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Phytochemical and Antifungal Activity of Leaf Extracts of *Prosopis africana* and *Anacardium occidentale* against Macrophomina Root Rot of *Sesamum indicum* L. in Benue State, Central Nigeria

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Abstract

This study investigated the antifungal activity of leaf extracts of *Prosopis africana* and *Anacardium occidentale* against *Macrophomina phaseolina*, the causal agent of root rot of *Sesamum indicum* L. Phytochemical analysis of the two plants showed the presence of alkaloids, saponins, tannins, flavonoids and anthraquinones in petroleum ether, ethyl acetate, methanol and water extracts. The effectiveness of the two medicinal plants viz: *P. africana* and *A. occidentale* was tested against the causative agent of root rot of *Sesamum indicum* L. The effect of plant leaf extracts on mycelia growth of the test organism shows that both *P. africana* and *A. anacardium* reduced the mycelia growth significantly as compared to the control (plate, 2, 3, 4). The antifungal property of *P. africana* and *A. occidentale* makes these plants of potential interest for the control of the fungi *Macrophomina phaseolina*.

Keywords

Phytochemical, Antifungal Screening, *Prosopis africana*, *Anacardium occidentale*, Leaf Extracts, *Macrophomina Phaseolina* (Tassi) Goid

1. Introduction

Plants have long served mankind as source of medicinal agents. These have been found to possess fungicidal properties against various phytopathogenic fungi [1]. Elaigwu, *et al.* [2] earlier reported that the extracts of *Prosopis. africana* and

Anacardium occidentale significantly reduced the incidence on the development of *M. phaseolina* root rot disease both in pre-yield and yield parameters. Similarly, the extracts of pulverized bark of *Prosopis africana* and leaves of *N. latifolia* inhibited both radial mycelia growth and sclerotial formation of *Macrophomina phaseolina* by 100% [3].

Akash [4] reported that petroleum ether extract and ethanolic extracts of *Anacardium occidentale* leaves exhibited significant antimicrobial and antifungal activity. Similarly, Ezike [5] reported that almost all parts of *Prosopis africana* tree are used in medicine; the leaves in particular are used for the treatment of headache and toothache as well as various other head ailments. Leaves and bark are combined to treat rheumatism. Remedies for skin diseases, caries, fevers and eyewashes are obtained from the bark. The roots are a diuretic and are used to treat gonorrhea, tooth and stomach-ache, dysentery and bronchitis. In Mali, the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps. In Ghana, boiled roots serve as a poultice for sore throat, root decoction for toothache, and bark as a dressing or lotion for wounds or cuts. In Ghana, the pod ashes of *P. africana* are source of potash for soap making [5].

The fruits or seeds of the cashew are consumed whole, roasted, shelled and salted, in Madeira wine, or mixed in chocolates. Bark is used in tanning. Stems exude a clear gum used in pharmaceuticals and as substitute for gum Arabic. Juice turns black on exposure to air and provides an indelible ink [6].

Among the fungi diseases, the root rot caused by *M. phaseolina* remains to be a challenging task in terms of management, since it is soil borne in nature. Many synthetic fungicides have shown promise in the control of Sesame disease [7]; however, the high cost of such chemicals forbids their use by poor resource farmers. Furthermore, continuous use of these chemicals may pose serious health hazards to the applicator as well as to consumer of the treated material as toxic forms persist in soil and contaminate the whole environment besides from development of resistant strains [8].

These facts necessitate the search for alternatives in plant products that are environmentally friendly, many of which have been reported in the control of several plant diseases [2] [9] [10].

2. Materials and Methods

2.1. Collection and Identification of Plant Materials

Fresh leaves of *Prosopis africana* and *Anacardium occidentale* were collected from the premises of Federal University of agriculture Makurdi, Nigeria in March, 2012. Taxonomic identification of the plant samples were authenticated at the Department of Biological Sciences University of agriculture Makurdi. Voucher specimens were preserved in the Herbarium collection of the Department of Biological Sciences, University of Agriculture, Makurdi.

