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Spatial Distribution of Phytochemical and Antifungal Evaluation of Six Medicinal Plants in South-West, Nigeria

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Abstract

The phytochemical and antifungal evaluations of the leaves of six plant species (*Chromolaena odorata, Euphorbia hirta, Ficus asperifolia*, *Momordica charantia*, *Nicotiana tabacum* and *Spondias mombin*) in Nigeria were studied. The leaves of these plants were collected from the wild, air-dried and pulverized into fine powder for the phytochemical constituents and minimum Inhibitory concentrations. The powdered leaves were screened and results were properly recorded as observed. Based on MIC, water extract of sample A *Euphorbia hirta* (6,620 mg/100 g, sample B *Spondias mombin* (6,51 mg/100 g), sample C *Nicotiana tabacum* (7,210 mg/100 g), sample D 97,010 mg/100 g), sample E *Chromolaena odorata* (5,420 mg/100 g), sample F (6,960 mg/100 g), sample G *Momordica charantia*, sample H *Ficus asperifolia* (65030 mg/100 g), sample I (7,480 mg/100 g). For acetone extract of sample, A (4,978 mg/100 g), Sample B (5,470 mg/100 g), sample C (4,450 mg/100 g,) sample D (4,560 mg/100 g), sample E (3,870 mg/100 g), sample F (4810 mg/100 g). sample G (4,560 mg/100 g, sample H (4,780 mg/100 g), sample I (4,700mg/100g), For hexane extract of sample A (3,640 mg/100 g), sample B (4125 mg/100 g), sample C (3,37 mg/100 g). sample D (3,335 mg/100 g), sample E (3,310 mg/100 g), sample F (3,648 mg/100 g), sample G (3,425 mg/100 g, sample H (3,940 mg/100 g), sample I (3,560 mg/100 g). For bacitracin extract of sample, A (3,927 mg/100 g), sample B (3, 926 mg/100 g), sample C (3,775 mg/100 g), sample D (3,877 mg/100 g), sample E (3,7825 mg/100 g), sample F (3,810 mg/100 g), sample G (3,606 mg/100 g), sample H (3,676 mg/100 g), sample I (3,810 mg/100 g). Phytochemical constituuents present includes tannin, phenol, saponins, alkaloids, phylate, oxalate, cyanogenic glycosides, trypsin inhibitor and flavonoids. It is therefore recommended that *Euphorbia hirta*, *Ficus asperifolia*, *Momordica charantia*, *Nicotiana tabacum*, *Spondias mombin* and *Chromolaena odorata* can be used as source for antibiotics substances for possible treatment of ailments like malaria, asthma, diabetes, fibroids, hypertension, epilepsy, fever and some mycotic infections.

Keywords: Antifungal evaluation; Plant extracts; Phytochemicals; Nigerian medicinal plants; Botanical description; Constituuents *Euphorbia hirta; Ficus asperifolia*; *Momordica charantia*; *Nicotiana tabacum; Spondias mombin*

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Introduction

Medicinal plants are of great importance to the health of individuals and communities. In developing countries, 80% of the population continues to use medicinal plants and plant products in handling medical problems due to their accessibility, availability and affordability. In these countries, a variety of plants had been reported to have bioactive ingredients, while a few have been tested for such phytochemicals. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, cyanogenic glycosides and phenolic compounds. Many of these indigenous medicinal plants are used as spices, food plants, and also sometimes added to foods for pregnant and nursing mothers. Our environment is endowed with different types of plants and

animals therefore, plants serves as important part of culture and tradition in Africa. The use of local plants for various medicinal purposes had been the oldest form of health care in the history of mankind, and medicinal tropical plants are widely collected for its drug value to take care of man and animal health [1,2].

Plant secondary metabolites are potential sources of effective antifungal agents. Plant-derived compounds such as hydroquinones and naphthoquinones (lapachol, juglone), sesquiterpenes (cinnamodial, capsidiol) and alkaloids (berberine) had shown antimicrobial and antifungal activities. An advantage to the approach of using ethnobotanical leads to identify compounds with antimicrobial activity for instance, leaves of *Euphorbia hirta* and fruits of *Spondias mombin* are rich in tannins, flavonoids, procyanidins and also contain organic acids, lipids, enzymes and vitamins. New antifungal compounds with distinct modes of action need to be identified because of increasing incidence of fungal resistance to existing antibiotics [3].

