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Antifungal screening of *Mimosa pudica* plant extracts against phytopathogenic fungi

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Abstract

The antifungal properties of the leaf and stem of *Mimosa pudica* plant (Touch me not) was investigated using agar incorporation method. The activity was tested against *Trichophyton verrocusum, T. mentagrophytes, Microsporum nanum, Aspergillus niger and A. flavus* at 10, 20 and 40 mg/ml. The extracts inhibited the growth of the above mentioned fungi at 10, 20 and 40 mg/ml except *A. niger.* The highest growth inhibition (90%) was observed at 40 mg/ml of leaf methanolic extract against *T. verrocusum.* Generally, the results showed significant difference ($P<0.05$) in the percentage growth inhibition of leaf, stem and mixture of *M. pudica* on all the fungal isolates at the different concentrations of the extracts. Antifungal activity against the isolates were observed to increase significantly ($P<0.05$) with increasing concentrations of plant extracts. The highest antifungal activity of the extracts was recorded at 40mg/ml concentration, which was significantly higher than that of the positive control (Greseofulvin, 10mg/ml), but lower than Greseofulvin at 40 mg/ml concentration. The plant extracts of the leaves, stem and mixture did not show any antifungal activity against *A. niger* at the various concentrations used in this study. It is therefore suggested that the local ethnomedical preparation and prescription of the plant sources should be scientifically evaluated and then disseminated properly and the knowledge about botanical preparation of traditional sources of medicinal plants should be extended for future investigation for drugs discovery.

Keywords

Antifugal Activity, *Mimosa Pudica* Plant, Aqueous Extract, Methanol Extract, Ethanol Extract

1. Introduction

Plants have long served mankind as source of medicinal agent. Natural products have once served as source of all drugs (Shale, *et al.*, 1999)

Herbal medicine involves the use of leaves, stem, flowers, fruits, seeds, roots rhizome and bark for healing of diseases. There can be little doubt that, the use of plants for healing purpose is the most ancient form of medicine known. The quest of the plant with medicinal properties continues to receive attention as scientists are in need of plants particularly of ethno botanical significance for a complete range of biological activities, which ranges from antibiotic to anticancerus. Several plants and herbs species used traditionally have potential antimicrobial and antiviral properties (Shelef, 1983; zaika, 1988) and this has raised the optimism of scientists about the future of phyto antimicrobial agent (Das, *et al*., 1999).

Mimosa pudica is among the medicinal herbal plant. It is a creeping annual or perennial herb often grown for its curiosity values; as the compound leaves fold inward and droop when touched and reopens within minutes later. It belongs to Fabaceae family. The species is a native to South America and Central America, but is now a pan tropical weed. The other names given to this plant are

humble plant, shame plant, touch me not (Dininger, 1984). This plant has history for use for the treatment of various ailments and the most commonly used plant part is the root, but leaves, flowers, fruit and bark can also be utilized (Ahmad and Beg, 2001;Deininger,1984) and also about the antimicrobial activity of the plant(Placios, *et al.,*1991;Ajalla, *et al.,*1999).

1.1. Classification and Morphology of *MIMOSA Pudica*

M. pudica belongs to kingdom; plantae, division; magnoliophyta, class; magnoliopsida, Order; Fabale, Family; Fabaceae, subfamily; mimosoideae, Genus; Mimosa, Species; *M. Pudica*, English Name: Sensitive Plant. (Stevia and Reb, 2008). The plant is commonly called sensitive plant. It is also known as a source of fascination to adults and children alike. When you gently touch the narrow fern-like leaf lets they almost instantaneously fold together and leaf stalk droops (Bigal, 2010).

Fig. 1. *Mimosa pudica in its natural habitat (with leaves opened)*

M. pudica is well known for its rapid plant movement. In the evening the leaflets will fold together and the whole leaf droops down ward. It then re-opens at sunrise. This type of motion has been termed nyctinastic movement. (as shown in the real picture below). The leave also closes up under various other stimuli, such as touching, warming, or shaking (Bigal, 2010).

Fig. 2. *Mimosa pudica in its natural habitat (with leaves closed when touched)*

1.2. Chemical Constituents

The leaves of *M. pudica* contain alkaloid mimosine. Root contains tannins, ash, calcium oxalate crystals and mimosine. "It is susceptible to several herbicides, including dicamba, glyphosate, picloram and triclopyr"
(Stevia and Rebs, 2008).

1.3. Toxicology

The seeds and other parts of *M. pudica* contain mimosine, a non-proteins alpha-amino acid that is known to cause hair loss and depressed growth in mammals. However an unlikely large dose would be necessary to cause such problems in humans. *Mimosa pudica* and other related species can become troublesome weed in tropical crops. The variety in Hawaii is reported to be toxic to livestock (Bigal, 2010 :Ahmad and Beg, 2001*).*

1.4. Antimicrobial Agents

Antibiotics are chemical compounds used to kill or inhibit the growth of infectious organisms. Originally the term antibiotics referred to only to organic compounds, produced by bacteria or molds that are toxic to other micro-organisms. The term now used loosely to include synthetic and semi-synthetic organic compounds. Antibiotics refer generally to antibacterial; however, because the term is loosely defined, it is preferable to specify compounds as being antimalarial, antifungal, antiviral, or antiprotozoal. All antibiotics share the properties of selective toxicity; they are more toxic to an invading organism than they are to an animal or human host. Penicillin is the most well known antibiotic and has been used to fight many infectious disease s, including; syphilis, gonorrhea, tetanus and scarlet fever. Antibiotic streptomycin has been used to combat tuberculosis (Dale *et al., 2009*).

In facts, antibiotics have become a risk to public health. Over prescription of antibiotics is harmful to children, to medical patients and to the environment. When never antibiotics is used, a few bacteria always survive. They reproduce and share their ability to survive the drug with other bacteria. Soon many bacteria have this ability and the drugs don't work anymore. Some infectious bacteria can survive almost every antibiotic known and the process of developing new antibiotics take years (Dale *et al.,* 2009.) Essential oil obtained from the herb of *Santolina chamaecyparissus* showed that significant anti fungal activity both in vitro and in vivo (Suresh *et al.,* 1994).

The present research work, intends to study the antifungal properties of leaves, stem and mixture, of *M. pudica* (Touch me not) plant extract in aqueous, ethanol and methanol suspensions. The objectives of this study are;

1. To evaluate the antifungal activity of the leaves, stem and the mixture *Mimosa pudica* plant extracts.
2. Compare and evaluate the percentage of growth inhibition at varying concentrations of the extracts.

2. Materials and Methods

The Following sample materials and methods were used to assess the screening of antifungal properties of *Mimosa pudica*.

2.1. Collection and Preparation of Sample Materials

Fresh samples of leaves and stems of *M. pudica* were collected from Kwalkwalawa area, Usmanu Danfodiyo University, Sokoto permanent site at the bank of the stream. The identification and verification of the plants species was conducted at the herbarium, Botany unit, Department of Biological Sciences, Usman Danfodiyo University, Sokoto, where the voucher specimens were deposited.

