

ANTIFUNGAL EFFECT AND PHYTOCHEMICAL ANALYSIS OF *SOLANUM MACROCARPON* LIN. LEAF EXTRACT IN SOUTHEAST NIGERIA

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ABSTRACT

The study investigated the antifungal activities and phytochemical composition of *Solanum macrocarpon* Lin. Leaf extract. The research was carried out at the Microbiology Laboratory, Department of Microbiology, Faculty of Biological Sciences, Imo State University, Owerri, Nigeria. The phytochemical revealed that alkaloid, phenol, saponin, flavonoid and tannin were present while steroid and terpenoid were absent. Quantitatively, flavonoid, constitutes the highest substance at 9.44% while phenol was lowest with 0.145. Antifungal investigation reveals that aqueous and ethanol extract of *Solanum macrocarpon* Lin was able to exhibit strong inhibitory activity against fungal isolates. (*Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum* and *Botrodipodia theobromae* tested). Ethanol extract showed higher inhibitory effect than aqueous extract. In aqueous extract the inhibitory effect is independent of the extract concentration. For 0.5ml aqueous, *Fusarium oxysporum* had 2.3cm mycelium growth while it

was 2.93cm in 1ml. *Botrodipodia theobromae* had 3.95cm mycelium growth in 0.5ml but 2.75cm in 1ml. The ethanol extract inhibition increases with increase in concentration. In *Geotrichum candidum* at 0.5ml, mycelium growth was 1.60cm but 1.02cm in 1ml. The antifungal activity of these extract is possibly linked to the presence of flavonoids, saponins and tannins. Further analysis should be carried out to determine the precise active bioactive compounds from *Solanum macrocarpon* L. that could inhibit fungi growth.

Key words: Antifungal, Effect, Phytochemical analysis, *Solanum macrocarpon*, Leaf extract

INTRODUCTION

Solanum macrocarpon Lin. (Eggplant) are said to possess ayurvedic medicinal properties and also called the father of modern medicine (Sekara *et al.*, 2007). The fruit is used to cure diabetes, bronchitis, asthma. Dysuria, dysentery, high blood pressure, osteoporosis, arthritis, Alzheimer's, some types of cancer (skin, colon, stomach), heart disease and stroke (Matsubara *et al.*, 2005). Additionally, the mature fruits are used to cure stomach pains, poultice for abscesses and cracked nipples (Murray, 2004 and Small, 2009). The fruit stalk is also used to cure fistula and piles whilst the fruit juice (sometimes with pounded leaves) is used for the treatment of syphilitic eruptions of the hands (Murray, 2004). The leaves and roots are juiced and boiled for tonic and then used to cure skin diseases, rheumatism, inflammation, intestinal hemorrhages, foot pain, cough, obits, anorexia, tooth ache, burns, general stimulant, piles, throat and stomach troubles. *Solanum macrocarpon* Lin. especially cherry eggplant, turkey berry, pea eggplant and Thai eggplant are recommended for people with low iron content in their body especially anemic patients as well as expectant and nursing mothers. It is also needed for all human development as natural food supplements (Asiedu-Addo, 2014 and Putra, 2011.). As fresh *Solanum macrocarpon* Lin. are used to kill germs, control diabetes, strengthen bones, cure paralysis,

prevent gas trouble and prevent teeth related diseases, dry eggplants are used to cure stomach bloating, treat piles, relieve stomach gas and gout related problems (Cassidy *et al.*, 2013 and Krisban, 2013).

Still on the usefulness of the roots, they are boiled, blended with sour milk and grain porridge for syphilis treatment. Roots are decocted and used to cure asthma, skin diseases, and hemorrhages and as a general stimulant (Mak, 2013 and Murray, 2004). More so, the skin of *Solanum macrocarpon* Lin. called nasunin is used to protect the brain cell from damage. An electrolyte leakage from the pulp of *Solanum macrocarpon* Lin. is used to measure the membrane damage of a person (Concellòn *et al.*, 2005).

The fruits are used for the control of series of microbial activities. It has been discovered by (Akobundu and Agyakwa, 1998) that, in India, the leaves which contain glycol alkaloids solasodine is used to manufacture steroidal sex hormones for oral contraceptives. Still in India, then in Central America and Gabon, the leaves are applied on cuts and wounds. As *Solanum macrocarpon* Lin. fruit is used for cough medicine for children in Sierra Leone, the people of Senegal use it to treat sore throat and stomach ache (Sharma *et al.*, 2013). In most villages around the globe, as the plant of *Solanum macrocarpon* Lin. (leaves and stalk) are dried for fuel (Bhasker and Ramesh, 2015), the shoots are also dried and used as fuel. The leaves, stalk and shoots are sundried so that they become free from moist. These are easily lighted and used as fuel for the preparation of food, medicinal concoctions and for incense (Bhasker and Ramesh, 2015).

Generally, spoilage fungi are considered toxigenic or pathogenic; fungi could cause infections or allergies. *Aspergillus* species are known to produce toxic metabolites that are hazardous to human and animal health. In order to reduce the high cost of agriculture produces and the risk of getting fungi infection, fungicides have been used to control fungi infection. These chemical fungicides has proven to be harmful to man and the environment, hence need for alternative approach.