Problems associated with the scanty information on antifungal activities of *Chromolaena odorata*, *Euphorbia hirta, Ficus asperifolia*, *Momordica charantia*, *Nicotiana tabacum* and *Spondias mombin* prompted this study which aimed at investigating and evaluating the antifungal activities of leaf extracts of the six medicinal plants against four economically important fungi with a view to determining the importance of the inhibitory effects of plant extracts with phytochemical screening.

Chromolaena odorata (Siam weed Linn.) R.M. King and H. Rob. is a tropical and subtropical species of flowering plants in the family *Asteraceae*. It inhabits waterways (riparian areas), bushland, forest margins, roadsides, disturbed sites, waste areas, neglected pastures, crops and plantations. It also grows on a wide range of soils and grows in a range of vegetation types, e.g. forests (annual rainfall 1500 mm), grassland and arid bushveld (annual rainfall less than 500 mm). It is also a nuisance weed in agricultural land and commercial plantations. *C. odorata* shows marked morphological variability in terms of flower colour, leaf shape and hairiness, pungent, aromatic smell of the crushed leaves, and plant architecture. The plant is hairy, glandular and the leaves are 4 cm-10 cm long by 1 cm-5 cm wide (up to 4 inches×2 inches), opposite-decussate, flaccid membranous, velvety-pubescent, obovate to deltoid-ovate **(Plate 1)** [4,5].

Euphorbia hirta Linn. (Asthma herb) is a small prostrate herb of about 20 cm-35 cm tall with milky latex, which belong to the family Euphorbiaceae. It is native to Central America and a common weed of the tropics and subtropics, particularly tropical Africa. It grows in human disturbed areas of Northern and Southern parts of Nigeria including roadsides, fields and yards. The stem is slender, reddish in colour, covered with yellowish bristly hairs especially in young parts. Leaves are opposite, distichous, simple; stipules linear, up to 2.5 mm long; petiole up to 3.5 mm long; blade ovate, (1 cm-4 cm) × (0.5 cm-2 cm), unequal base, one side cuneate, the other side rounded, apex almost acute, margin finely toothed, often with a purple blotch near the mid vein. The flowers and fruits are found throughout the year Seeds are oblong, conical, slightly wrinkled, pinkish brown without caruncle **(Plate 2)**.

Ficus asperifolia Miq**.** (Sand paper tree) of the family Moraceae is a small or average size tree, terrestrial or epiphyte which can reach 12 m in height. It is abundant in the forest and savannah regions, especially along river banks and marshy areas. The leaves are enormous and displayed spirally, while the limb is largely oval

