Journal of Biopesticides and Environment
ISSN: 2449-1284 (Online); 2449-1292 (Print)
http://www.biopjournal.com

PHYTOCHEMICAL CONSTITUENTS AND *IN VITRO* INHIBITORY EFFECT OF TEN PLANT EXTRACTS ON FUNGI ASSOCIATED WITH *GMELINA ARBOREA* ROXB IN CROSS RIVER STATE, NIGERIA.

Umana, E. J[1]., *Akwaji, P. I.[1], Markson, A. A.[1], and Udo, S. E.[2]
1. Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.
2. Department of Biological Sciences, Cross River State University of Technology, Calabar, Cross River state, Nigeria.
Correspondence: akwajiisnever@yahoo.com

ABSTRACT

The phytochemical constituents and inhibitory properties of the ethanolic leaf extracts of *Carica papaya, Vernonia amygdalina, Chromolaena odorata, Ocimum viridis, Zingiber officinale, Azadirachta indica, Costus afar, Ocimum gratissimum, Magnifera indica and Alium cepa* on fungal pathogens isolated from *Gmelina arborea* (root, bark, leaf, fruit and soil) were investigated. The pathogens were *Aspergillus flavus, Aspergillus niger, Apodachlya pyrifera, Botryodiplodia theobromae, Bovularia sp, Ceratocystis fimbriata, Cercospora appi, Chalaropsis sp, Dacromyces deliquescens, Fusarium oxysporum, Geotrichum sp, Mucor mucedo, Penicillium vermiculatum, Penicillium thomii, Phorma herbarum, Rhizopus stolonifer, Thielaviopsis brasicola, Trichoderma viride, Trichosporonoide oedocephalus* and *Graphium penicilliodes*. 1ml of each concentration of the plant extract was added to 20ml of basal medium in 150ml Erlenmeyer flasks and each inoculated with 4mm mycelia disc using a cork borer from a 7 days old culture and incubated for 7 days. Each of the fungi had five replicates per treatment. The basal medium with distilled water served as the control. After the period of observation, the mycelia were filtered over a pre dried and weighed filter paper in a Millipore filter connected to suction pump. The filter papers with the content were dried to a constant using hot air oven for 24 hours and the dry weights of the mycelia obtained. Results showed that, the extracts had a significant ($P < 0.05$) effect on the mycelial dry weight of the fungal pathogens and the rate of antifungal activity differed from one extract to the other. The *in vitro* antifungal activity of these extracts on the mycelial dry weight of the fungi indicated that there can be used for the control of *G. arborea* diseases. Phytochemical screening of the plant extracts revealed strong presence of cardiac glycosides in *C. papaya, O. viridis, Z. officinale, O. gratissimum* and *M. indica* while *C. papaya, V. amygdalina, C. odorata* and *A. indica* showed strong presence of polyphenol. Phlobatannins were heavily present in *C. papaya* and *O. viridis* while saponins, tannins and flavonoids were lightly present in most of the plant extracts.

Key words: *Gmelina arborea*, plant extracts, fungi, inhibitory effect.

1. INTRODUCTION

Gmelina arborea Roxb (Family: Lamiacea) is reported to be a widely grown deciduous tree of moderate to large size with an arborescent habit hence, the specific name "*arborea*" (Cromer *et al.*,1993). It is a fast growing tree, which grows faster than some exotic species under the same conditions. It is medium-sized, reaching a height of about 30 – 40m, with a bole averaging 40cm in diameter but sometimes attaining 50cm. The leaves are more or less heart-shaped, 10 - 25cm × 5 - 18cm and globrous or velvety beneath, the corolla is bright yellow and the ovary glabrous (Anon, 2002). Drupes are ovate or pyriform, 2 - 2.5cm long, smooth, becoming orange-yellow,pulpy with large egg-shaped stone, having 1 - 4cells, seeds 1 - 4 (Duke, 2002). The drupes are reported to contain butyric acid, traces of tartaric acid resinous and saccharine matter. Resinous and saccharine matter and benzoic acid are also found in roots (Julian, 1982).

Gmelina arborea is native to tropical moist forest from India, Burma and Sri Lanka to Southern China. It is widely introduced in Brazil,

Gambia, Honduras, Ivory Coast, Malaysia, Malawi, Nigeria and Sierra Leone (Duke, 2002). Ademiluyi and Okeke (1973) described *G. arborea* as one of the widely grown plantation species in Nigeria. Best development of *G.arborea* in Nigeria occurs where air temperature ranges from 18˚C - 35˚C, with distinct dry season, and relative humidity above 40%. The occurrence of these climatic features in West Coast of Africa accounts for the success of *G. arborea* in Nigeria, Cote d'Ivoire, Sierra Leone and Ghana (Chijoke, 1986, FAO, 1989). *G. arborea* can be propagated by seeds or cuttings (wildings and root cuttings) (Enemuoh, 1970).