The main objective of the research was to evaluate the effectiveness of *Solanum macrocarpon* L. leaf extract in controlling fungal infested crop for

sustainable food security. Specific Objectives are: (a) to investigate the various phytochemical present in *Solanum macrocarpon* L. leaf extract. (b) to ascertain the concentrations of aqueous and ethanol extracts that inhibits mycelium growth of certain fungi.

MATERIALS AND METHODS

The research was carried out at the Microbiology Laboratory, Department of Microbiology, Faculty of Biological Sciences, Imo State University, Owerri, Nigeria. Imo State University is sited at latitude 5°10' N and 6° 0' N and Longitude 4° 3.5' E and 7° 0' N in Nigeria.

Source of Materials

The plant sample *Solanum macrocarpon* L. was collected from Imo Agricultural Development Centre (ADP) Owerri, Imo State and will be authenticated by a taxonomist. Fungi organism used was isolated from rotten yam that was obtained from Ekeonuwa market, Owerri, Imo State.

Medium Preparation

A semi synthetic potato dextrose agar (PDA) medium was used for the fungi isolate. The PDA was prepared from commercially dehydrated medium following the manufacturer's instruction. The method to be used was as described by Oviasogie *et al.*, (2015) with slight modification. Twenty (20) grams of powdered (PDA) was weighed and dissolved in 500ml of distilled water in a sterile conical flask and covered. The mixture was autoclaved for 15 minutes at a temperature of 121°C and 1.055 kg/cm² pressure and allowed to cool. The warm medium was dispensed into sterile petri discs aseptically and allowed to solidify at room temperature. Two drops of lactic acid was added to exclude bacterial growth (Okigbo and Odurukwe, 2009). Awurum and Nwaneri (2011) two concentrations of both aqueous and ethanol extracts (0.5ml, and 1ml) was prepared from the extracts for antifungal test. The effect of the plant extract on mycelium growth of the

isolates was determined using the food poisoning method technique (Sungoyomi *et al.*, 2009).

Phytochemical Test

Qualitative secondary phytochemical analysis was carried out, such as, test for Alkaloids using Wagner's test, Flavonoids using Shinoda test, Phenols using Ferric chloride test, Saponins using Foam test, Steroids using Liebermann Burchard test, Terpenoids using Salkowski test, Tannins using Ferric chloride test and Cardiac glycosides using Keller-Killani test.

Quantitative Secondary Phytochemical Estimation was also carried out to obtain, Total Alkaloid Estimation by using Harborn(1973) Method, Total Flavonoids Estimation by Using Boham and Kocipai (1994) Method and Total Saponin Estimation By Using Nahapetian and Bassiri (1975) Method.

Isolation and Identification of Fungi Isolates

The isolation adopted from Ugwuja and Chiejina (2015) was used with modification. The section was cut from periphery of disease yam and surface was rinsed in three changes distilled water and plated in potato Dextrose Agar (PDA) plates. The plates was incubated at room temperature ($28 \pm 2^{\circ}\text{C}$) for 3 -4 days. Subculture of colony growth was made to obtain a clean culture. Identification was based on observed culture wraith pattern, mycelium colour and microscopic examinations of vegetative and reproductive structures of the fungi isolates using Barnett and Hunter (1999) and Alexopoulos *et al.*, (2002).

Extraction of Plant Samples

Fresh and Healthy leaves of *S. macrocarpon* was obtained , washed and allowed to dry in room temperature ($28 \pm 2^{\circ}\text{C}$) for two weeks. The dried leaves was grounded using milling machine. The grounded sample was sieved through a 1mm muslin cloth to obtain powdered processed samples used for the extraction. Aqueous and ethanol extracts was obtained using cold solvent extraction method as described by Awurum and Nwaneri (2011). Two concentration of both aqueous and ethanol extract (0.5ml, and

1ml) was prepared from the extract for antifungal test. The effect of the plant extract on mycelium growth of the isolates was determined using the food poisoning method techniques (Sungoyomi *et al.*, 2009).

Determination of Total Phenol by Folin-Reagent Method

Total phenol content was determined by folin-ciocalten reagent method with modification. From each crude extracts (1ml) was dissolved with methanol (1ml). A total of 10% folin-ciocalten reagent was prepared by adding Folin-ciocalten reagent (10ml) in water (90ml). Then, 5% Na₂CO₃ was prepared by dissolving Na₂CO₃(3g) in water (50ml). Each crude sample (200ml) was taken in a test tube and added 10% Folin-Ciocalten reagent (15ml). Then all the test tube was kept in a dark place for 5 min. Finally, 5% Na₂CO₃(1.5ml) was added to the solution and mixed well by hand constant wavelength 750nm.

Determination of Tannin

1 gram of sample was dissolved in 10ml distilled water and agitated, left to stand for 30mins at room temperature. Each sample was centrifuged and the extract recovered 2.5ml of the supernatant was dispensed into 50ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50ml flask; a 1.0ml folin-dennis reagent was measured in each flask followed by 2.5ml of saturated Na₂CO₃ solution. The mixture was diluted to 50ml in the flask and incubated for 90mins at room temperature. The absorbance of the sample was measured at 700nm with the reagent blank at zero. The % tannin was calculated.