The plant materials (Leaves and stems were freshly dried under the shade and later pulverized into coarse powder using cleaned mortar and pestle and sieved into fine particles and then stored in a cool dry place until required.

2.2. Extraction and Preparation of Materials for Antifungal Screening

One hundred grams (100g) each of the powdered leaves and stems from *M. pudica* were separately extracted with water, 95% ethanol and methanol. The extracts were obtained by 2000 ml of distilled water, 95% ethanol and methanol in 2000 ml beakers. The solutions were stirred, cupped with aluminum foils and kept for twenty four hours (24 hrs). The solutions were filtered using muslin cloth and kept in the beakers and later concentrated to dryness using thermo plate instrument set at 40^{o}c. The extracts were then labeled as: LPW and SPW signifying; leaves and stems of *M. pudica* (aqueus extraction) while LPE and SPE stand for leaves and stems (ethanol extraction), while LPM and SPM symbolise leaves and stem (methanol extraction) of the same plant.

The extracts were reconstituted in sterilized distilled water at different concentrations of 10, 20, and 40 mg/ml using the sterilized conical flasks. The varying concentrations which are to be used for anti fungal screening were stored in a freezer to inhibit the growth of bacteria.

2.3. Isolation and Identification of Dermatophytes

2.3.1. Microscopic Examination

Microscopic examination was carried out in accordance with Markie and Cartney, (1999). The hair, nail and skin scrapings were examined microscopically for the characteristics of macroconidia and microconidia, Presence of hyphae and arthroconidia. Samples were treated with 20% potassium hydroxide (KOH) solution by flooding on slides cover slips were used under low power and subdued light. Infected hair and skin were seen encased in regular sheath of arthrospores that doubled their normal thickness.

2.3.2. Inoculation and Isolation of Dermatophytes from Sample

Scrapings of skin and nail were reduced in size to pieces approximately 1mm across and the hair roots were cut in to similar sized fragments. Both samples were planted on the surface of selected medium that is sabouraud dextrose agar containing chlorophenicol at 500 mg/ml. The culture media were incubated at 30 C^{o} for up to 21 days. After isolation the cultures were transferred to freshly prepared (SDA) media to obtain pure cultures. The test dermatophytes were identified by their cultural morphology and microscopic characteristics (Hartman and Rohde, 1980; Cheesbrough, 2003).

2.3.3. Identification of Dermatophytes

Identification of dermatophytes was done in accordance with Hartman and Rohde, (1980). The identification was based on colonial appearance and microscopic morphology of the spore produced (cultures were examined at 4 to 5 days intervals from onset. Characteristics such as texture, colour and shape of the upper thallus as well as the production of pigment on the underside were noted. The identified isolates were *Trichophyton verrocuson, T. metangrophyte and Microsporum nanum.*

However during subculture pure isolates of *Aspergillus niger and A. flavus* were identified under microscope, hence forth, microscopic and colonial appearance were used as criteria for identification of *Aspergillus species* as presented in table 1.

2.4. Test Organisms

The fungi used in this study are as follows:-

i. *Trichophyton verrocuson*
ii. *T. metangrophyte*
iii. *Microsporum nanum*
iv. *Aspergillus niger*
v. *flavus.*

2.5. Screening Procedure

The preliminary screening for the antifungal properties of the extracts was done by using Agar incorporation method Dwivedy and Dubey (1983).The strains were cultivated on sabaraud dextrose Agar (SDA) in 9cm sized petri-dishes.

Five 5 ml of the varying concentration (10, 20 and 40 mg/ml) of the extracts (water, ethanol and methanol) were aseptically mixed with 15 ml of sabaroud dextrose Agar (SDA) to give final volume of 20 ml.

After solidification the seeding was carried out by inoculating the media with the fungal isolates separately in the middle of the petri-dishes. The petri-dishes were then be incubated at room temperature (i.e. 30^{o}C) for 72 hrs in the case of *Aspergillus niger* and *A. flavus*, but for the *Trichophyton verrocuson, Trichophyton mentagrophyte* and *Microsporum nanum* observation is between (10 – 21 days). Each concentration is replicated three times; the negative

control used is plane (SDA) media for each set up. While for positive control, greseofulvin drugs were used. The experiment was terminated when the negative control of each test fills up the petri-dishes

2.6. Minimum inhibitory Concentration (M.I.C) Test

When there is total inhibition at minimum or least concentration used, then minimum inhibitory concentration (M.I.C.) test must be carried out, for example if the minimum inhibitory concentration is (5mg/ml) and it happens that there is total inhibition at this level, then (M.I. C.) test is set e.g .2.5 mg\ml, 1.25 mg\ml and 0.625 mg\ml respectively. This is to be done in order to know where the inhibition starts.

The (MIC) test was not carried out in this research because throughout the concentration 10 - 40 mg/ml and even the standard drugs Greseofulvin does not completely inhibited the growth.

2.7. Media Preparation

All media used were prepared according to the manufacturer's instruction: The media used is sabaroud dextrose agar (SDA). Purchased from Antect Diagnostic product Company Clarion Medicals Ltd Lagos 62g of SDA mixed in 1litre distilled water.

2.8. Sterilisation of Petridishes

All the dishes were washed with detergent and allowed to dry before they are to be placed in an oven at 160^{o}C for one hour to sterile.

2.9. Agar Preparation

Sixty two grams (62g) of sabouraud dextrose agar (SDA) was dissolved in 1000 ml of distilled water and 3g of chlorophenicol antibiotics was added in order to inhibit the growth of bacteria in the media. The mixture was heated to ensure complete dissolution and the pH of 5.6, this pH level ensures effective growth of the organism (fungi).

15 ml of the agar solution was then transferred from the volumetric flask to 100ml conical flasks.

Each conical flask was covered with cotton wool plugged and cupped with Aluminium foil to prevent the inlet and outlet of air and moisture during sterilization in the autoclave at 121^{o}C, per square inch for 15 minutes. The pointer is allowed to drop back to 0^{o}C before the flask were removed and allowed to cool to 45^{o}C at room temperature. Then their contents are each transferred into a petri-dish and allowed to solidify.

2.10. Agar Incorporation

Agar incorporation method was used; this method involves the mixing of each extract with (SDA) agar before the fungi organisms are transferred into both mixtures for culturing to determine the extracts ability. Two types of incorporation methods could be used and these include: hot and cold incorporations. But for this research cold incorporation method was employed.

2.11. Cold Incorporation

Five millilitres (5ml) each of 10, 20 and 40 mg/ml respectively, of the extracts were kept in different petri-dishes and 15 ml of sterilized SDA solution was added to each. The dishes were stirred carefully before the mixture allowed to solidify. Five organisms were inoculated on each extract in the incubation room. While in hot incorporation method the known quantity of the extracts and that of media solution will be autoclave in conical flasks before the context are transferred each into Petri-dishes and allows solidifying before the fungi organisms are transferred into a dish in the incubation room.