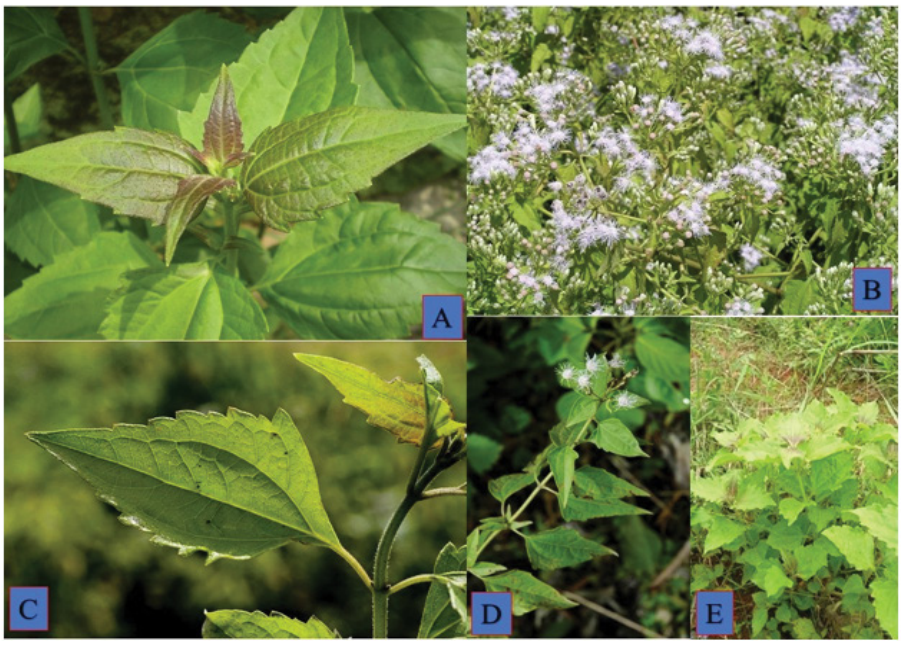

Plate 1 Photographs of *Chromolaena odorata* (Linn.) R.M. King and H. Rob. (a): showing the close-up of leaves, leaf type (simple) and leaf shape (ovate); (b): showing the close-up of flowers and habit in flowers;(c): showing the younger hairy stem and paired leaves with base (obtuse or subtruncate) and apex (acute);(d): showing the phyllotaxy (opposite-decussate), flowers and leaves with long petioles;(e): showing the plant habit (Shrub).

or has a form of ellipse and the roots are most often fibrous. Sand paper trees produce three types of flower; male, a long-styled female and a short-styled female flower, often called the gall flower **(Plate 3)** [6,7].

Momordica charantia Linn. (Bitter Melon) is an annual to perennial monoecious climbing or sprawling herb, 3 m-5 m (16 ft.) tall with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils. It requires tropical conditions for growth, and may be either hairless or slightly hairy. The fruit looks like a warty gourd, usually oblong and resembling a small cucumber. The young fruit is emerald green, turning to orange-yellow when ripe. At maturity, the fruit splits into three irregular valves that curl backwards and release numerous reddish-brown or white seeds encased in scarlet arils. The Latin name *Momordica* means "to bite," referring to the jagged edges of the leaves, which appear as if they have been bitten **(Plate 4)** [8].

Nicotiana tabacum Linn. (Tobacco) is a robust annual herb up to 2.5 m (8.2 ft) high with large green leaves and long trumpet-shaped white-pinkish flowers. It is a native of tropical and subtropical America but now commercially cultivated worldwide as ornamental plants or weed. All the parts are sticky, covered with short viscid-glandular hairs, which exude a yellow secretion

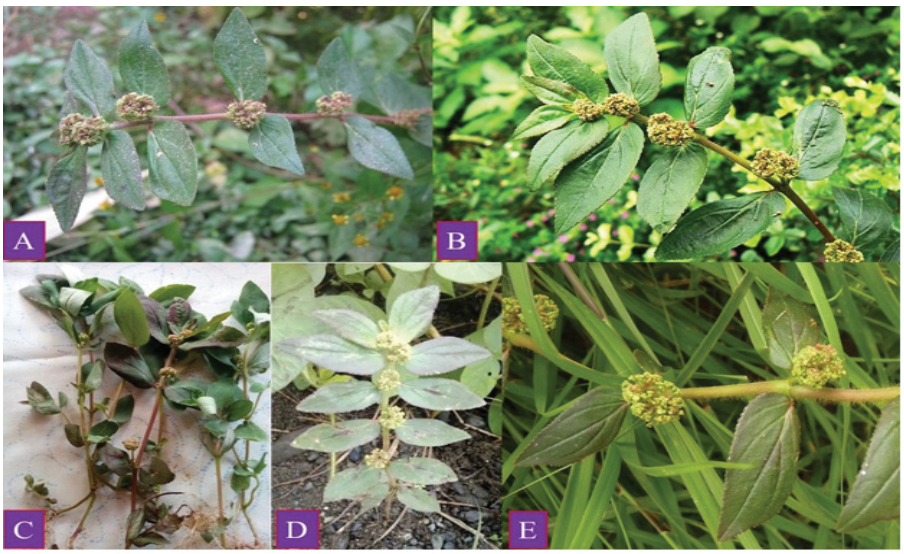

Plate 2 Photographs of *Euphorbia hirta* Linn. (a) and (b): showing the leaf arrangement (opposite), leaf type (simple) and blade shape (ovate) and apex (almost acute); (c): showing the plant habit (herb), younger hairy stem and paired leaves with leaf apex (acute), roots and flowers; (d): showing the stem type (erect), habit (prostrate herb), flowers and leaves with short petioles; (e): showing the leaf margin (finely toothed or serrated), hairy stem (pubescent) and flowers of *Euphorbia hirta*.