Statistical Analysis

Standard errors of the mean values were calculated for the separate readings and data was subjected to analysis of variance (ANOVA) to compare the means at 0.05 confident intervals (Obi, 2002).

RESULT

The result of antifungal and phytochemical analysis of *Solanum macrocarpon* leaves were studied and summarized in fig.1 to Fig. 3 and table 1 to 2. The fungi *Fusarium Oxysporum schlecht*, *Botryodiplodia theobromae_Pat* *Geotrichum candidum_* link and *Aspergillus niger. Van Tieghem* were isolated and used for antifungal test (Table 1) and Plates 1 to 6).

Table 1: Macro and Micro features of isolated fungi.

S/N	Macroscopy	Microscopy	Isolate
A	Colonies are whitish with purple tinge mycelium extensive and cotting in culture. Growth is fast	Micro-condia, ovoid to ellipsoidal in shape are borne on simple phialide on phialides on branched conidiopore. Septated fujsi from, slightly cured and pointed at both ends.	<i>Fusarium Oxysporum schlecht</i>
B	Colonies are white, moist yeast-like' submerged hyphacare seen later at the periphera. Aerial mycelium fast growing	Hyphac are hualine, septate, branched, conidia. One celled smooth, subglobose to cylindrical anthroconidia.	<i>Geotrichum candidum link</i>
C	Whitish when young, gradually turns black/gray back is black. Fast growing mycdelium	Mycelium septate chlamydospores are intercalary and terminal, conidia are two celled.	<i>Botryodiplodia theobromae pat</i>
D	Wool, at first. White, yellow, then turning black as it ages	Condiophore stripe are smooth. Walled; conidia heads are biseriate with the phialides borne or globose to subglobose black and rough walled.	<i>Aspergillus niger. Van Tieghem</i>