2.12. Negative Control Sample

Twenty millilitres (20ml) each of the plane SDA media solution was autoclaved in conical flasks and then transferred when cool into the petri-dishes. After solidification, each fungal isolates was transferred on the SDA surface with an inoculation needle.

2.13. Positive Control

Griseofulvin Clarian Medicals Ltd Lagos Nigeria was measured from the pulverized 500 mg tablets. 5 ml of water solution of Griseofulvin at concentration 10 and 40 mg/ml were aseptically mixed with 15 ml of SDA. After cooling and solidification of the medium, the seeding was carried out by inoculation of all the fungal isolates in the middle of Petri-dishes. The treated and control Petri-dishes were incubated at ambient laboratory conditions, for 72 hrs for *Aspergilius species* and 21 days for dermatophytes three replicates for each concentration were made. Growth was observed after 7 days for dermatophytes and 72 hours for *Aspergillus s*

2.14. Statistical Analysis

For this research, an analysis of variance (ANOVA) was adopted for comparing means. The results of the optional "Descriptive" bottom line of the procedure are a table of the means and standard deviations. The results of the (ANOVA) were presented in an (ANOVA) table. This table contains columns labeled SS or sum of squares, DF–for degree of freedom, MS- for mean square, F or F- ratio p. probability or sig. of F. The only column that was critical for interpretation is that of sig. of F. (Bailey, 2008).

If the number (or numbers) found in this column is (are) less than the critical value (0.05) set by the experimenter, then the effect is said be significant while any value greater than this value will result in non significant effects. If the effect is found to be significant using the above procedure, it implies that means differ more than expected by chance alone. In terms of the experiment it would mean that, the treatments were not equally effective. If the effects are

found to be non significant, than the differences between the means are not great enough to allow the researcher that they are different, in that case no further interpretation is attempted (Montgometry, 2001; Bailey, R. A. 2008).

3. Results and Discussion

Table 1. *Colonial and microscopic description of the test isolates*

Colonial description	Microscopic features	Organism
It is waxy, smooth, tortuous windings and grows upwards. Also they are velvety and have a disc-like shape. The upper side is grey-white-yellow to ocre and the underside is brownish yellow. It has a periphery with light marginal zone with fine extensions.	The hyphae is ramified and sepate. microconidia are roundish, oval and pear-shaped, macrosnidia are longish with smooth-walled and are multicellular. chlamydospores are numerous.	*Trichophyton verrucoson*
Chalky to grainy and forms groves; the upper side is white to light yellow or pink and the under side is red-brown to grey-yellow it has a periphery with fine saw teeth	Ramified and sepate hyphae; straight some are curved and spiral. Microconidia are spherical, also pear-shaped; some are like a bunch of grapes. Macroconidia are longish, smooth –walled, roller-shaped with blunted ends and are multicellular.	*Trichophyton mentagraphyte*
It is suede like to powdery, thin and spreading. The upper side is creamy to buff and becoming dark beige and the underside is red brown	Ramified and sepate hyphae. The microconidia are club shaped. The microconidia are pear to egg shaped, rough and thin walled with a truncated base many borne on stalks.	*Microsporum nanum*
It is black and powdery with a raised surface. The upper side and under side are black in colour	Conidiophores terminate in vesicles and the conidia are in Chains	*Aspergillus niger*
It is light green and powdery with a slight raised surface.	Conidiophores are coarsely rough	*Aspergillus flavus*

Table 2. *Antifungal activity of leaf, stem and mixture, aqueous extract in varying concentrations of Mimosa pudica*

Plant parts	Fungi/Percentage Growth Inhibition					
	Conc. (mg/ml)	*Tricoophyton verrocuson*	*Trichophyton mentagrophyte*	*Microsporum nanum*	*Aspergillus niger*	*Aspergilus flavus*
Leaves	10	$61.11^{d} \pm 3.21$	$44.07^{d} \pm 0.37$	$43.52^{e} \pm 0.49$	0.00 ± 0.00	$32.04^{c} \pm 16.15$
	20	$81.85^{bc} \pm 0.98$	$49.44^{cd} \pm 0.32$	$47.59^{d} \pm 0.67$	0.00 ± 0.00	$58.33^{bc} \pm 3.21$
	40	$86.67^{b} \pm 1.28$	$77.59^{b} \pm 0.67$	$70.37^{b} \pm 0.37$	0.00 ± 0.00	$67.78^{ab} \pm 1.95$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{c} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{bc} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stem	10	$57.41^{e} \pm 1.85$	$27.96^{e} \pm 0.49$	$42.22^{e} \pm 1.28$	0.00 ± 0.00	$25.56^{b} \pm 25.56$
	20	$62.78^{d} \pm 1.95$	$42.59^{d} \pm 0.98$	$48.33^{d} \pm 0.96$	0.00 ± 0.00	$34.63^{b} \pm 17.32$
	40	$81.67^{b} \pm 0.32$	$73.15^{b} \pm 0.49$	$65.93^{b} \pm 0.74$	0.00 ± 0.00	$53.33^{ab} \pm 3.78$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{c} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{ab} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mixture	10	$57.59^{e} \pm 0.74$	$26.67^{e} \pm 0.64$	$37.59^{e} \pm 0.49$	0.00 ± 0.00	$31.48^{c} \pm 16.88$
	20	$59.63^{d} \pm 0.19$	$38.70^{d} \pm 0.49$	$49.63^{d} \pm 0.37$	0.00 ± 0.00	$38.52^{bc} \pm 5.07$
	40	$73.52^{c} \pm 0.49$	$71.85^{b} \pm 0.98$	$64.81^{b} \pm 0.37$	0.00 ± 0.00	$64.63^{b} \pm 4.09$
Greseofulvin (Positive control)	10	$76.85^{b} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{c} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{bc} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Legend

Values are mean ± standard error of 3 replications

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

The result of colonial and microscopic description of the test isolates was presented in Table 1. The percentage growth inhibition of aqueous extracts of leaf, stem and mixture of *M. pudica* has been presented in Table 2.The result showed significant difference (P<0.05) in the percentage inhibition of leaf, stem and mixture of leaf and stem of *M. pudica* on all the fungal isolates at the varying concentrations of the *M. pudica* extracts. *T. verrocuson* was found to be more susceptible towards the aqueous extract of leaf, stem and mixture at 40 mg/ml concentration with percentage growth inhibition of 86.67% (leaves), 81.67% (stem) and 73.52% (mixture) respectively, followed by *T. mentagrophyte* (77.59, 73.15 and 71.85%), *M. nanum* (70.37, 65.93 and 64.81%) and *A. flavus* (67.78, 53.33 and 64.63%) respectively. The highest activity was observed at 40 mg/ml concentration of the leaf extract, which produced percentage inhibition of 86.67% against *T. verrocuson*, 77.59% against *T. mentagrophyte,* 70.37% against *M. nanum,* and 67.78% against *A. flavus*, as indicated in Fig. 1, 2 and 3 respectively. However, the positive control (Greseofulvin) at 40 mg/ml concentration had the highest activity against T. *verrocuson* compared to the other test

isolate. It is worthy of note that, the leaf extract at 40 mg/ml concentration showed a significantly higher (P<0.05) activity against *T. verrocuson*, *T. mentagrophyte, M. nanum,* and *A. flavus* than the positive control (Greseofulvin) at 10mg/ml concentration.