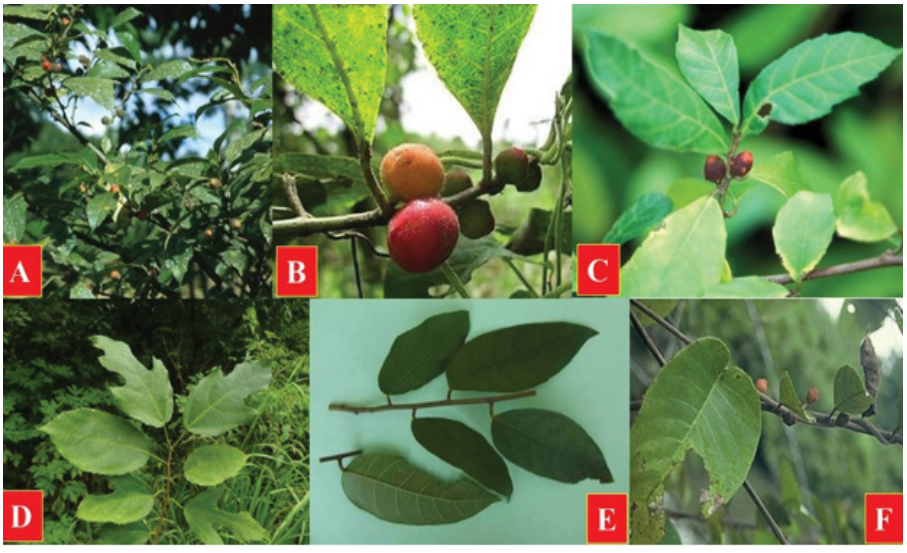

Plate 3 Photographs of *Ficus asperifolia* Miq. (a): showing the close-up of leaves, stems and flowers that look like fruits; (b) and (c): showing the flowers leaf blade with long petioles and venation (reticulate);(d) and (e): showing the two varieties of *Ficus asperifolia* with the leaf arrangement (spiral) and apex (acute); (f): showing the prominent lateral nerves on the leaf blade.

containing nicotine [9]. The ovate to lanceolate leaves are alternate, spiral around the stem. The tubular flowers, which range in colour from white or cream to pink to carmine red, grow in a large, branching terminal clusters, with individual flowers 3.5 cm to 5 cm (1.25 to 2 in) long. The flowers may be attractive and aromatic. Many related species of *Nicotiana* are grown as ornamentals, but in commercial cultivation, the inflorescence is generally cut off before fully developed to encourage greater leaf growth. Fruits are oval to elliptical capsules that contain several to numerous small brown seeds (0.5 mm) **(Plate 5)** [10].

Spondias mombin Linn. (Hog plum) is a fructiferous deciduous tree of the family Anacardiaceae, and grows up to 25 m (66 ft) high and 60 cm-75 cm in diameter. It is native to the tropical Americas, but having its habitat in Nigeria, Brazil and several other tropical forests in the world. This plant is commonly used as folk medicine in South West of Nigeria. Its bark is conspicuously thick, deeply fissured, and corky characters which confer a protection of the tree against savanna fires. When slashed, it is pale pink, darkening rapidly. Branches are low and branchlets are glabrous. The leaves are pinnate, with 5-10 leaflets alternate pairs with a terminal leaflet (20 cm-45 cm long), oblong or oblong lanceolate, broadly acuminate, glabrous. The flowers are in large, lax terminal panicles of small white flowers. Fruits are nearly 4 cm (1.5 in) long, ovoid or ellipsoidal drupe, 1-seeded, yellow-skinned when ripe and wrinkled when dry. The fruits have sharp acidulous

