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Research article

Phytochemical screening and antifungal activities of five plant species

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Abstract: *Aspergillus niger* and *Rhizopus oryzae* are two common pathogens of post-harvest fruits diseases in Mkpat Enin area of Akwa Ibom State in Nigeria. Traditional control measures of these pathogens have emphasized the use of agro-chemicals with high environmental toxicity. The search for alternative control methods using local plant products which are environmentally friendly is therefore, necessary. Antifungal properties of leave extracts of five plant species; *Mimosa pudica, Phyllanthus amarus, Emilia sonchifolia, Bryophyllum pinnatum* and *Amaranthus lhybridus* leave extracts were investigated using disc diffusion method while screening for bioactive compounds was carried out using standard phytochemical procedures. Antifungal activities were tested against the two pathogens at 2%, 4%, 6%, 8% and 10% concentrations. All extracts indicated significant growth inhibition on the two pathogens with 10% recording the highest. Phytochemical screening showed the presence of tannin in all the plant extracts tested while saponin was found in all except *Bryophyllum pinnatum* extract. Flavonoids were present in all excluding *Bryophyllum pinnatum* extracts. Leave extracts of the tested plants can be explored as potential crop disease control measures being natural products which are less hazardous.
Keywords: Plant extracts - *In-vitro* analysis - Diseases.

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INTRODUCTION

Although the screening of plants for bioactive agents is one of the most intensive areas of natural product research currently, the field is far from being exhausted. Only around ten percent of plants have been investigated (Sandberg & Bruhn 1979). Also, the WHO (2010) report noted that although, traditional medicines represent 60% of the world primary health care system, the plant species with possible biological activities remain largely unexplored. Natural products play a significant role in the drug discovery and development process (Newman & Cragg 2012, Mehra *et al.* 2014, Truven 2015, Bajpai *et al.* 2015). There are no doubts that plants represent an unlimited source of novel chemical entities with potential as drug leads.

While the extraction of natural products from plants may appear simple, Sticher (2008) reported that, the presence of numerous inactive components makes the isolation and screening of target compounds extremely cumbersome. It is therefore, necessary to modify existing protocols and develop new ones for effective screening. Consequently, choosing plants for scientific evaluation of biological activities will depend on a number of criteria such as ethno-pharmacological usage by local population, species availability, mode of preparation and administration (Van der Watt & Pretorious 2001). A number of secondary products such as tannins, phenolics, flavonoids, alkaloids, essential oils, steroids, saponins have been reported as possessing anti-microbial activities (Liu 2003, Akindele & Adeyemi 2007, Okey 2014)

The objective of this study is therefore, to evaluate five indigenous plant species (*Emilia sonchifolia, Mimosa pudica, Phyllanthus amarus, Bryophyllum pinnatum* and *Amaranthus hybridus*) for their bioactive properties through phytochemical screening and *invitro* assays on fungal pathogens. These plant species are selected for their availability and associated traditional healing properties.

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MATERIAL AND METHODS

Collection and identification of plant materials

Five plant species were collected from the Botanic garden of the Department of Biological Sciences, Akwa Ibom State University and identified by taxonomic characters as *Emilia sonchifolia (*Red tassel flower*), Mimosa pudica* (Sensitive plant), *Phyllanthus amarus* (Stone-breaker), *Bryophyllum pinnatum* (Life plant) and *Amaranthus hybridus* (Female finger). These plant species were selected based on availability and historic medicinal applications.

Preparation of Plant Extracts

Fresh leaves were collected from each species, washed thoroughly and air-dried at room temperature for one week. These were separately triturated into fine powder in a mortar and pestle. The powders were stored separately in sterile containers, kept away from light and moisture for further analysis. 5g of each powder leaves were then treated with 500 ml of hexane and allowed to stand for 14 days at room temperature with frequent agitation. The extracts were filtered using Whatman filter paper No 1 and were then concentrated in a water bath at 50^{o}C. These were subsequently brought to dryness at 60^{o}C in an oven. The crude extracts were then phytochemically screened for the presence of flavonoids, tannins and saponins, using modified protocols of Edeoga *et al.* (2005).

Bioassay

The effects of leaf extracts on two fungal pathogens were assessed based on zone of inhibition. In vitro antifungal activity was conducted on Potato Dextrose Agar (PDA) plates. Plates of Potato Dextrose Agar (PDA) were prepared following standard procedures. 3-day old fungal cultures were used in this experiment. 0.5 ml of each extract at 2%, 4%, 6%, 8% and 10% concentrations were separately added to sterile filter paper disc measuring 5mmin diameter. The plates were incubated at room temperature for 72 hours and the activities of the extracts were determined by measuring the diameter of zone of inhibition. For each plate standard antibiotic ketoconazole (10µg ml^{-1}) was used as control and each experiment replicated three times.

Statistical Analysis

Data was subjected to ANOVA (Analysis of Variance) to determine the significance of treatment effect.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of plant extracts revealed the presence of favonoids, saponins and tannins (Table 1). Tannin was present in all the plant extracts tested while saponin was found in all except *A. hybridus* extract. Flavonoids were present in all except *P. amarus* and *B. pinnatum* extracts. Results also showed that *E. sonchifolia* and *M. pudica* contained all three constituents tested. These constituents have been reported in several plant species including those presently tested in different parts of the world (Vander & Pretorious 2001, Okwu & Josiah 2006).

Table 1. Phytochemical constituents in five plant species leaf extracts.

Plant Species	Constituents		
	Saponin	**Tannin**	**Flavonoids**
Phyllantus amarus	+	+	-
Emilia sonchifolia	+	+	+
Mimusa pudica	+	+	+
Bryophyllum pinnatum	+	+	-
Amaranthus hybridus	-	+	+

Note: + = Present; - = Absent.