2.2. Isolation of the Fungi Pathogen

The pathogen, M*acrophomina phaseolina* (Tassi) Goid used for this experiment was isolated according to the method of Oluma & Elaigwu [3] and confirmed by (International Mycological Institute (IMI), Kew Survey, England.

2.3. Preparation of Czapeck-Dox-Agar Medium (CDA)

The Czapeck-dox-agar medium (CDA) comprised of the following; Sodium nitrate ($NaN0_3$) 3.0 gm, Ferrous sulphate ($FeS0_4{\cdot}7H_20$) 0.01 gm, Di-potassium hydrogen orthophosphate (K_2Hp0_4) 1 gm, Magnesium sulphate ($MgS0_4{\cdot}7H_20$) 0.5 gm, Potassium chloride (Kcl) 0.01 gm, 20 gm each of glucose and plain agar and sterile distilled water 1000 cm^3 and autoclaved at 15 p.s.i. (121˚C for 15 minutes).

2.4. Preparation of Pure Culture

Pure cultures of the pathogens were obtained by sub-culturing colonies growing from the plated root tissues on Czapecks agar (CDA) medium.

2.5. Inoculation of the Test Organism

To study the effect of plant extracts on the growth and sporulation of the fungus, the CDA-plant extracts was inoculated respectively at the centre of the plates with 2 mm mycelia discs of the test fungus. The mycelia plugs were obtained with 2 mm cork borer lifted from the margin of actively growing culture of the test fungi. These were placed upside down in centre of each plate. The inoculated plates were incubated at 28˚C ± 2˚C for 6 days. Plates of medium without plant extracts served as control.

2.6. Effect of Plant Extracts on Mycelium Growth

The poisoned food method was used in the preliminary screening of aqueous extracts for their antifungal properties evaluation. First, the mycelia growths were evaluated in 60 mm Petri dishes filled with CDA solid medium amended with 20% aqueous extracts of each plant. Next, the center of each Petri dish was inoculated with 5 mm diameter disc of fungal mycelium, taken from pure culture (7 days old). The petridish without plant extract served as control. Then, all inoculated dishes were incubated at 25˚C for 6 days (plate 1, 2, 3, 4).

2.7. Extraction of Plant Materials

The two plant materials (leaves of *A. occidentale* and *P. africana*) were sun-dried for 2 weeks, after which it was ground to a uniform powder.

2.8. Petroleum Ether Extraction

Two hundred g each of *Anarcadium occidentale* and *Prosopis africana* were separately extracted with petroleum ether in 450 ml. Each of the plant material

was dissolved in 450 ml of the solvent in a sterile conical flask at room temperature for 24 hours. The conical flask was covered with cotton wool, wrapped with aluminium foil. The extracts were filtered after 24 hours, first through cheese cloth and then through a Whatmann filter paper No. 42 (125 mm). The dark brownish, light brown and brownish filtrate were obtained and transferred into a labeled specimen bottle. The powdered plant materials was removed and spread on the laboratory bench to dry for two weeks.

This experiment was similarly performed for ethyl acetate and methanol extraction (Table 1).

2.9. Water Extraction

The specimens were washed with several changes of sterile distilled water and were later pulverized using pestle and mortar according to the method of [11]. The crushed leaves were separately plunged in required quantity of water (1:1 w/v) in a beaker and boiled at 100˚C for 10 minutes. Crude extracts of the leaves were obtained after 24 hours in the laboratory at 28˚C ± 2˚C by filtering the infusions through cheese cloth and then through a Whatmann filter paper No. 42 (125 mm) which formed a standard plant extracts (100%).

3. Phytochemical Screening of the Leaf Extracts

Phytochemical screenings were performed using standard procedures [12] [13] with petroleum ether, ethyl acetate, methanol and aqueous leaf extract for both *Prosopis africana* and *Anarcadium occidentale* extract as shown in Table 1.

Table 1. Petroleum ether extract of Prosopis africana (leaves).