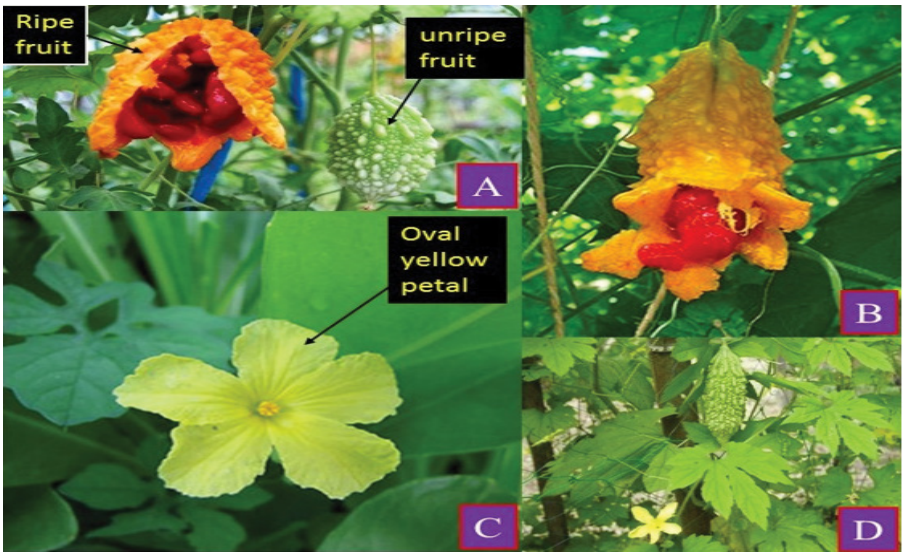

Plate 4 Photographs of *Momordica charantia* Linn. (a): showing the ripe and unripe fruits; (b): showing the ripe fruit with its long stalk;(c): showing the leaves and close-up of petals (five oval yellow petals) with five central stamens; (d): showing the plant habit (climbing herb), leaf type (simple) and unripe fruit.

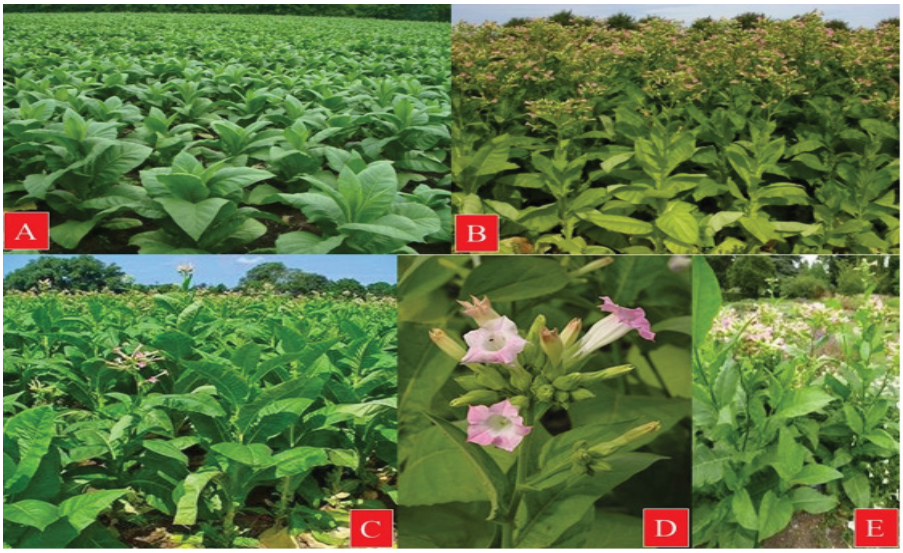

Plate 5 Photographs of *Nicotiana tabacum* Linn. (a): showing the younger leaves of *Nicotiana tabacum*;(b) and (c): showing the plant habit (herb) and close-up of flowers; (d): showing the colour of flowers, flower buds and leaf arrangement (spiral); (e): showing the leaf type (simple) and leaf shape (ovate to lanceolate).

taste and commonly sold in West African markets. The seed has an oil content of 31.5% (**Plate 6)** [11].

Aspergillus niger van Tieghem is a filamentous fungus and one of the most common species of the genus *Aspergillus* which belongs to the family Trichocomaceae**.** *A. niger* has a saprophytic lifestyle and plays an important role in the degradation and recycling of dead plant material. Many of the secreted enzymes of *A. niger*, like amylases, proteases, pectinases, xylanases and lipases find applications in the baking, starch, textile, food and feed industry. In human beings and animals, *A. niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane. *Aspergillus niger* has been useful in research and industry for several decades.