Gmelina arborea Roxb is an economic tree with vast uses as timber and is a major source of raw material for the construction, instrument and paper industry (Duke, 2002). However, in spite of its vast economic potentials, there have been reports of attack by various fungi on both the seedling and matured stages. In this study, a total of twenty fungi were isolated from different parts of *G. arborea* (root, bark, leaf, seed) and soil. Of the twenty fungal isolates, *Aspergillus flavus* was isolated from infected (root, bark, leaf and seed) of *G. arborea*, while *A. niger* was isolated from infected (root, bark, seed) and soil. *Apodachlya pyrifera* was isolated from infected (bark, leaf, seed and soil), *Botryodiplodia theobromae* (root, bark) and soil, *Bouvularia sp* (leaf and soil), *Ceratocystis fimbriata* (bark) and soil, *Cercospora appi* (root, leaf and seed), *Chalaropsis sp* (leaf, seed) and soil, *Dacromyces deliquescens* (root, bark) and soil, *Geotrichum sp* (root, leaf, seed) and soil, *Mucor mucedo* (root and seed), *Fusarium oxysporum* (root, bark) and soil, *Penicillium vermiculatum* (root and seed), *Penicillium thomii* (root, seed) and soil, *Phoma herbarum* (bark and leaf), *Rhizopus stolonifer* (root, leaf, seed) and soil, *Thielaviopsis brasicola* (root) and soil, *Trichoderma viride* (seed) and soil, *Trichosporonoide oedocephalos* (bark) and *Graphium penicilloides* (root, bark, seed) and soil. Inyang (1990) reported that soil borne fungi causes butt and root rot, stem and root diseases of *G. arborea*, such fungi clog the water-conducting tissues of the stem and uptake of nutrients by the roots from the soil causing about 50% seedling mortality rate if unchecked. According to Julian (1982) fungi associated with the leaves of seedlings and in some cases matured trees causes tissues to rot near the collar region affecting cellular activities of the plant. Duke (2002) reported that mould infection on leaves and stem causes foliage disease which affects the rate of photosynthesis. Those associated with the fruit cause deterioration of the fruit and seeds and as such affects germination of seeds (Anon, 2002). Nair and Sumardi (2000) reported that fungi infection on *G. arborea* bark or stem obstructs fiber arrangement which affects the wood during usage in construction of doors, ply woods etc, moisture content at the seedling and mature stages, stem height and girth, fresh and dry weight, wood texture, colour, smell, taste, lignin components, cellulosic materials, micro-fibrils and causes sharp differentiation between its hard wood and sap wood region.

Phytochemical screening of the ten plant extracts used in this study was carried to determine their exact phytochemical contents. Phytochemicals, as compounds which occur naturally in plants, form part of plants defense mechanisms against diseases (Eleazu *et al.*, 2012). They are classified into primary and secondary, based on their activity in plant metabolism. The primary ones comprise of sugars, amino acids, proteins and chlorophyll (Krishnaiah *et al.*, 2007), while secondary ones include the phenolic compounds such as tannins, flavonoids, alkaloids, saponins, anthraquinones, phlobatannins, proanthocyanidins, etc. (Eleazu *et al.*, 2013). These phenolic compounds have been reported to possess considerable antimicrobial properties, which is attributed to their redox properties (Molan and Faraj, 2010, Zongo *et al.*, 2011). Thus the antimicrobial properties of plants have been attributed to the presence of these secondary metabolites (Prakash and Hoschi, 2010).

Due to complaints received from the management and staff of the Cross River State Forestry Commission during a field survey and

the observed disease incidence/severity at the Awi, Oban and Ovonum *Gmelina* Forestry Project, and in view of the role played by this plant in the economy of Cross River State, Nigeria. It became necessary to isolate and identify the causative agents of the infections and to find out a better control measure that is environmentally friendly to control the infections. This work involved extensive investigation of the fungal pathogens that affect *G. arborea* in Cross River State, Nigeria and the possibility of using available plant extracts in their control. It is a follow up research on our earlier work titled *Gmelina arborea* Roxb: Associated mycoflora and diseases in Cross River State, Nigeria.

2. MATERIALS AND METHODS

Sources of materials

The diseased plant parts comprising of the fruits, roots, stems and leaves were collected from different and widely spread locations in Cross River State, Nigeria namely Awi and Oban *Gmelina* plantations in Akamkpa Local Government Area and Ovonum in Obubra Local Government Area. Wildlings and seeds for planting (used for pathogenicity test) were collected from *G. arborea* plantations in Awi, Akamkpa L.G.A. Soils were also collected from the rhizosphere of the plantation from the three locations. The soils were put in polyethylene bags. The leaves of *Azadirachta indica*, *Vernonia amygdalina*, *Carica papaya*, *Costus afar*, *Mangifera indica*, *Ocimum viridis*, *Zingiber officinalis*, *Ocimum gratissimum*, *Chromolaena odorata* and *Allium cepa* were obtained from the Botanic Garden of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria. The research work was carried out in the Green house and Laboratory of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.

2.1 Isolation and identification of fungi associated with *G. arborea*

Isolations were made from diseased plant parts (leaf, stem, fruit, and root) and rhizosphere (soil) using the method of Richter and Dallwitz (2000). Pieces of the diseased parts were cut with a sterile scalpel and placed separately. These were then later washed several times with distilled water and sterilized with 95% ethanol. Sterile inoculating needle was used to pick the parts and placed on Potato Dextrose Agar (PDA), then incubated for seven days at 28 ± 1˚C. These were sub-cultured until pure cultures were established for identification. Isolation from soils was carried out by dilution plate and soil washing method as described by Halverson *et al.,* (1993) and Tsao (1983). For soil dilution plate method, 20grams of soil from the different locations (*Gmelina arborea* plantations) were collected in polyethylene bags. Ten grams (10g) of each soil was suspended in 90ml of distilled water. Ten-fold dilution series was made and 1ml of each was incorporated into PDA in Petri dishes (9mm). The plates were incubated at room temperature 28 ± 1˚C for seven days and fungal counts made from 10^{-1} dilution plates and recorded in percentages. For soil washing method, ten grams (10g) of each soil sample were separately suspended in 90ml of sterile distilled water. The supernatant was in each case decanted and the settled particles washed into 250ml flasks with 50ml of sterile distilled water. The soil was shaken and allowed to settle at an angle of 45˚C for five minutes, and then the liquid was again decanted. The washing was repeated five times after which 1gram of the soils was each aseptically transferred into Petri dishes (9mm) containing 20ml of molten PDA and incubated at 28 ± 1˚C for seven days. The percentage occurrence of each fungus was recorded and the pure culture of each prepared by aseptically transferring the mycelia to newly prepared PDA and incubated at 28 ± 1˚C for seven days. Fungi identification was carried out by microscopic studies of the isolates. Identification of the isolates were based on morphological characteristics, described in 1998 Illustrated Genera of Fungi by Barnett and Hunter and with literature on the Identification of Pathogenic Fungi by Dugan (2006). Confirmation was made by comparing with cultures identified by International Mycological Institute, Egham, UK. Stock cultures of the isolates were prepared for further use.