Extract of *M. pudica* did not show any activity in water against *A. niger* in all three plant parts (leaves, stem and mixture). *M. pudica* extracted from the stem produced significant (P<0.05) activity against the fungal isolates. The activity was observed to increase significantly (P<0.05) with increasing concentrations of *M. pudica* extract. The highest antifungal activity of the test isolate was recorded at 40 mg/ml concentration of the extract, significantly higher than that of the positive control (Greseofulvin, 10 mg/ml), but lower than Greseofulvin at 40 mg/ml concentration. Furthermore, extract of mixture, of *M. pudica* in aqueous also produced significant (P<0.05) antifungal activity against fungal isolates at the various concentrations. Percentage inhibition was positively andsignificantly (P<0.05) influenced by concentration of the *M. pudica* extract. The antifungal activity of the 40 mg/ml concentration of the extract of mixture of leaf and stem was significantly (P<0.05) higher than the positive control (Greseofulvin) at 10 mg/ml against *T. mentagrophyte* (71.85 vs. 50.66%), *M. nanum* (64.81 vs. 51.30%) and *A. flavus* (64.63 vs. 50.37%) respectively.

Table 3. *Antifungal activity of leaf, stem and mixture, ethanolic extracts in varying concentrations of Mimosa pudica*

Plant parts	Conc. (mg/ml)	Fungi/Percentage Growth Inhibition				
		Trichoophyton verrocuson	*Trichophyton mentagrophyte*	*Microsporum nanum*	*Aspergillus niger*	*Aspergilus flavus*
Leaves	10	$57.96^{e} \pm 0.19$	$30.00^{e} \pm 0.64$	$39.81^{e} \pm 0.49$	0.00 ± 0.00	$23.52^{b} \pm 23.52$
	20	$75.00^{d} \pm 0.56$	$39.81^{d} \pm 0.19$	$47.96^{d} \pm 0.19$	0.00 ± 0.00	$24.63^{b} \pm 24.63$
	40	$86.85^{b} \pm 0.19$	$70.37^{b} \pm 0.37$	$69.26^{b} \pm 0.49$	0.00 ± 0.00	$65.74^{ab} \pm 3.05$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{c} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{ab} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stem	10	$59.44^{e} \pm 0.56$	$29.44^{e} \pm 0.85$	$48.52^{b} \pm 6.33$	0.00 ± 0.00	$11.11^{c} \pm 5.78$
	20	$66.85^{d} \pm 0.19$	$42.41^{d} \pm 1.03$	$51.11^{b} \pm 0.64$	0.00 ± 0.00	$17.41^{c} \pm 17.41$
	40	$83.89^{b} \pm 0.56$	$70.00^{b} \pm 0.64$	$72.59^{a} \pm 0.37$	0.00 ± 0.00	$35.93^{bc} \pm 1.30$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{b} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{b} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mixture	10	$55.74^{d} \pm 0.19$	$27.41^{e} \pm 0.98$	$42.96^{e} \pm 0.37$	0.00 ± 0.00	$41.11^{b} \pm 20.56$
	20	$62.22^{c} \pm 0.64$	$42.78^{d} \pm 0.85$	$47.78^{d} \pm 0.64$	0.00 ± 0.00	$36.67^{b} \pm 18.38$
	40	$76.30^{b} \pm 0.74$	$65.37^{b} \pm 0.67$	$69.81^{b} \pm 0.67$	0.00 ± 0.00	$62.41^{ab} \pm 0.81$
Greseofulvin (Positive control)	10	$76.85^{b} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{c} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{ab} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Legend
Values are mean ± standard error of 3 replications
Means in a column with different superscripts are significantly different (P<0.05)

The result of the percentage growth inhibition of extract of leaf, stem and mixture of leaf and stem of *M. pudica* in ethanol is shown in Table 3. Percentage fungal growth inhibition of the extract from the leaf of *M. pudica* at concentrations 10 to 40 mg/ml ranged from

57.96-86.85% against *T. verrocuson,* 30.0-70.37% against *T. mentagrophyte,* 39.81-69.26% against *M. nanum* and 23.52-65.74% against *A. flavus*, as indicated in Fig. 4, 5 and 6 respectively. The test isolates showed no activity against *A. niger*

There was significant increase (P<0.05) in the percentage growth inhibition with increase in concentration of *M. pudica* extract from the stem as seen in Table 3. The percentage growth inhibition of the stem extract at 10 mg/ml were 59.44% against *T. verrocuson,* 29.44% against *T. mentagrophyte,* 48.52% against *M. nanum* and 11.1% against *A. flavus*; while at 40 mg/ml concentration of the extract, the inhibition increased significantly (P<0.05) to 83.89% against *T. verrocuson,* 70.0% against *T. mentagrophyte,* 72.59% against *M. nanum* and 35.93% against *A. flavus*. These activities of the 40 mg/ml stem extract was significantly higher than that of the positive control (Greseofulvin, 10 mg/ml) against all the fungal isolates except *A. flavus*, where the inhibition was lower (35.93 vs. 37%) with P>0.05. The antifungal activity of *M. pudica* extract from the stem at 40mg/ml concentration produced statistically the same effect (P>0.05) as that of Greseofulvin (40 mg/ml) against *M. nanum* (72.59 vs. 75.93%). Extract from the stem in ethanol also did not show any activity against *A. niger* at the various concentration.

The positive control (Greseofulvin) at 40 mg/ml concentration, produced the highest activity against the fungal isolates which was significantly higher (P<0.05)

than that of the test isolates. However, the activity of the 40 mg/ml extract from the leaf of *M. pudica* in ethanol was significantly higher (P<0.05) than that of the positive control (Greseofulvin) at 10 mg/ml concentration.

With respect to the extracts from the mixture of leaf and stem of *M. pudica* in ethanol, significant increase (P<0.05) in percentage growth inhibition with increase in concentration of the extract was recorded; the 40 mg/ml test sample producing the highest activity compared to the 10 and 20 mg/ml test isolates. The activity of the 40 mg/ml sample compared to Greseofulvin (10 mg/ml) was significantly higher (P<0.05) against *T. mentagrophyte* and *M. nanum*. Greseofulvin (40 mg/ml) produced statistically the same (P>0.05) activity against *A. flavus* as that of the 40 mg/ml test sample.