Plate 1: a =Macro and b= Micro features of *Fusarium oxysporium*

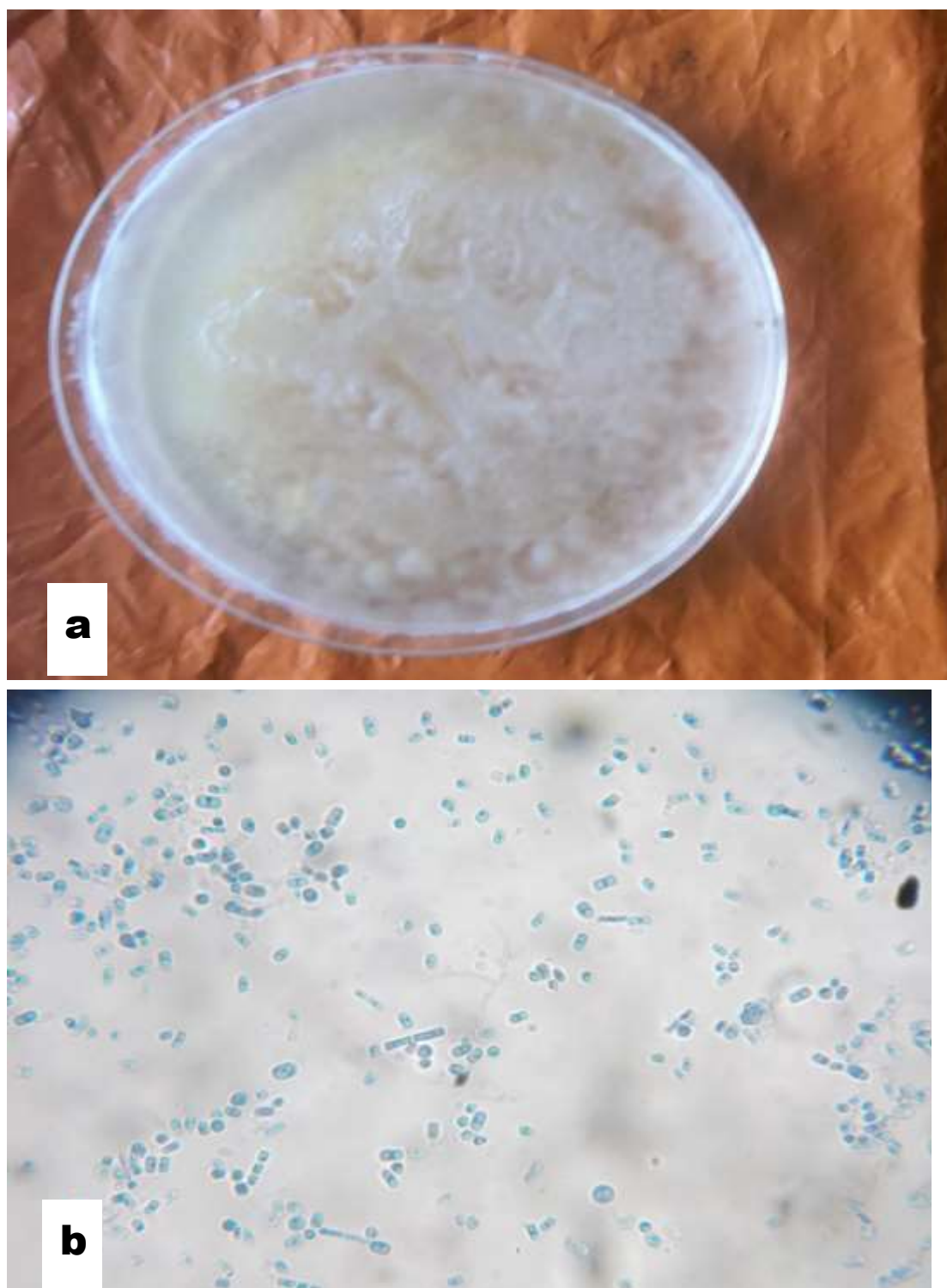


Plate 2: a =Macro and b= Micro features of *Geotrichum candidum*

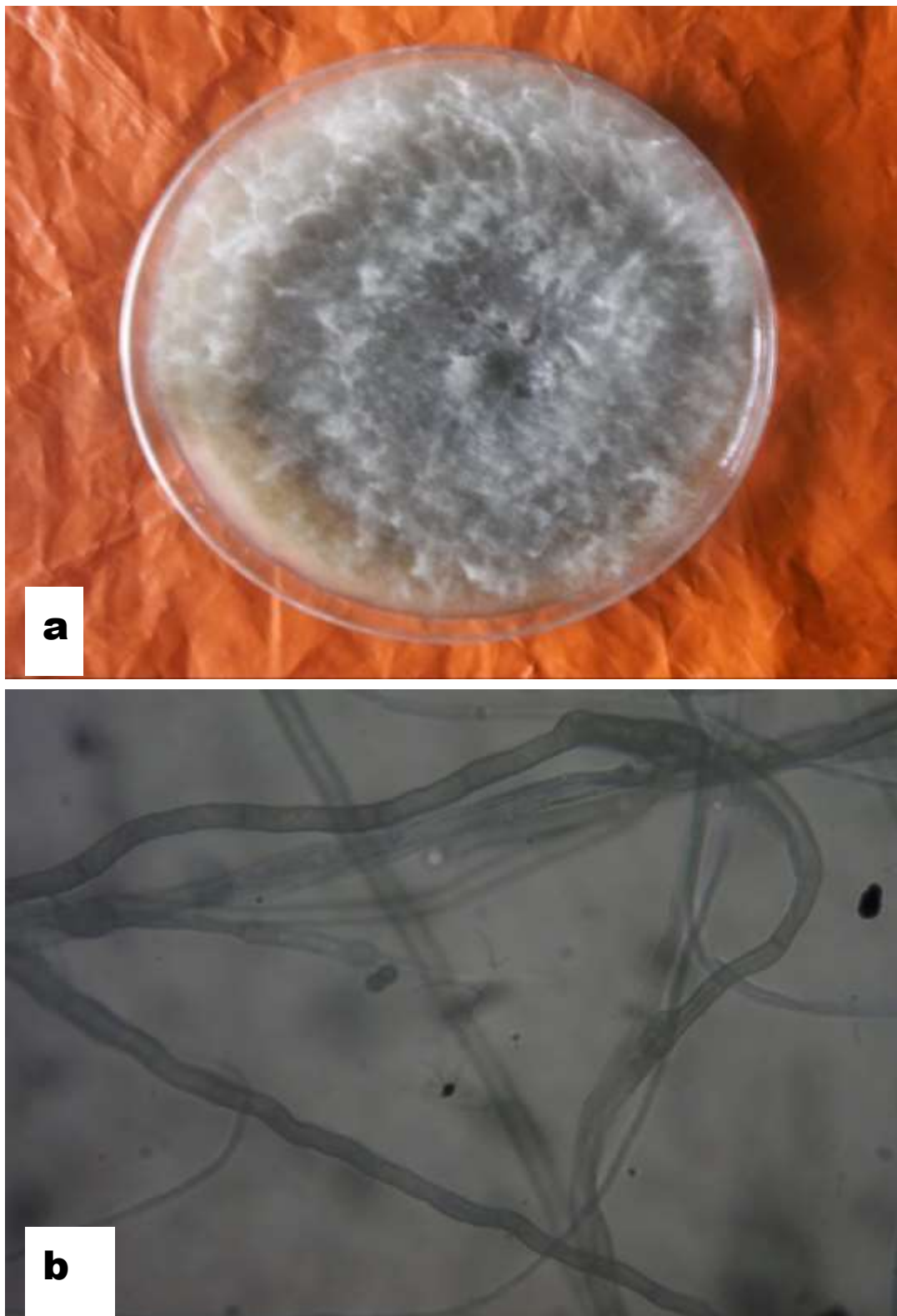


Plate 3: a =Macro and b= Micro features of *Botryodiplodia theobromae*

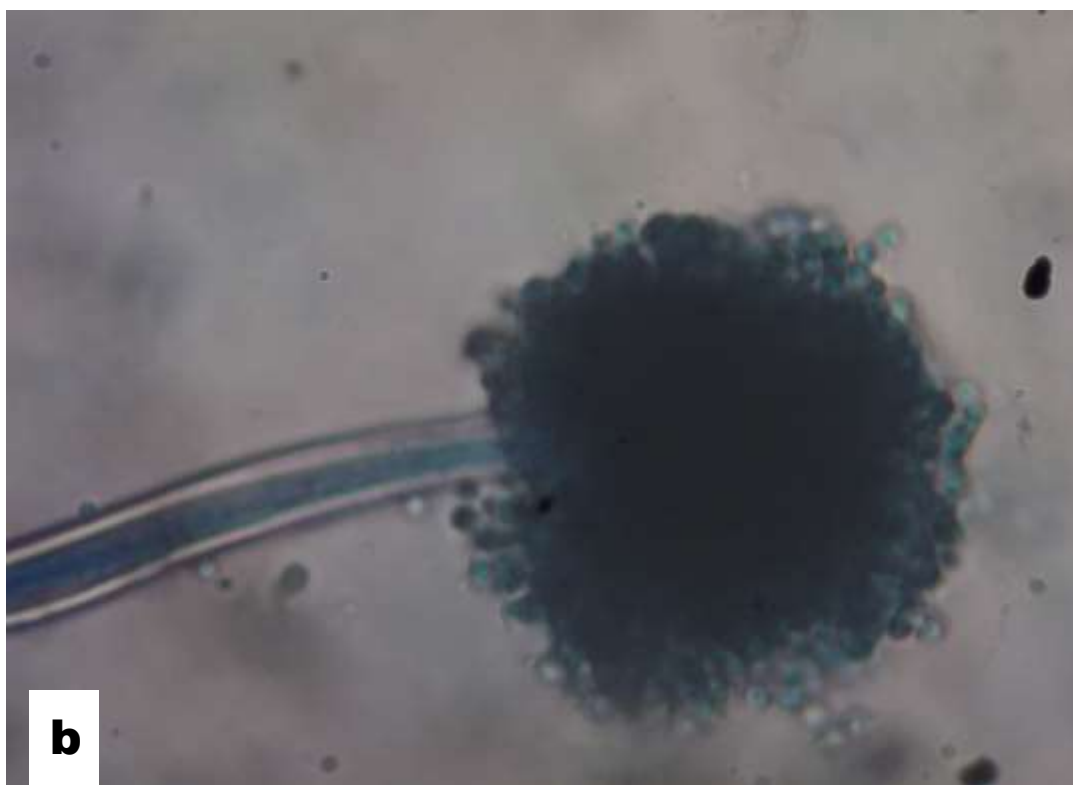


Plate 4: a =Macro and b= Micro features of *Aspergillus niger*



Plate 4: Antifungal effect of *Solanum africana* on *Fusarium oxysporium* and





Plate 6: Antifungal effect of *Solanum africana* on a = *Botryodiplodia theobromae* and b = *Aspergillus niger*

For aqueous extract, at 0.5ml, *F. Oxysporum* has a mycelium growth of 2.32cm, *G. Candidum* had 1.47cm, *B. theobromae* has 3.95cm while *A. niger* had 1.52cm. at 1 ml, mycelium growth was 2.75cm in *F. oxysporum*, 1.38cm in *A. niger* (Fig 1).

For ethanol extract, at 0.5ml, mycelium growth diameter was 1.62cm in *F. oxysporum*, 1.60cm in *G. candidum*, 2.93cm in *B. theobromae* while in *A. niger* 2.00cm; *G. Candidum* 1.02cm ; *B. theobromae* 2.93cm while 2.32 was recorded in *A. niger* (Fig 2).

For the control (0ml); *F. Oxysporum* had 4.30cm; for *G. Candidum* 5.68cm; for *B. theobromae* 7.72cm while for *A. niger* 3.67cm (Fig 1-2).