S/NO	TEST	OBSERVATION	INFERENCE
1	Draggen dorff's and Mayer's reagents	No turbidity or precipitation	Alkaloids absent
2	Frothing test	Frothing did not persist on warming	Saponins absent
3	Ferric chloride reagent test	No blue-green precipitate formed	Tannins absent
4	Dil. Ammonia and conc. H_2So_4 test	No yellow colouration formed on standing	Flavonoids absent
5.	H_2SO_4, chloroform and Ammonia test	The resulting solution changes from brown to colourless	Anthraquinones present

4. Results

Table 2. Phytochemical screening of PEE, EAE, MEE and ALEfor P.africana extracts (leaves).

S/NO	Test	Reagent	PEE	EAE	MEE	AWE
1	Alkaloids	Dragendorff's and Mayer's Reagents	−	+	+	+

Continued

2	Saponins	$NaHC0_{3aq}$ Emulsion foaming test.	−	+	+	+
3	Tannins	5% $FeCl_{3aq}$10% KOH_{aq}	−	+	+	−
4	Flavonoids	Dil. Ammonia and conc.H_2SO_4	−	−	+	+
5	Anthraquinones	H_2SO_4, Chloroform and Ammonia	+	−	+	−

Table 3. Phytochemical screening of PEE, EAE, MEE and ALE for Anarcadium occidentale extracts (leaves).

S/NO	Test	Reagent	PEE	EAE	MEE	AWE
1	Alkaloid	Dragendorff's and Mayer's Reagents	−	+	+	−
2	Saponins	$NaHC0_{3aq}$ Emulsion foaming test	−	+	+	−
3	Tannins	5% $FeCl_{3aq}$ and 10% KOH_{aq}	−	−	+	+
4	Flavonoids	Dil. Ammonia andconc.H_2SO_4	−	+	+	+
5	Anthraquinones	H_2SO_4, Chloroform and Ammonia	+	−	+	+

Legend: + = positive. − = negative. PEE = Petroleum ether extract. EAE = Ethyl acetate extract. MEE = Methanol extract. ALE = Aqueous Leaf extract.

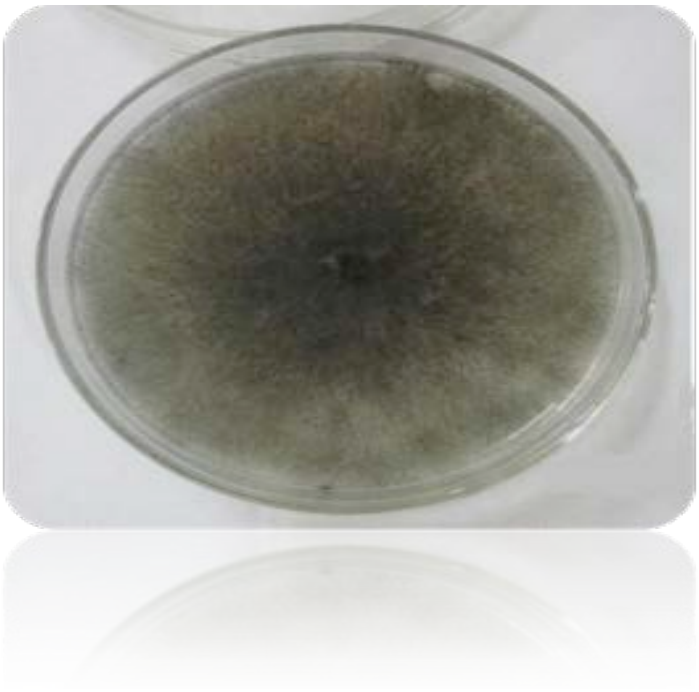

Figure 1. A pure culture of the test organism (*Macrophomina phaseolina*).