Table 4. *Antifungal activity of leaf, stem and mixture, methanolic extracts in varying concentrations of Mimosa pudica*

Plant parts	Fungi/Percentage Growth Inhibition					
	Conc. (mg/ml)	*Trichophyton verrocuson*	*T.mentagrophyte*	*Microsporum nanum*	*Aspergillus niger*	*A. flavus*
Leaves	10	$57.41^{e} \pm 1.85$	$32.04^{e} \pm 0.81$	$54.44^{d} \pm 0.64$	0.00 ± 0.00	$42.41^{b} \pm 21.25$
	20	$73.33^{d} \pm 0.64$	$42.78^{d} \pm 0.85$	$62.22^{c} \pm 0.64$	0.00 ± 0.00	$49.63^{b} \pm 0.19$
	40	$90.00^{b} \pm 0.64$	$71.85^{b} \pm 0.37$	$73.70^{a} \pm 0.37$	0.00 ± 0.00	$73.33^{ab} \pm 4.55$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{e} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{b} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{b} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stem	10	$58.52^{d} \pm 3.87$	$30.37^{e} \pm 0.74$	$50.37^{d} \pm 0.37$	0.00 ± 0.00	$20.93^{c} \pm 20.93$
	20	$72.96^{c} \pm 0.37$	$43.33^{d} \pm 0.64$	$52.04^{c} \pm 0.49$	0.00 ± 0.00	$37.41^{bc} \pm 19.10$
	40	$88.52^{b} \pm 0.98$	$65.56^{b} \pm 0.64$	$70.93^{b} \pm 0.49$	0.00 ± 0.00	$65.74^{ab} \pm 4.81$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{cd} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{abc} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mixture	10	$58.15^{e} \pm 1.93$	$28.89^{e} \pm 0.64$	$32.04^{e} \pm 0.67$	0.00 ± 0.00	$21.67^{c} \pm 13.77$
	20	$72.41^{d} \pm 0.49$	$43.33^{d} \pm 0.64$	$43.89^{d} \pm 0.56$	0.00 ± 0.00	$55.93^{b} \pm 1.93$
	40	$87.04^{b} \pm 0.98$	$62.04^{b} \pm 0.67$	$67.96^{b} \pm 0.49$	0.00 ± 0.00	$62.59^{b} \pm 5.33$
Greseofulvin (Positive control)	10	$76.85^{c} \pm 0.67$	$50.56^{c} \pm 4.10$	$51.30^{c} \pm 0.19$	$55.37^{b} \pm 0.19$	$50.37^{b} \pm 7.13$
	40	$97.22^{a} \pm 0.32$	$93.52^{a} \pm 0.81$	$75.93^{a} \pm 0.37$	$84.26^{a} \pm 0.19$	$86.11^{a} \pm 0.56$
SDA (-ve) control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Legend

Values are mean ± standard error of 3 replications

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

Table 4. shows the result of the percentage growth inhibition of extract of leaf, stem and mixture of leaf and stem of *M. pudica* in methanol. There were significant differences (P<0.05) in the percentage growth inhibition of the test isolates from the leaves, stem and mixture of leaf and stem against the fungal isolate *M. pudica* extracts from the leaves at 40mg/ml concentration was very active against *T. verrocuson* producing 90% growth inhibition, *T. mentagrophytes* (71.85%), *M. nanum* (73.70%) and *A. flavus* (73.33%) respectively.

These activities were significantly (P<0.05) higher than that of Greseofulvin (10 mg/ml) but lower than that of Greseofulvin (40 mg/ml). However, the antifungal activity at 40 mg/ml concentration of the test isolate and the Greseofulvin (40 mg/ml) against *A. flavus* was statistically comparable.

Percentage growth inhibition of *M. pudica* extracted from the stem in methanol also showed very high activity at 40 mg/ml concentration of the extract against the fungal isolates. Percentage fungal growth inhibition at 10 and 40 mg/ml concentrations of the extract ranged from 58.52-88.52% against *T. verrocuson,* 30.77-65.56% against *T. mentagrophyte,* 50.37-70.93% against *M. nanum* and 20.93-65.74% against *A. flavus*. The extract at 40 mg/ml concentration produced higher activity than Greseofulvin (10 mg/ml) against *T. verrocuson, T. mentagrophyte, M. nanum* and *A. flavus*. However, its activity was comparable (P>0.05) against *A. flavus.* The stem extract in methanol produced no antifungal activity against *A. niger*

The test isolates at 40 mg/ml concentration from the mixture of leaf and stem of *M. pudica* extract showed its highest activity of 87.04% against *T. verrocuson.*

There was significant difference (P<0.05) in the percentage growth inhibition of all the treatments, with Greseofulvin (40 mg/ml) having the highest antifungal activity followed by the sample extract at 40 mg/ml concentration, Greseofulvin (10 mg/ml), extract at 20 mg/ml and 10mg/ml as shown in Fig. 7, 8 and 9 respectively.

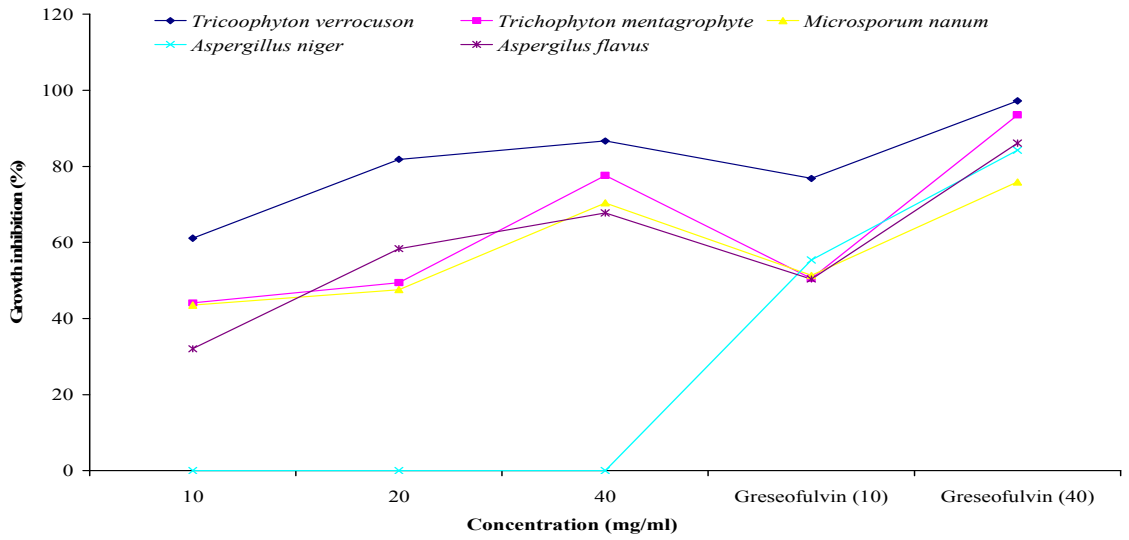

Fig. 1. *Growth inhibition effect of aqueous leaf extract of M. pudica against various fungi*