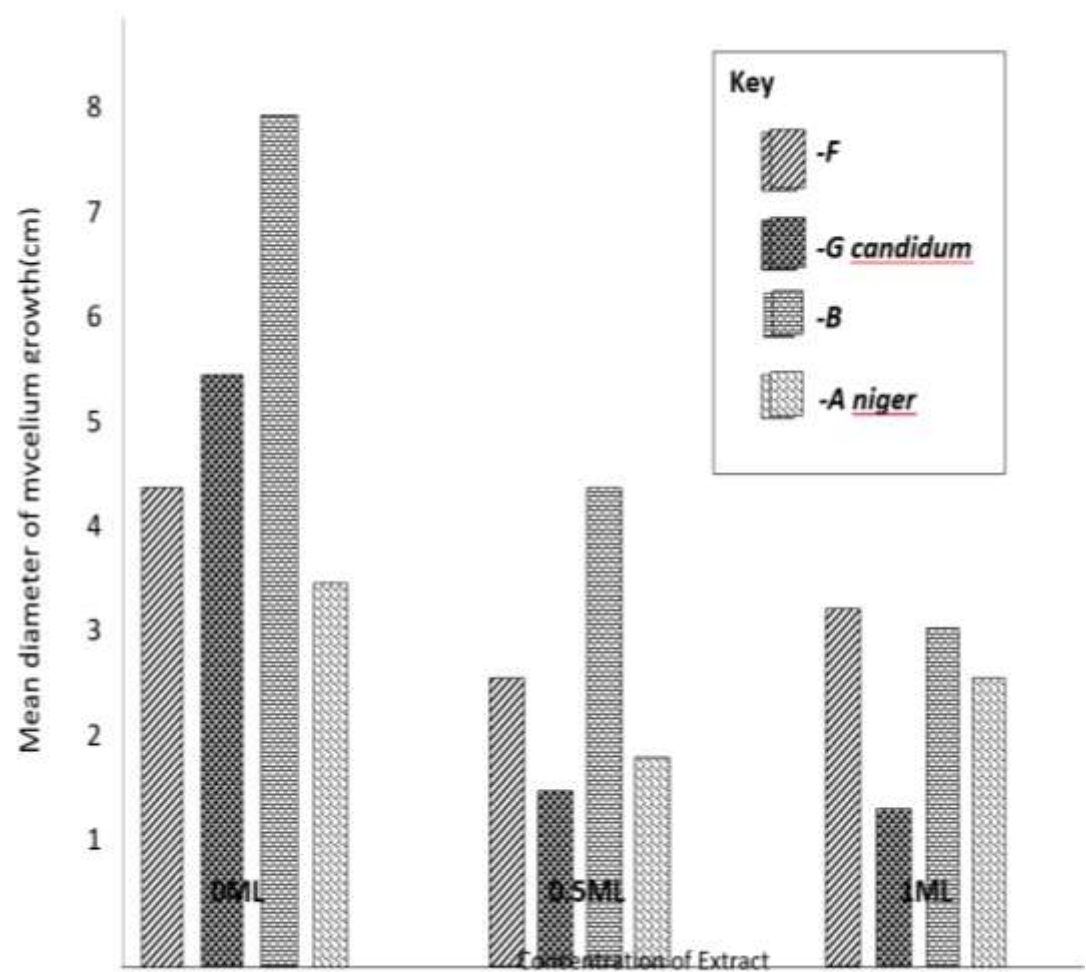


Fig. 1 Antifungal effect of aqueous leave extract of *S. macrocarpon* on fungi isolates

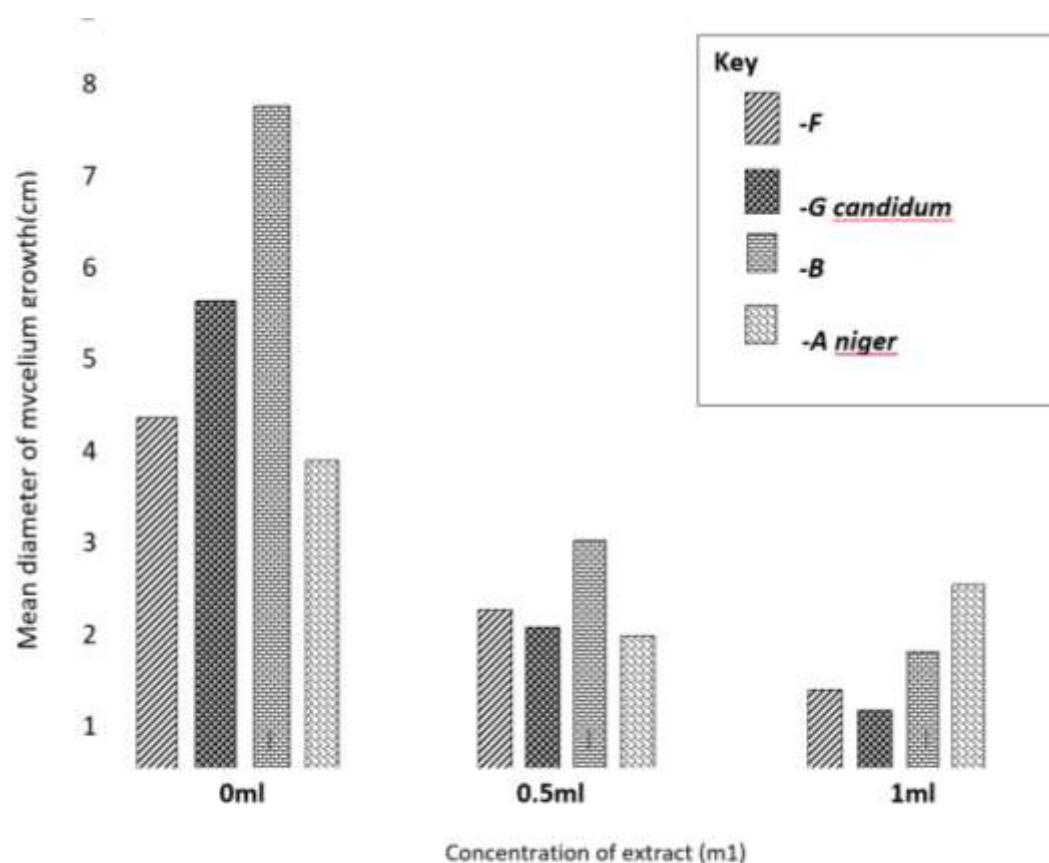


Fig. 2 Antifungal effect of ethanol extraction of *S. macrocarpon* L. leaf on fungi isolates

The results of phytochemical screening were shown in fig.3 qualitative determination of Photochemical indicated the presence Tannins, Flavonoids, Saponins, Alkaloid and Phenol(Table 2) while steroid and Terpenoid were absent (Table 2).

Table 2 Qualitative phytochemical screening of the leaf extract of *Solanum macrocarpon*

Constituent	<i>S. macrocarpon</i>
Alkaloids	+
Flavonnoids	+
Phenols	+
Saponins	+
Steroid	-
Tannin	+
Terpenoid	-

Quantitative estimation of the percentage composition of each of the phytochemicals detected showed that Flavonoid had the highest percentage composition of 9.44%; Alkaloid has 1.96% composition, Tannin had 1.43%, Saponin has 0.61%, while Phenol has 0.14% composition (Fig. 3).