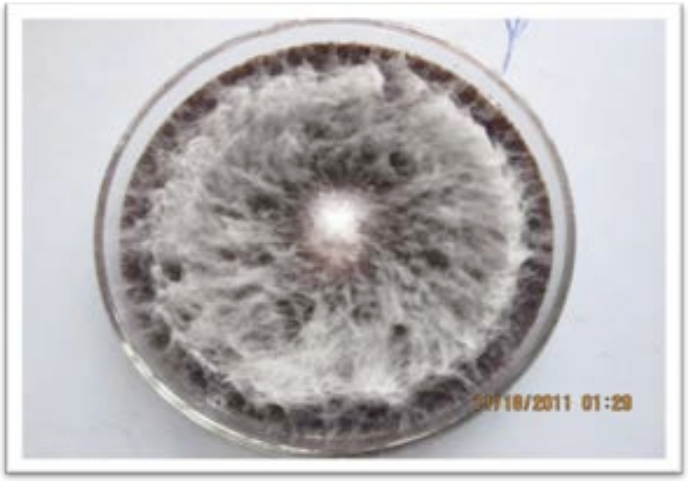

Figure 2. Control experiment.

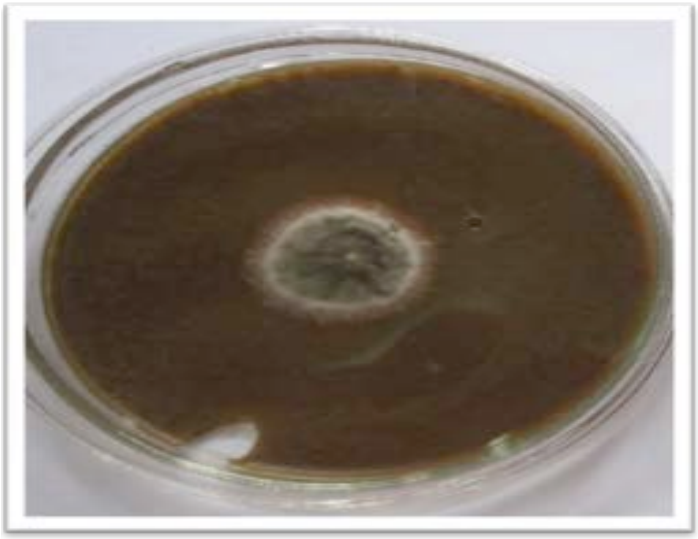

Figure 3. *Prosopis africana.*

Figure 4. *Anacardium occidentale.*

5. Discussion

The study has demonstrated the antifungal activity of *Prosopis africana* and *Anarcadium occidentale* against *Macrophomina phaseolina*. This indicates their broad range of activity. These results agree with those reported by Elaigwu *et al.* [2] that extracts of *P. africana* and *A. occidentale* significantly reduced the incidence on the development of *M. Phaseolina* root rot disease both in pre-yield and yield parameters. This is consistent with the earlier reports that many plant products contain fungitoxic constituents that have the potential to control plant diseases [9] [14] [15]. Apart from this indication of antifungal activity here, in Nigeria, the decoction of root and stem of *Anacardium occidentale* has been used as anti -inflammatory agent and anti-diarrhoea [16]. Similarly, the antimicrobial activities of *Anacardium occidentale* extracts have been confirmed [17] [18]. Omojasola and Awe [19] observed the antimicrobial activity of the leaf of *Anacardium occidentale* and *Gossypium hirsutum* against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typimurium*, *Staphylococcus aureus and pseudomonas aeruginosa*. The phytochemical analysis of the two plants extracts indicated that alkaloids, saponins, tannins, flavonoids and anthraquinones were detected in various components of plant products. For instance, Lale [20] reported that the bioactivity of plant products was related to the chemical nature of their active constituents, such as alkaloids, saponins, tannins, flavonoids and glycoside. These finding confirms the work of Tewari & Singh [21] that the valuable medicinal properties of different plants are due to presence of several constituents *i.e.* saponines, tannins, alkaloids, alkenyl phenols, glycol alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters. The presence of high amount of tannins and moderate saponins in *Anacardium occidentale* agrees with the report of Okorie *et al.* [22]. The methanolic extracts of both plants show the presence of all the active ingredients. This might be due to the ability of the solvent to extract more of the active ingredients (bioactive compounds) from the plant materials. This is similar to the findings of Arekemase *et al.* [23] that the high potency of ethanol extracts might be connected with the extraction solvent and that ethanol has been shown to have a greater extractive power than water.

