synthesize secondary metabolites as part of their normal programme

of growth and development, often sequestering them in tissues for protection against microbial attack.

From this work, *E. cylindricum* is seen to possess antifungal activity against *C. lunatus*, *F. solani, A. niger, A. flavus* and *T. longibrachyatum* (Table 3). The activity of the various extracts varied in their inhibitory effect with the different species of fungi. The inhibition of *C. lunatus* to all the extracts was significantly ($P < 0.05$) different compared to that of the other fungi. Ethanol extract exhibited the strongest fungitoxicity against the fungi tested. The observed variation in the susceptibility of the fungi to the extracts may be due to the difference in the chemical, physiological and structural integrity of the fungi (Alade and Irobi, 1993). The difference in the inhibition of the extracts may be because the various solvents extracted to varying degrees (Qasem and Abu-Blan, 1996; Onifade, 2000).

Table 1: Percentage yield of the *Entandrophragma cylindricum*stem bark extracts using different solvents

Solvent	Weight of material extracted (g)	Weight of extract (g)	Yield (%)
Ethanol	650	29.3	4.51
Chloroform	650	14.1	2.17
Petroleum ether	650	19.7	3.03
Water	650	10.4	1.60

Table 2: Phytochemical analysis of the various extracts of *Entandrophragma cylindricum* stem bark

Phytochemical	Ethanol	Chloroform	Petroleum ether	Water
Alkaloids	+	+	+	+
Tannins	+	-	+	-
Steriods	-	-	-	-
Flavonoids	+	+	+	+
Saponins	-	-	-	-
Anthraquinones	-	-	-	-
Reducing sugar	-	-	-	-
Cardiac glycosides	-	-	-	-
Phenols	+	+	-	-
Terpenoids	-	-	-	-

Key: + = Present; - = Absent

Table 3: Antifungal activities of extracts of stem bark of *Entandrophragma cylindricum* against some plant pathogenic fungi (%)

Fungi	Ethanol	Chloroform	Petroleum ether	Water	Control
Curvularia lunatus	72.87[a]	64.34[a]	50.39[a]	41.86[a]	0
Fusarium solani	44.96[c]	39.54[c]	25.58[c]	16.28[c]	0
Aspergillus niger	34.32[d]	30.10[d]	8.61[e]	14.58[c]	0
Aspergillus flavus	28.65[e]	27.51[d]	15.12[d]	13.19[c]	0
Trichoderma longibrachyatum	57.36[b]	51.16[b]	42.64[b]	36.43[b]	0

Means in the same column with different superscripts are significantly different (P < 0.05)

Conclusion

This investigation has shown that *Entandrophragma cylindricum* bark extracts contained fungitoxic substances and were effective against *C. lunatus, A. niger, A. flavus, F. solani* and *T. longibrachyatum.* The study also revealed that *C. lunatus* was the most susceptible while *A. flavus* was the least. Ethanol extract exhibited the strongest fungitoxicity against the test pathogens. These findings suggest that *E. cylindricum* stem bark extract could be used as biological fungicides to control the test pathogens in plants.

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