2.2 Koch's postulates and pathogenicity test (Disease severity)

To confirm pathogenicity of fungal isolates obtained from (leaf, stem, root, bark and soil), a seven-day old culture of isolates filtrates from the different location were grown on basal medium supplemented with pectin as the only carbon source. This was done by pouring 100ml of inoculums at the base of the plants. The plants were earlier wounded with a sterile inoculating needle to facilitate entry of spores (Udo *et al.*, 2001). The base of the plant stands were then covered with polyethylene bags for one day to prevent moisture loss and entry of other pathogens. Pathogenicity tests were carried out at intervals and in sets when the plants were 4 months, 8 months and 12 months old and the symptoms observed if there were similar to those earlier observed in the field.

2.3 Preparation of plant extracts

The leaves of *Azadirachta indica*, *Vernonia amygdalina*, *Carica papaya*, *Costus afar*, *Mangifera indica*, *Ocimum viridis*, *Zingiber officinalis*, *Ocimum gratissimum*, *Chromolaena odorata* and *Allium cepa* were collected, washed initially with tap water, rinsed with distilled water, allowed to air dry then oven dried for 48 hours at 60˚C. One hundred (100g) grams each of the dried leaves were cut into tiny pieces macerated separately in a blender and put into sterilized black plastic containers to prevent light reducing the potency. The specimens were stored until required. For the control, 500ml of distilled water without the extracts was added to the basal medium in a flask. The contents of the flask were subjected to mild heat treatment in Gallenkamp water bath at 80˚C for 20 minutes allowed to cool and refrigerated at 4˚C. The ethanolic extracts of the leaves from the ten plants were obtained by Soxhlet extraction Hambone (1973). 20grams of the powdered products were extracted with 100ml of 95% ethyl alcohol (intermediate solar solvent by using a continuous extraction) apparatus (Soxhlet) for two or three times for 40 minutes. The extracts were then stored in a refrigerator in reagent bottles for use when needed.

2.4 Evaluation of the *in vitro* inhibitory effect of the different plant extracts on mycelia dry weight (g) of fungi associated with *G. arborea*.

This study was carried out using basal medium described by Punja and Jenkins (1984). 1ml of each concentration of the plant extract or distilled water was added to 20ml of the basal medium in 150ml Erlenmeyer flasks and each flask was inoculated with 4mm mycelia disc obtained by means of cork borer from 7 day old cultures. Each flask was then incubated at 26˚C ± 1˚C for 7 days. Each of the fungi had five replicates per treatment. The basal medium without the extracts was similarly inoculated. The flask with distilled water served as the control. After the period of observation, the mycelia were filtered over a pre dried and weighed filter paper in a Millipore filter connected to suction pump. The filter papers with the content were dried to a constant weighed at 85˚C at hot air oven overnight. The dry weights of the mycelia were then obtained from the difference of the two weights. The experiment was carried out in five replicates with the P^H of the medium adjusted to 6.5 before inoculation. The dry weights of the mycelia were calculated thus:

$W_m = W_3 - W_2$

W_1 = Weight of dry filter paper

W_2 = Weight of filter paper + agar

W_3 = Weight of dry mycelium + filter paper + agar

The percentage exhibitions of each extracts and fungi were calculated by comparing it with the control. This was achieved with the formula:

$$\text{Percentage exhibition} = \frac{C - X}{C} \times \frac{100}{1}$$

(Abdusalem *et al.*, 1990)

Where C = Growth in control

X = Growth in treated samples

Phytochemical screening of plant extracts

Phytochemical screening of the plant extracts was carried out at the Department of Biochemistry, University of Calabar, Calabar, Nigeria using standard methods by Sofowora

(1984), Culei (1982) and Trease and Evans (1973).

3. RESULTS

3.1 Fungal isolates of different parts of *G. arborea* including rhizosphere (soil).

Twenty pathogenic fungi were isolated from different parts of *G. arborea* and soil. Of the twenty fungal isolates, *Aspergillus flavus* was isolated from infected (root, bark, leaf and seed), while *A. niger* was isolated from infected (root, bark, seed) and soil. *Apodachlya pyrifera* was isolated from infected (bark, leaf, seed) and soil, *Botryodiplodia theobromae* (root, bark) and soil, *Bouvularia sp* (leaf) and soil, *Ceratocystis fimbriata* (bark) and soil, *Cercospora appi* (root, leaf and seed), *Chalaropsis sp* (leaf, seed) and soil, *Dacromyces deliquescens* (root, bark) and soil, *Geotrichum sp* (root, leaf, seed) and soil, *Mucor mucedo* (root and seed), *Fusarium oxysporum* (root, bark) and soil, *Penicillium vermiculatum* (root and seed), *Penicillium thomii* (root, seed) and soil, *Phoma herbarum* (bark and leaf), *Rhizopus stolonifer* (root, leaf, seed) and soil, *Thielaviopsis brasicola* (root) and soil, *Trichoderma viride* (seed) and soil, *Trichosporonoide oedocephalos* (bark) and *Graphium penicilloides* (root, bark, seed) and soil.

3.2 Koch's postulates and pathogenicity test (Disease severity)

Results showed that all the isolated fungi were pathogenic on *G. arborea*. Symptoms similar to those earlier identified in the field were observed. The affected root and stem showed a marked reduction in fresh and dry weight when compared with the control, the lengths and girth of the stem were shorter in the affected ones, and there were also dead spots on the stems. In the case of the leaf, the numbers of leaf spot depended on the fungus applied, and this varied significantly. The pathogenicity of the different fungi on the fruit showed significant differences based on the fungus applied and the rate of deterioration.