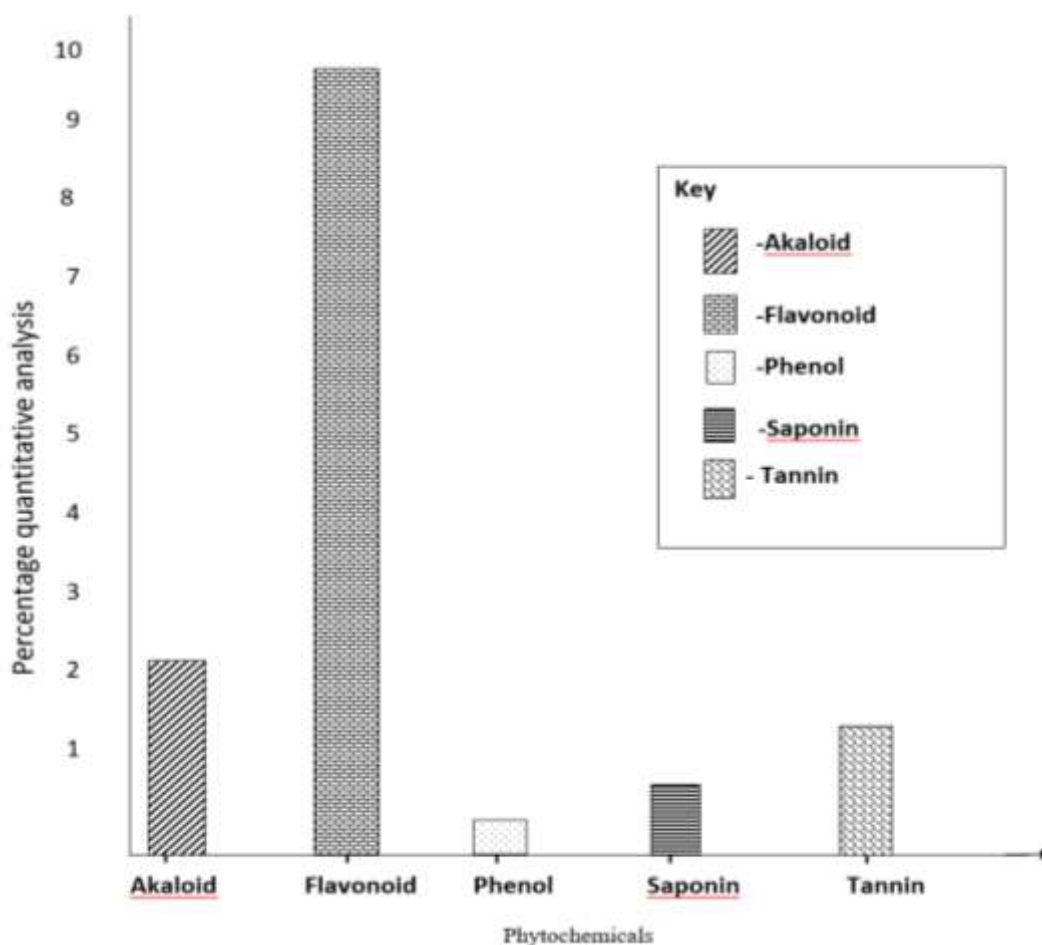


Fig. 3 Quantitative analysis of *S. macrocarpon L.* leaf phytochemicals

Table 3 antifungal effect of aqueous and ethanol extract of *Solanum macrocarpon L.* leaves on fungi

ORGANISM	AQUEOUS			ETHANOL		
	0ml	0.5ml	1ml	0ml	0.5ml	1ml
<i>F. Oxysporum(cm)</i>	4.30	2.32	2.93	4.30	1.62	1.22
<i>G. candidum(cm)</i>	5.68	1.47	1.38	5.68	1.60	1.02
<i>B. theobromae(cm)</i>	7.72	3.95	2.75	7.72	2.93	1.57
<i>A. niger (cm)</i>	3.67	1.52	2.13	3.67	2.00	2.32

DISCUSSION, CONCLUSION AND RECOMMENDATION

The fungal associated with post harvest spoilage of yam (*Discoriea alata*) were identified in this study as *Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum* and *Botrodipodia theobromae*. These organisms are commonly implicated in the post harvest deterioration of many food items. (Amadi *et al.*, 2012, Amadi, 2005, Oyetunji *et al.*, 2012, Oviasogie *et al.*, 2015).

Generally, spoilage fungi are considered toxigenic or pathogenic fungi could cause infections or allergies. *Aspergillus* species are known to produce toxic metabolites that are hazardous to human and animal health (Akinmusire 2011, Petzinger and Werdenbach 2002). In order to reduce the high cost of agriculture produces and the risk of getting fungi infection, fungicides have been used to control fungi infection. These chemical fungicides has proven to be harmful to man and the environment, hence need for alternative approach. (Yusuf *et al.*, 2011, Al-Rahmah *et al.*, 2013).

The use of botanicals as an alternative means of controlling fungi effect has been widely reported by researchers as been effective (Al-Rahmah *et al.*, 2008, Pareth *et al.*, 2006, Mohana *et al.*, 2008, Jalanda and Gachande 2012).