These compounds have also been implicated in the control of both animals and plant diseases such as; anticonvulsant (Asije *et al.* 2006), anti-inflammation (Akindele & Adeyenmi 2007), hepatoxicity (Adeneye *et al.* 2006). Thus, the antifungal activities of the extracts reported in this study could be associated with the presence of favonoids, tannins, and saponins. It is therefore, not surprising why these plants have been used by both traditional healers and contemporary medicine (Liu 2003). The fact that all plant species tested did not contain the same constituents implies different levels of antifungal activities. Hence, the need for screening to determine most efficient plant species.

Effect of plant extracts of Pathogenic fungi

Results of the effect of five plant extracts indicate growth inhibition of *A. niger* at different concentrations (Table 2). The level of inhibition significantly increased with increase in concentration, 10% being the highest in all the extracts tested. Inhibitory effects of *E. sonchifolia* and *M. pudica* extracts were the highest at 10% concentration and were not significantly different from those of the control (ketoconazole). Extracts of *P. amarus* recorded the least inhibition at 10% concentration. The significant high inhibitory effects of *E. sonchifolia* and *M. pudica* could be associated with the fact that these extracts contained all the constituents identified compared to the other extracts tested (Table 1). These results imply that all extracts tested can be employed as biocontrol agents against *A. niger*, although, *E. sonchifolia* and *M. pudica* more recommended.

Table 2. Antifungal activities of extracts on *Aspergillus niger*.

Plant Extracts	Zone of inhibition at different concentrations (cm)				
	2%	**4%**	**6%**	**8%**	**10%**
Phyllantus amarus	$5.0^{a}\pm0.00$	$6.0^{a}\pm0.00$	$8.0^{b}\pm0.00$	$10.0^{c}\pm0.00$	$14.6^{e}\pm0.00$
Emilia sonchifolia	$6.5^{a}\pm0.50$	$12.0^{d}\pm1.00$	$15.0^{e}\pm1.00$	$17.0^{f}\pm0.70$	$18.6^{g}\pm5.10$
Mimusa pudica	$6.8^{a}\pm0.70$	$11.5^{d}\pm0.80$	$14.6^{e}\pm0.00$	$16.9^{f}\pm0.60$	$16.9^{f}\pm0.60$
Bryophyllum pinnatum	$5.6^{a}\pm0.10$	$9.0^{b}\pm0.00$	$9.2^{b}\pm0.60$	$11.2^{c}\pm0.30$	$17.5^{f}\pm0.00$
Amaranthus hybridus	$6.0^{a}\pm0.10$	$9.0^{b}\pm0.10$	$10.5^{c}\pm1.00$	$13.5^{d}\pm0.50$	$17.4^{f}\pm0.00$
Control	$6.7^{a}\pm0.00$	$12.8^{d}\pm0.00$	$15.5^{e}\pm0.60$	$17.2^{f}\pm0.00$	$19.9^{g}\pm0.00$

Note: Different letters are significantly different ($p<0.05$).

Table 3. Antifungal activities of extracts on *Rhizopus oryzae*.

Plant Extracts	Zone of inhibition at different concentrations (cm)				
	2%	**4%**	**6%**	**8%**	**10%**
Phyllantus amarus	$5.0^{a}\pm0.00$	$6.5^{b}\pm1.00$	$8.6^{c}\pm1.00$	$10.0^{d}\pm0.00$	$17.0^{f}\pm0.50$
Emilia sonchifolia	$7.9^{c}\pm0.00$	$12.8^{e}\pm0.70$	$16.0^{f}\pm0.00$	$18.0^{g}\pm0.40$	$19.6^{h}\pm0.30$
Mimusa pudica	$8.1^{c}\pm0.00$	$13.0^{e}\pm0.00$	$16.2^{f}\pm0.40$	$17.6^{g}\pm0.20$	$19.7^{h}\pm0.70$
Bryophyllum pinnatum	$5.0^{a}\pm0.00$	$6.0^{b}\pm0.00$	$8.5^{c}\pm0.60$	$10.0^{d}\pm0.00$	$18.0^{g}\pm0.00$
Amaranthus hybridus	$5.0^{a}\pm0.00$	$7.0^{b}\pm1.00$	$10.0^{d}\pm0.00$	$16.0^{f}\pm0.50$	$18.4^{g}\pm0.30$
Control	$8.0^{c}\pm0.00$	$13.5^{e}\pm0.40$	$16.3^{f}\pm0.20$	$18.2^{g}\pm0.50$	$20.0^{h}\pm0.00$

Note: Different letters are significantly different ($p<0.05$).

Results of growth inhibition effects of the extracts on *R. oryzae* are shown in table 3. The trends were generally similar to those recorded on *A. niger* with 10% concentration having the highest inhibition in all the extracts tested. Extracts of *E. sonchifolia*, *M. pudica*, were significantly higher than the others tested. These results support previous reports by (Asije *et al.* 2006, Akindele & Adeyenmi 2007, Adeneye *et al.* 2006, Agrawal *et al.* 2004). There is collaborative evidence that the antifungal activities of the tested extracts are associated with the presence of saponin, tannin and flavonoids. Formulations of these plants can therefore, be recommended for both plant and animal disease control. The advantages of these plants as biocontrol measures are enormous. Apart from being environmentally friendly, these plants are common weeds that can easily be obtained and hence reducing the cost burden.

CONCLUSIONS

Amaranthus hybridus and *Mimosa pudica*, are the most recommended plant species to develop pharmaceutical products against plant and animal diseases, while *Phyllanthus amarus*, *Bryophyllum pinnatum* and *Emilia sonchifolia* can be further processed in this regard.

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