3.3 *In vitro* effect of ethanolic plant extracts on the mycelia dry weight (g) of fungal pathogens of *G. arborea*

Results showed that the ethanolic plant extracts had a significant effect ($P < 0.05$) on the mycelia dry weight of the fungal pathogens used. Results showed that, out of the thirteen fungi isolated from the root of the assay plant, three fungi, *Botryodiplodia theobromae*, *Fusarium oxysporum* and *Thielaviopsis brasicola* were found to be most pathogenic as shown in (Figure 1). When extracts of the ten plants were tested on these fungi, the mycelia dry weights of *F. oxysporum* (0.45g) and *B. theobromae* (0.73g) were most affected by extract of *Allium cepa*. While the ethanolic leaf extract of *Carica papaya* was found to be more potent on *B. theobromae* than any other fungi when compared with the control (4.08g). Ten fungi were isolated from the stem (bark) of *G. arborea*. Out of the ten fungi, the three most pathogenic ones were used for the assay. When the ten plant extracts were tested on *Apodachlya pyrifera*, *Dacromyces deliquescens* and *Ceratocystis fimbriata*. The mycelia dry weight of *Dacromyces deliquescens* was most affected (0.78g) by extract of *Carica papaya* when compared with the control (4.00g). Ethanolic extract of *Chromolaena odorata* was found to be more potent on *A. pyrifera* (0.92g) than any other fungus tested when compared with the control (4.00g) as shown in (Figure 2). Out of the eight fungi isolated from the leaf of *G. arborea*, the three most pathogenic ones were chosen for the bioassay. When aqueous extracts from ten medicinal plants were tested for their antifungal activity on these fungi, the mycelia dry weight of *Chalaropsis sp* was most affected (0.78g) by extract from *Vernonia amygdalina*. Extract of *Zingiber officinale* was found to be most potent on *Cercospora appi* (1.03g) while extract of *Ocimum gratissimum* was most effective on *Geotrichum sp* (1.11g) when compared with the control (4.26g) as shown in (Figure 3).

Thirteen fungi were isolated from the fruit of *G. arborea*. Seven of the fungi were found to be pathogenic but the three most pathogenic of the fungi were chosen for the test. When extracts from the ten plants were tested on these fungi for their antifungal efficacy, the mycelia dry

weight of *Trichoderma viride* was most affected (0.74g) by extract from *Azadirachta indica*. The extract of *Ocimum gratissimum* was found to be potent on *Bouvularia sp* (0.89g) while that of *Mangifera indica* was most effective on the mycelia dry weight of *Graphium penicilloides* (0.95g) when compared with the control (4.11g) as shown in (Figure 4).

3.4 Phytochemical screening of plant extracts

Results (Table 8) showed that tannins were absent in ethanolic leaf extracts of *Vernonia amygdalina*, *Ocimum viridis*, *Zingiber officinale*, *Allium* cepa and *Azadirachta indica*. Saponnins were absent only in *Azadirachta indica*. Flavonoids was absent in *Carica papaya* and *Chromolaena odorata*. Phlobatannins were absent in *Vernonia amygdalina*, *Chromolaena odorata*, *Zingiber officinale*, *Ocimum gratissimum* and *Mangifera indica*. Cardiac glycosides were heavily present in *Carica papaya*, *Ocimum viridis*, *Zingiber officinale* and *Ocimum gratissimum*, alkaloids were heavily present in only *Costus afar*. Reducing compounds were heavily present in *Zingiber officinale* and *Costus afar*. Phlobatannins were higher only in *Carica papaya* and *Ocimum viridis*. Hydroxymethyl anthraquinones was present only in *Zingiber officinale*, *Allium cepa* and *Ocimum gratissimum*. Anthraquinones were present only in *Carica papaya*, *Zingiber officinale*, *Azadirachta indica* and *Costus afar*.

4. DISCUSSION

In this study, twenty pathogenic fungi were isolated from roots, bark, leaf, fruit and rhizosphere (soil) as the causative agents of various fungal diseases of *G. arborea* in the field. The fungal isolates were: *Aspergillus flavus, Aspergillus niger, Apodachlya pyrifera, Botroyodiplodia theobromae, Bouvularia sp, Ceratocystis fimbriata, Cercospora appi, Chalaropsis sp, Dacromyces deliquescens, Fusarium oxysporum, Geotrichum sp, Mucor mucedo, Penicillium vermiculatum, Penicillium thomii, Phoma herbarum, Rhizopus stolonifer, Thielaviopsis brasicola, Trichoderma viride, Trichosporonoide oedocephalus* and *Graphium penicilliodes*. Similar reports have been given by Tsao (1983), Inyang (1990) and Duke (2000). Tsao (1983) observed that some of these isolated fungi were isolated from the root of rubber plant (*Hevea brasiliensis*) and were pathogenic to the plant. Duke (2002) reported on the pathogenicity of most of the isolated fungi on different parts of *G. arborea* and concluded that the isolated pathogens were responsible for the various diseases of *G. arborea* in the wild or in organized forestry plantations.

Phytochemical screening of the ethanolic plant extracts revealed strong presence of cardiac glycosides in *C. papaya, O. viridis, Z. officinale, O. gratissimum* and *M. indica* while *C. papaya, V. amygdalina, C. odorata* and *A. indica* showed strong presence of polyphenol. Phlobatannins were heavily present in *C. papaya* and *O. viridis* while saponins, tannins and flavonoids were lightly present in most of the plant extracts. These results are similar to those of Mbadianya *et al.,* (2013) and Umana *et al.,* (2014) who reported the presence of flavonoids, alkaloids, glycosides, polyphenols, cardiac glycosides and anthraquinones in ethanolic extracts of *Carica papaya*, *Azadirachta indica* and those of other medicinal plants.