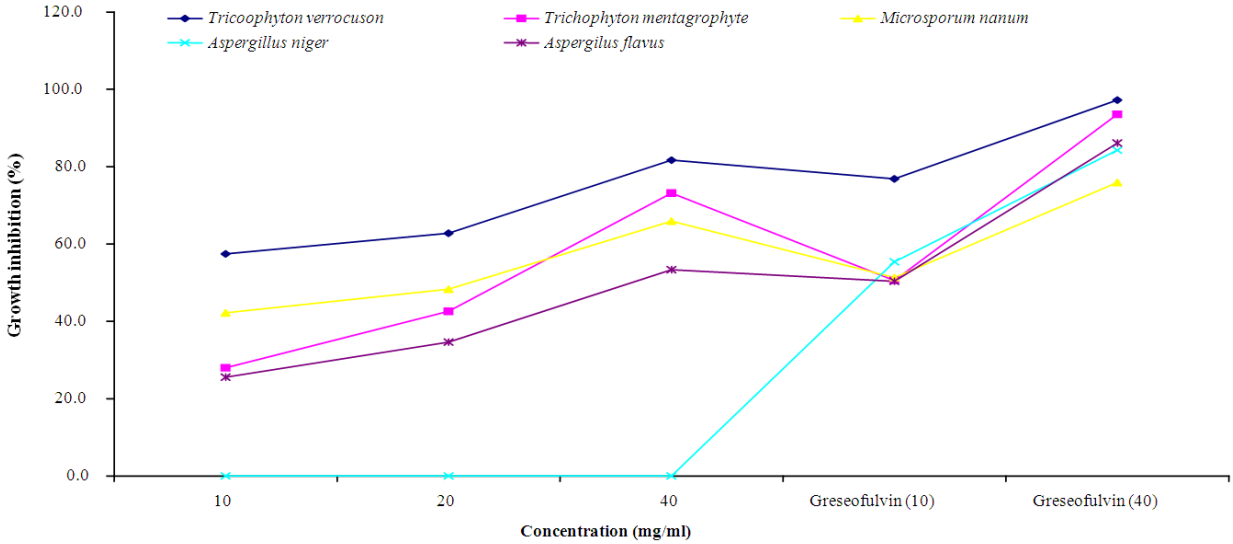

Fig. 2. *Growth inhibition effect of aqueous stem extract of M. pudica against various fungi*

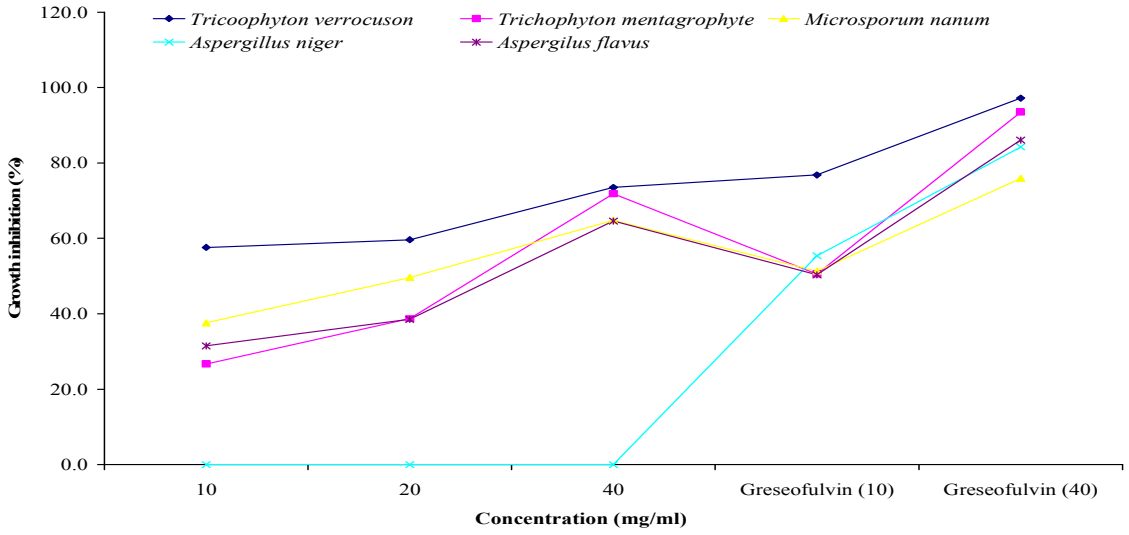

Fig. 3. *Growth inhibition effect of aqueous mixture extract of M. pudica against various fungi*

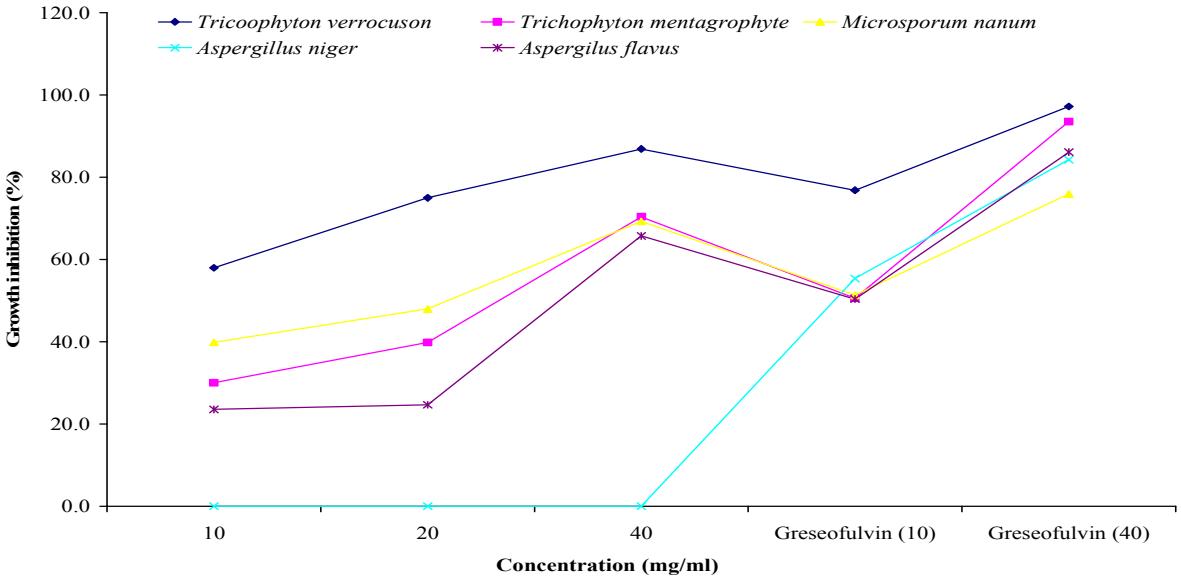

Fig. 4. *Growth inhibition effect of leaf ethanolic extract of M. pudica against various fungi*