This study has revealed that all the plant extracts has significance effect in reducing the disease caused by the organisms isolated. The aqueous extract exhibited antifungal quality inhibited myceline growth that is not concentration dependent. At 0.5ml, it was observed that *F. oxysporum* growth was more inhibited than in 1ml. but other fungal isolates showed more inhibitory effectiveness with an increase in extract concentration. The use of aqueous extract of plant materials has received great attention as researchers have applied it mostly in their work.

Awurum and Nwaneri (2011) reported successful the growth of *Choanephora cucurbitarum*, the fungal responsible for wet rot in *Amaranthus hybridus* using aqueous extract of four plant species. Karthika *et al.*, (2016) reported the antimicrobial efficacy of aqueous extract of *Senna alata* in controlling bacteria and fungi. Furthermore, ethanol extract of *S. macrocarpon* leaves in this study showed that fungicidal activities of extract

could be based on the concentration. When compared, all the 1ml extracts assessed for antifungal activity exhibited more fungicidal effect to those of lesser concentration (0.5ml) in three fungi pathogens tested. The most effective was in *G. candidum*: where mycelium growth was reported as 1.60cm in 0.5ml and 1.02cm in 1ml. On the contrary 0.5ml showed more antifungal effect in *A. niger* than in 1ml. This indicates that the antifungal potency of ethanol extract could be either based on concentration or the fungi pathogen involved. The efficiency of ethanol extract fungal control has been reported by numerous authors. (Okigbo and Odurukwe, 2009, Ilondu 2013, War *et al.*, 2014, Al-Rahmah *et al.*, 2013, Anjum *et al.*, 2016, Al-Hazmi 2013).

The higher inhibitory effect of ethanol extract on *F. oxysporum* recorded in this study similar to the report of Al Hazmi (2013) on ethanol extract of neem seed on the same fungi species. The antifungal potency of ethanol extract have shown to be more effective in three of the isolates, *G. candidum*, *F. oxysporum* and *B. theobromae* while aqueous is more effective in *A. niger*. This indicates that, ethanol extraction has the ability to extract more phytochemical compounds in plants which has antimicrobial properties than aqueous. Similar report was made by Jalander and Gachade (2012), in this report; they stated that the inhibitory effect of the plant extract might be attributed due to the presence of antifungal compounds.

Also, Timothy *et al.*, (2012) linked antimicrobial properties of plants to the presence of bioactive secondary metabolites like saponins, flavonoids, glycosides, diterpenes etc. The phytochemical components of plant such as alkaloids, anthraquinones, saponins, tannins, terpenes, steroids, flavonoids, carbohydrates have been investigated for their therapeutic potency (Owoyale *et al.*, 2005).

The systematic phytochemical screen of leaf extract of *S. macrocarpon* was conducted to determine the presence of some compounds. The result showed the presence of tannins, flavonoids, saponins, alkaloids and phenol while steroid and terpenoid were absent. According to Harbone (1973) phytochemicals constitute such as alkaloids, flavonoids, tannins, phenols,

saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against invasion by any microorganism, insects, and other herbivores. Quantitatively, the phytochemical components recorded in this table varies from flavonoid (9.44%) which is the highest to alkaloid (1.96%), tannin (1.43%), saponin (0.61%) and the least was phenol (0.14%). This show that flavonoid occurred more in *S. macrocarpon* leaf than any other compound.

Flavonoids tend to be most commonly known with regards to antioxidant nature (Madhu *et al.*, 2016). Many according to Etam and Ebong (2009) many plants display their characters for anticancer, anti-inflammatory, anti-bacteria and anti-allergic nature and could be useful in therapeutic roles (Anjum 2016). Alkaloids tend to be organic and natural ingredients that have nitrogen, and are also physiologically active together with sedative and analgesic roles. Tannins was reported to possess physiological astringent properties, which hasten wound healing and ameliorate inflamed mucus membrane (Tyler *et al.*, 1988), the high level of antifungal activities of *S. macrocarpon* leaf could be attributed to the phytochemical components especially the quantity of flavonoids. The presence of metabolites such as phenols, tannins, saponins, alkaloids, flavonoids has been reported to exert antimicrobial activity through different mechanisms (Owoyale *et al.*, 2005) The phytochemical components of *Allium sativum* has been attributed to its antifungal properties (Onwuliri and Wonang 2005, Ayodele *et al.*, 2009). Owoyale *et al* (2005) made same comment in *Senna alata*. Also Simon and Ezejiofor (2014) made same observation.

Conclusion

The phytochemical analysis of *S. macrocarpon* L. revealed the presence important secondary metabolites such as alkaloids, tannins, saponins, flavonoids, and phenols. Thus indicating the therapeutic potentials of the plant. The higher level level of flavonoids in the extract makes it a good antifungal, antioxidant and anti-inflammatory plant source.

Antifungal efficacy of *S. macrocarpon* leave extract from this study has offered a valuable source for discovering an alternative to use of synthetic chemical fungicides.

Recommendation

A further study of the extract should be carried out to isolate, characterized and elucidate the structure of the bioactive compounds present which were responsible for the potent antifungal activity *S. macrocarpon* leaf.

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