In this study, the antifungal activity of ten plant extracts was tested *in vitro* on fungi isolated from various parts of *G. arborea*. Results showed that the ethanolic extracts of the leaves of ten plants investigated, exhibited various antifungal activities against the species of fungi isolated. The antifungal activity of ethanolic extracts of *Azadirachta indica*, *Vernonia amygdalina*, *Carica papaya*, *Costus afar*, *Mangifera indica*, *Ocimum viridis*, *Zingiber officinalis*, *Ocimum gratissimum*, *Chromolaena odorata* and *Allium cepa* on the isolated pathogens are shown in (Figures 1- 4). The results showed that, the extracts had a significant ($P < 0.05$) effect on the mycelia dry weight of the fungal pathogens and the rate of antifungal activity differed from one extract to the other as seen in the mycelia dry weight. The differences in the fungitoxic potentials between these plant extracts may be attributed to the susceptibility of each of the fungal pathogens to the different plant extracts. This agrees with the results of some workers like Amadioha, (2000) and Okigbo and Nmeka, (2005). Ilondu *et al.,* (2001) reported that some plants contain phenolic substances and essential oils, which are inhibitory to micro-organisms. The presence of these compounds in these extracts has been reported to be responsible for their antifungal properties (Ahmed and Stoll, 1996). It is noteworthy that of all the tested plant extracts,

aqueous extracts of *A. cepa*, *C. papaya*, *C. odorata*, *V. amygdalina*, *A. indica*, *M. indica* and O. *gratissimum* had a more significant effect than all the other tested extracts. The inhibitory potency of the plant extracts may be attributed to the phytochemical compounds like tannins, alkaloids, flavonoids and saponins in them as reported by Chiejina and Ukeh (2013). This is also in agreement with the works of Amadioha and Obi, (1999) and Udo *et al.*, (2001) who reported that the high potency of plant extracts containing the same bio-active compounds could be use for the control of fungal pathogens of plants. The antifungal effects of the ten plant extracts were evaluated in order to develop the cheaper methods of controlling the different fungal diseases associated with *G. arborea* in the field. The greater efficiency of *A. cepa*, *C. papaya*, *A. indica*, *M. indica* and *O. gratissimum* may be due to the high contents of alkaloids, cardiac glycosides, polyphenols and reducing compound they contain (Chiejina and Ukeh, 2013), since alkaloids, cardiac glycosides and reducing compounds are ranked as the most efficient therapeutically significant plant substances (Okigbo, 2009).

Conclusion

Twenty pathogenic fungi were isolated as the causative agent of various fungal diseases of major *Gmelina arborea* plantations in Cross River State, Nigeria. Results showed that, the extracts had a significant effect on the mycelia dry weight of the fungal pathogens and the rate of antifungal activity differed from one extract to the other. These extracts are therefore, highly recommended for use as spray in controlling the fungal diseases of *G. arborea* in the field.

REFERENCES

Abdusalam, K. S., Rezk, M. A., Abdel, M. I. and Muasa, A. E. (1990). Non-target activity of certain pesticides against soil borne fungi. Annals of Agricultural Science, Ain Sham University Cairo 35 (1): 459 – 467.

Ademiluyi, E. and Okeke, R. E. (1973). Studies on the specific gravity and fibre characteristics of *Gmelina arborea* in some Nigerian Plantations. Nigerian Journal of Sciences 13: 132 – 242.

Ahmed, S. and Stoll, G. (1996). Biopesticides. In: Biotechnology; Building on Farmers' Knowledge. Macmillan Education Ltd, Bunders, J., B. Haverkort and W. Hiemstra (Eds.). London, pp: 52-79.

Amadioha, A. C. (2000). Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Archives of Phytopatholpflanzo 34: 1-9.

Amadioha, A. C. and Obi, V. I. (1999). Control of Anthracnose diseases of Cowpea by *Cymbopogon cunitus* and *Ocimum gratissimum*. Acto Phytopathology and Entomology 85: 89-94.

Anonymous. (2002). Gmelina: *Gmelina arborea* Roxb. ASEAN Regional Centre for Biodiversity Conservation. ASEAN Environmental Education Plan 2000 – 2005.

Chiejina, N. V. and Ukeh, J. A. (2013). Efficacy of *Afromomum melegueta* and *Zingiber officinale* extracts on fungal pathogens of tomato fruit. IOSR Journal of Pharmacy and Biological Sciences volume 4, issue 6, 13-16.

Chijoke, E. O. (1986). Soil factors and growth of *Gmelina arborea* in Omo Forest Reserve. Forest Ecology and Management 23: 245 – 251.

Cromer, R. N., Kriedemann, P. E., Sands, P. I. and Stewart, L. G. (1993). Leaf growth and photosynthetic response to nitrogen and phosphorus in seedling trees of *Gmelina arborea*. Plant Physiology, 20: 83 – 98.

Culei, I. (1982). Methodology for analysis of vegetable drugs: Practical Manuals on Industrial Utilization of Medicinal Plants. UNIDO, Bucharest.

Duke, J. A. (2002). *Gmelina arborea* Roxb. Purdue University, Centre for New Crops and Plant Products. http:/www.hot.purdue.edu/newcrop/Dukeenergy/*Gmelina arbrea*.html. Retrival Date: 3/10/2002.

Eleazu, C. O., Eleazu, K. C., Awa, E. and Chukwuma, S. C. (2012) Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. Journal of Biotechnology and Pharmaceutical Research 3: 42-46.

Eleazu, C. O., Iroaganachi, M. A. and Okoronkwo, J. O. (2013) Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: 'The effect of varying colours'. International Journal of Biomedical Research 4: 1.

Enemuoh, P. O. (1970). Development of Forest Resources in the Economic Advancement of Nigeria. In Proceeding of the Inaugural Conference of Forestry Association of Nigeria. October 22 – 28.

FAO (1989). Le *Gmelina arborea*. Bureau regionalde la FAO pours l'Afrique, Accra, Ghana.