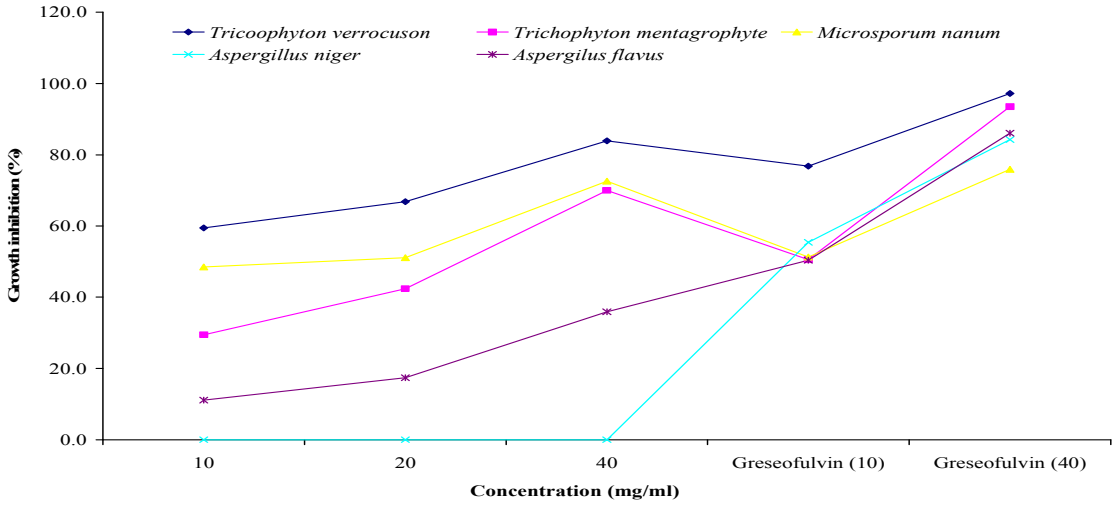

Fig. 5. *Growth inhibition effect of stem ethanolic extract of M. pudica against various fungi*

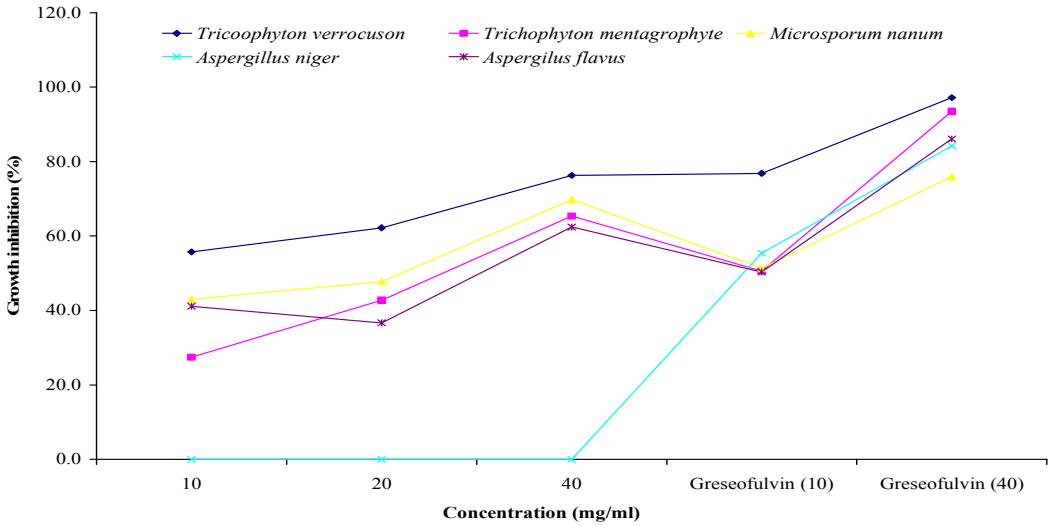

Fig. 6. *Growth inhibition effect of mixture of ethanolic extracts of M. pudica against various fungi*

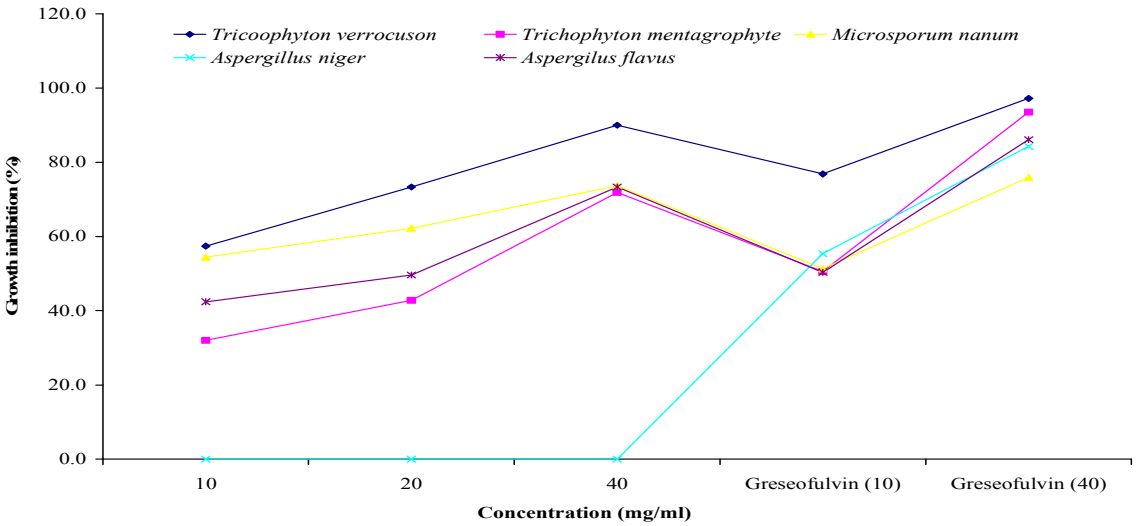

Fig. 7. *Growth inhibition effect of leaf methanolic extract of M. pudica against various fungi*

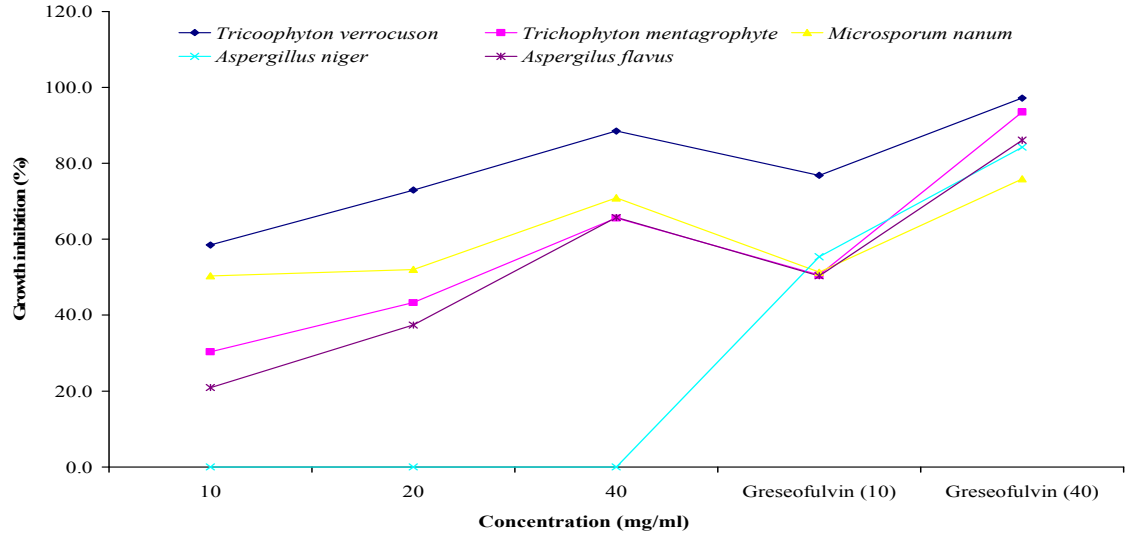

Fig. 8. *Growth inhibition effect of stem methanolic extract of M. pudica against various fungi*