The presence of flavonoids in methanolic extract of *Anarcadium occidentale* agrees with the earlier work of [24] that the ethanolic extract of cashew nuts revealed the presence of phytochemical compounds such as triterpenoids, phenolic, flavonoids, xanthoproptein and carbohydrate. The extracts obtained from the flowers, leaves and stem bark of *Anacardium occidentale* are rich in bioactive secondary metabolities, exerting a potential antimicrobial effects against Gram-positive and Gram-negative bacteria as well as fungi [25]. Similarly, it was also observed that *A. occidentale* has important biotechnological potential as a source of compounds with broad-spectrum antimicrobial activity and of antioxidant compounds to be used in the drug, food and cosmetic industry [25]. The chemical analysis of *Prosopis africana* is confirmed to contain alkaloids, be-

ta-phenethylamine (https://www.ncbi.nlm.nih.gov/pubmed/10904169) and tryptamine [26]. It was confirmed that phytochemical screening of *Prosopis africana* revealed the presence of tannins, saponins, anthraquinones, cardiac-glycosides, carbohydrate and steroids, these extracts showed antihelmminthic activity against the disease schistosomiasis [27]. Similarly, Badamasi [28] confirmed that phytochemical screening of the stem bark extract of *Prosopis africana* revealed the presence of alkaloids, tannins, glycosides,cardiac glycosides, saponin, volatile oil and steroids had anti-malaria potentials. Arshad & Rehman, [29] reported that a significant reduction in fungal biomass was recorded due to different concentrations of the leaf extracts of *E. citrodora* and that organic solvent extracts of allelopathatic especially chloroform contain antifungal constituents and can effectively be used for the management of *M. phaseolina*. Similarly, Jabeen [30] observed the fungal activity of alcoholic and chloroform extracts of leaves of *E. citriodora* against *A. rabiei*. Prince and prabakaran [31], demonstrated that *Vitex negundo* showed maximum antifungal activity against the pathogenic fungus *Colletotrichum falcatum*.

According to Shadab [32] *Cymbopogon citratus* (Lemon grass) oil is used as a pesticide (http://www.pjsir.org/documents/journals/01042011130700_PJSIR-VOL.35-(6)-1992-Abstract.pdf) and as preservative. Kareru [33] reported that *Thevetia peruviana* contains a milky sap containing a compound called thevetin (http://www.academicjournals.org/article/article1380870360_Kareru%20et%20al.pdf) in its natural form is extremely poisonous, as well as all parts of the plants, especially the seeds. Similarly, Ogunbosoye & Babayemi [34] confirmed that the leaves of ***Newbouldia leavis*** have antibiotic, bacteristatic and fungistatic properties. Arshad & Rehmen, [29] observed that the leaf extracts of allelopathatic trees especially ethyl acetate and chloroform extracts of *A. indica* contain natural fungicides which can be used for the management of *M. phaseolina*. Tannins isolated from medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance in future [35].

Further studies are, however, necessary to determine the minimum concentration of the extracts required for maximum disease control as well as the frequency and mode of application of the different plant extracts. Investigations into the active ingredients of the extracts and the mode of action are also necessary.

6. Conclusion

The effect of plant extracts on mycelia growth of the test organism shows that both *A. africana* and *A. anacardium* reduced the mycelia growth drastically as compared to the control (plate, 2, 3, 4). The phytochemical analysis of the plant extracts shows alkaloids, saponins products, tannins, flavonoids and anthraquinones. The activities of these antioxidants in the plant extracts probably contri-

bute to the effectiveness of these extracts and could be possible to be exploited for effective management of root rot diseases of Beniseed (*Sesamum indicum*) caused by *Macrophomina phaseolina.*

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