Halverson, I. J., Clayton, M. K. and Handelsman, J. O. (1993). Population biology of *Bacillus cereus* UW 85 in the rhizosphere of field-grown soy beans. Soil Biological Biochemistry 25 (4): 485 – 493.

Ilondu, E. M., Ejechi, B. O. and Souzey, J. A. (2001). Microbial stability of jam prepared from velvet tamarind and preserved by combined processes. Nigerian Journal of Microbiology 5: 93-96.

Inyang, L. E. D. (1990). Fungal butt and root diseases: threats to bioconservation of Nigeria forest. Nigerian Journal of Science Bioconservation 1: 98 – 115.

Julian, E. (1982). Plantation Forestry in the tropics. New York, Oxford University Press. P. 50.

Krishnaiah, D. R. and Sarbatly, B. A. (2007). Phytochemical antioxidants for health and medicine-A move toward nature. *Biotechnology and Molecular Biology Review* 1: 97-104.

Mbadianya, J. I., Echezona, B. C., Ugwuoke, K. I. and Wokocha, R. C. (2013). Phytochemical constituents of some medicinal plants. *International Journal of Scientific Research* (IJSR) 2, 4. ISSN: 2319-7064.

Molan, A. L., Faraj, A. M. (2010) The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (Nematoda: Trichostrongylidae). Folia *Parasitological* 57: 62-68.

Nair, K. S. S. and Sumardi, R. (2000). Insect pests and diseases of major plantation species. In Nair, KSS (ed.).Insect pests and diseases in Indonesia forests. CIFOR, Indonesia.Pp 15 – 37.

Okigbo, R. N. (2009). Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi state, Nigeria. American-Eurasian Journal of Sustainable Agriculture 3 (3): 407-409.

Okigbo, R. N. and Nmeka, I. A. (2005). Control of yam tuber with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. African *Journal of Biotechnology* 4: 804-807.

Onifade, A. K. (2000). Antifungal effect of *Azadirachta indica*. A. Juss extracts on *Colletotrichum lindemuthianum*. Global Journal of Pure and Applied Sciences, Volume 6, No.3: 425 – 428.

Prakash, G. and Hosetti, B. B. (2010). Investigation of antimicrobial properties of *Dioscorea pentaphylla* from mid-Western Ghats, India. Scientific World 8: 91- 96.

Punja, Z. K. and Jenkins, S. F. (1984). Influence of medium composition on mycelia growth and oxalic acid production in *Sclerotium rolfsii*. Mycologia, 7 (5): 950 – 957.

Richter, H. G. and Dallwitz, M. J. (2000). Commercial Timbers: Descriptions, illustrations, identification and information. Retrieval: English Version 4th May, 2002. http:/biodiversity.uno.edu/delta.

Sofowora, T. K. (1984). Medicinal plants and traditional medicine in Africa. New York, John Wiley and Sons Incoporation. Pp. 20 – 25.

Trease,G. E. and Evans, W. E. (1979). A Textbook of Pharmacognosy (11th ed). London, Bailliere Tindal. P. 55.

Tsao, P. H. (1983). Factors affecting isolation and quantification of Phytophthora fromsoil: Phytophthora, its biology, taxonomy and pathology (Eds Ewim, D. C., Barthnicki-gracia, S. and Tsao, P. H., St Paul American Phytopathological Society.

Udo, S. E., Madunagu, B. E. and Isemin, C. D. (2001). Inhibition of growth and sporulation of fungal pathogens in sweet potato and yam by garlic extracts. *Nigerian Journal of Botany*, 14: 35 – 39.

Umana, E. J., Akwaji, P. I., Markson, A. A., Udo, S. E. and Orok, E. E. (2014). Phytochemical composition, Antimicrobial effect of *Azadirachta indica* and *Carica papaya* extracts on fungi isolated from *Gmelina arborea* seedlings. International Journal of *Phytopathology* 03 (03): 109 – 115.

Zongo C, Savadogo K, Somda JM, Koudou J, Traore AS (2011) In vitro evaluation of the antimicrobial and antioxidant properties of extracts from whole plant of *Alternanthera pungens* H.B. & K. and leaves of *Combretum sericeum* G. Don. International Journal of *Phytomedicine* 3: 182-191.