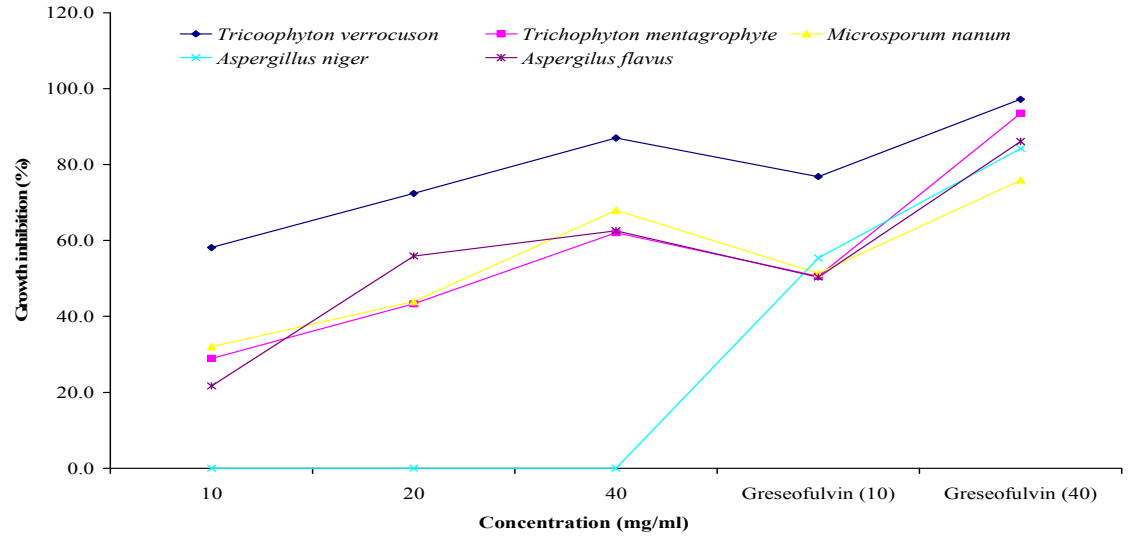

Fig. 9. *Growth inhibition effect of mixture methanolic extracts of M. pudica against various fungi*

Generally, the results showed significant difference ($P<0.05$) in the percentage growth inhibition of leaf, stem and mixture of *M. pudica* on all the fungal isolates at the different concentrations of the extracts. Antifungal activity against the isolates were observed to increase significantly ($P<0.05$) with increasing concentrations of plant extracts. The highest antifungal activity of the extracts was recorded at 40mg/ml concentration, which was significantly higher than that of the positive control (Greseofulvin, 10mg/ml), but lower than Greseofulvin at 40 mg/ml concentration. The plant extracts of the leaves, stem and mixture did not show any antifungal activity against *A. niger* at the various concentrations used in this study (tables 2, 3 and 4)

There was significant increase ($P<0.05$) in the percentage growth inhibition of the isolates with increase concentration of the leaf extract (tables 2, 3 and 4). Increase in susceptibility of fungal isolates (*Microsporum aduoinii, Candida albicans, T. rubrum, C. tropicalis, Penicillium notatum* and *Trichophyton mentagrophyte*) to aqueous extract of *Chrysanthellum americanum* with increasing concentrations has also been reported (Ofodile *et al.,* 2010). Leaf extract of *M. pudica* has also been reported to have strong antibacterial activity against *S. aureus* (76.67%), *B. subtilis* (73.89%) and *P. aeruginosa* (82.3%) (Doss *et al.,* 2011). From these results, it is possible that the extracts of *M. pudica* may be used as natural antimicrobial substance to replace antibiotics for controlling bacterial infections.

The antifungal effects of the leaves, stem, and mixture of leaf and stem from *M. pudica* indicated that the plant is very active against all the tested fungal isolates especially at concentration of 40mg/ml (tables 2, 3 and 4). This observation agrees with the reports of Leven *et al.* (1979), and Banso and Mann, (2008) both reports linked the antifungal properties of plants to the presence of phytocompounds such as tannins, alkaloids, flavonoids and saponins. The antifungal activity of extracts from *M. pudica* is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al.,* 1996).

Also, the observed range of percentage inhibition of *M. pudica* extracts against *A. flavus* at 40mg/ml concentration of the leave, stem and mixture of leaf and stem in methanol (62.59-73.33%) (Table 4) is comparable to the reported 70% percentage inhibition produced by *Tamarix dioica* against *A. flavus* as reported by Khan *et al.* (2004).

Furthermore, the high activity of *M. pudica* extracts from the leaf in methanol (table 4) is noteworthy since studies by Ofodile *et al.,* (2010) reported that *Chrysanthellum americanum* leaf extract in methanol was not active against all species of fungi studied (*Microsporum aduoinii, Candida albicans, T. rubrum, C. tropicalis, Penicillum notatum* and *Trichophyton mentagrophyte* respectively).

4. Conclusion

Plant resources are abundant, but these resources are dwindling due to the onward march of civilization (Vogel, 1991). Although a significant number of studies have been used to obtain purified plant chemicals, very few screening programmes have been initiated on crude plant materials. In the present investigation the antimicrobial activity of *Mimosa pudica* plant extract was tested against five potentially pathogenic microorganisms: *Tricophyton verrocuson, Trichophyton mentagrophyte, Microsporum nanum, Aspergillus niger* and *Aspergilus flavus* at different concentrations (10, 20 and 40 mg/ml) of the extract to determine the most effective activity. The maximum percentage inhibition was obtained for *Trichophyton verrocuson, T. mentagrophyte, Microsporum nanum* and *Aspergilus flavus* at a concentration of 40 mg/ml. While *A. niger* showed resistance against *Mimosa pudica* extract at all concentrations.

From the above findings, it could be concluded that the traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use

of plants in modern medicine.

Recommendations

Based on the results of the study, the following recommendations are hereby advanced:

1. The local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.
2. The sensitivity of other fungal strains to *M. pudica* extract should be tested.
3. Other effects such as antipyretic and hypoglycemic effects of *M. pudica* leaf extract should be tested.

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