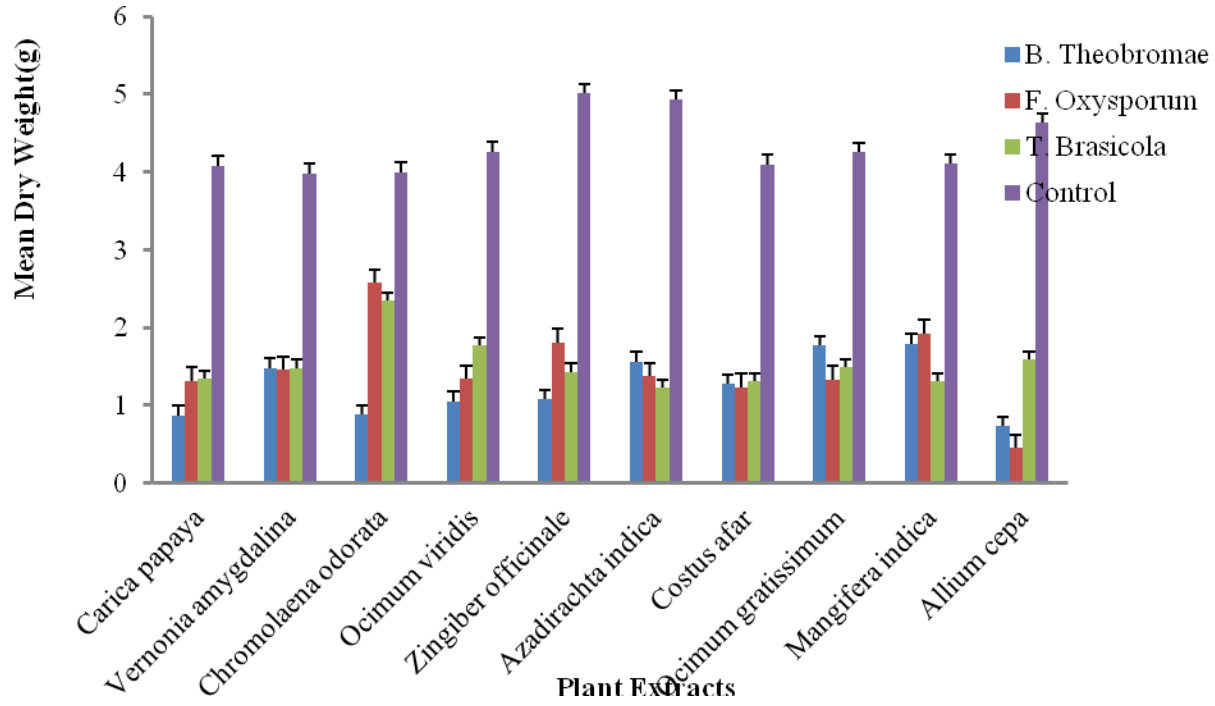

Figure 1: Effect of the different plant extracts on mycelia dry weight (g) of pathogenic fungi isolated from *G. arborea* root.

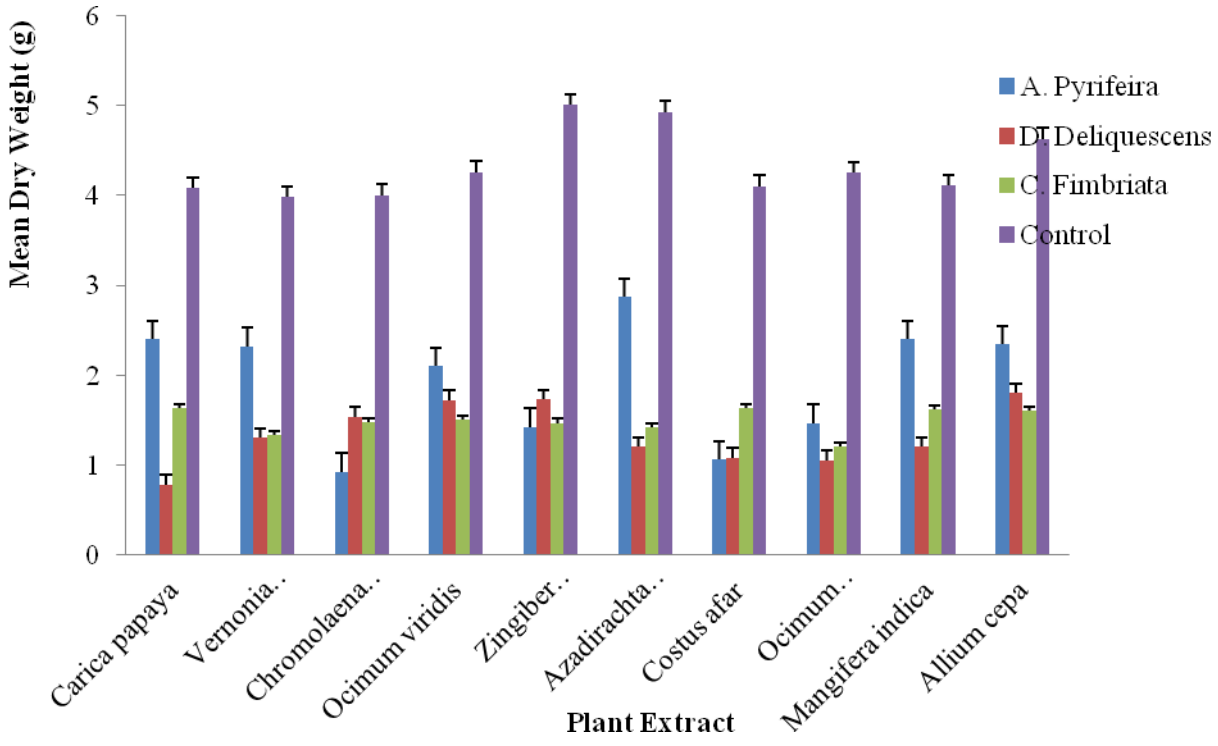

Figure 2: Effect of the different plant extracts on mycelia dry weight (g) of pathogenic fungi isolated from *G. arborea* stem

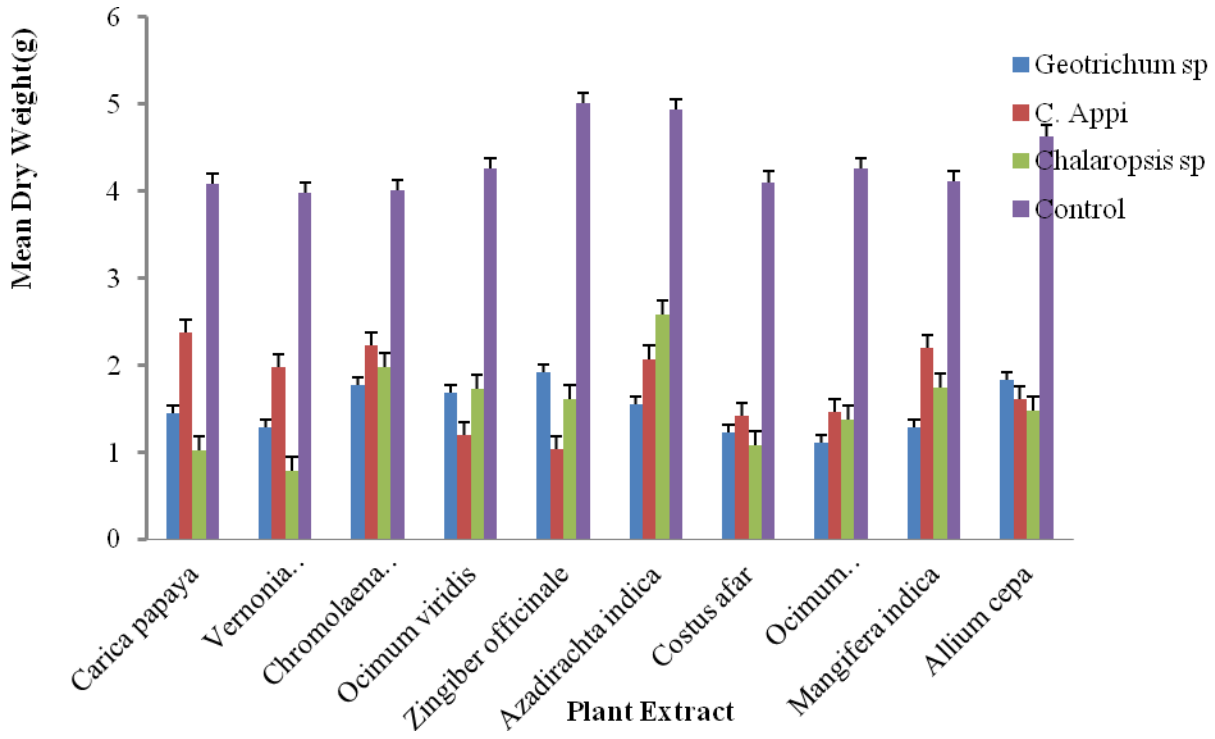

Figure 3: Effect of the different plant extracts on mycelia dry weight (g) of pathogenic fungi isolated from *G. arborea* leaf.

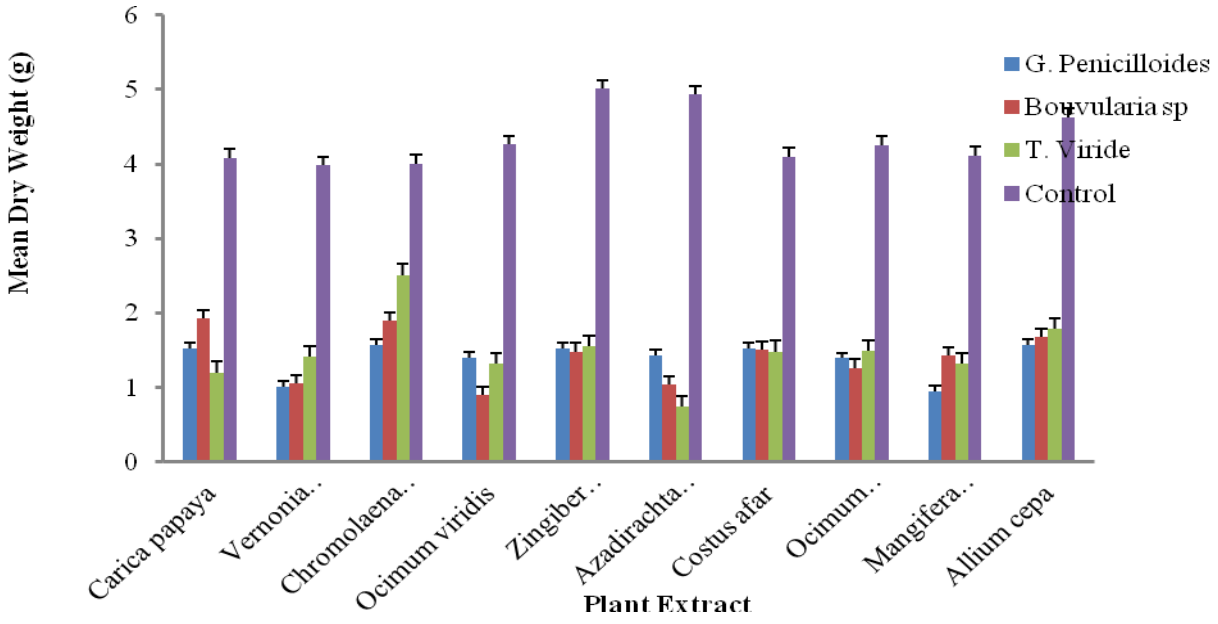

Figure 4: Effect of the different plant extracts on mycelia dry weight (g) of pathogenic fungi isolated from *G. arborea* fruit.

Table 8: Phytochemical screening of test plants

Phytochemicals	*C.p.*	*V.a.*	*C.a.*	*O.v.*	*Z.o.*	*A.i.*	*C. af*	*O.g.*	*M.i.*	*A.c.*
Alkaloids	+	+	+	+	+	+	++	+	+	+
Cardiac glycosides	++	+	+	++	++	+	+	++	+	++
Saponnins	+	+	+	+	+	-	+	+	+	+
Tannins	+	-	-	+	-	-	+	+	+	-
Flavonoids	-	+	+	-	+	+	+	+	+	+
Reducing compound	+	+	+	+	-	+	+	+	++	++
Polyphenol	++	++	++	+	+	++	+	+	+	+
Phlobatannins	++	-	-	++	+	-	+	+	+	+
Anthraquinones	+	-	-	-	+	+	+	-	-	-
Hydroxymethyl anthraquinones	+	-	-	-	-	-	-	+	-	+

Note: ++ (Heavily present or positive), + (Lightly present or positive) and – (Absent or negative).
C.p. – Carica papaya; *V.a.* - *Vernonia amygdalina*; *C.o.* - *Chromolaena odorata*; *O.v.* - *Ocimum viridis*; *Z.o.*- *Zingiber officinale*; *A.i.* - *Azadirachta indica*; *C.af.* - *Costus afar*; O.g. - *Ocimum gratissimum*; M.i. - *Mangifera indica*; *A.c.* - *Allium cepa.*

****