Aspergillus tamarii Kita are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. These fungi commonly grow on carbon-rich substrates like monosaccharides (such as glucose) and polysaccharides (such as amylose). Their colonies grow rapidly as dark brown. Microscopically, Conidial heads are compact and spherical or loosely radiate, 500 m-600 m diam. Conidiophore stipes usually 1 mm-2 mm in length, hyaline, usually roughened. Vesicles spherical, 10 m-50 m diam. *Aspergillus tamarii* are common contaminants of starchy foods such as bread and potatoes, and grow in or on many plants and trees. The species was implicated in a case of eyelid infection, and reported as an onychomycosis in a child [12,13].

*Fusarium oxysporum (*Schlecht.) Snyder and Hansen is an ascomycete fungus, comprising of all the varieties and forms recognized by Wollenweber and Reinking within an infrageneric grouping called section Elegans. It is part of the Nectriaceae family. *F. oxysporum* strains are ubiquitous soil inhabitants which exist as saprophytes, and degrade lignin and complex carbohydrates associated with soil debris. Although, the predominant role of *F. oxysporum* in native soils may be harmless or even beneficial plant endophytes or soil saprophytes, while many strains are pathogenic to plants [14].

Penicillium oxalicum Currie and Thom. species are group of fungi capable of producing high activities of cellulase and hemicellulose. They have higher β-glucosidase activity and better thermal stability of FPase activity than *Trichoderma* species [15]. *Penicillium oxalicum* is used for gene cloning of a novel disruptive activity of its swollen on crystalline cellulose and its characteristics in enhancing cellulosic hydrolysis.

Materials and Methods

The study area

The study area consists of five local government areas in Oyo State, Nigeria, including Akinyele, Ibadan South East, Ibadan North East and Ibadan North West. Its geographical location extends approximately from longitude 3°48' to 3°58' East of the Greenwich Meridian, and latitude 7°18' to 7°28' North of the Equator. Ibadan, the capital city of Oyo State of Nigeria, is the largest indigenous city in West Africa. It is located in south

Plate 6 Photographs of *Spondias mombin* Linn. (a): showing the glabrous leaflets and yellow-skinned fruits;(b): showing the ripe fruits in ovoid or ellipsoidal drupe shape; (c): showing the fruiting stage and foliage of *Spondias mombin* with phyllotaxy (alternate), leaf type (compound) and leaf shape (oblong or oblong lanceolate); (d): showing the plant habit (tree); (e): showing the branches with yellow-skinned fruits and leaflets.

western part of Oyo State of Nigeria in a hilly settlement with urban and rural sectors covering a total land area of 3,123 km^2. of which about 15 per cent is urban and the remaining 85 per cent is classified as peri-urbar (United Nations Environment Programme (UNEP). The mean annual rainfall of about 1,205 mm, falling in approximately 109 days with two rainfall peaks in June and September [16,17].The mean temperatures are highest at the end of the Harmattan (averaging 28°C), that is from the middle of January to the onset of the rains in the middle of March.During the rainfall months, average temperatures are relatively high, between 24°C and 25°C, with annual temperature fluctuation of about 6°C. The main land use of the study area is dominated by built up land (all residential, commercial and industrial areas, settlement and infrastructures) and less vegetation cover (trees, shrub land, natural and semi-natural vegetation. (United Nations Environment Programme (UNEP), (**Figure 1**).

Plant collection and preparation of crude extracts

Fresh leaves of *Chromolaena odorata*, *Euphorbia hirta, Ficus asperifolia*, *Momordica charantia*, *Nicotiana tabacum* and *Spondias mombin* were collected from wild in four local governments of Oyo State, Nigeria including Akinyele, Ibadan South East, Ibadan North East and Ibadan North West. The lists of plant samples are presented in **Table 1**. The plant samples were identified and authenticated at the Department of Botany, University of Ibadan Herbarium (UIH), Nigeria. The morphological and ecological features of each specimen colleted from the field were described. Photographs of the specimens were taken during field trips with 18 MP Digital Camera (TECNO SPARK: K7-H8019A) for the picture database. The leaves were plucked, washed with distilled water and air dried at room temperature (25.3°C) because drying at a higher temperature could decompose its bioactive constituents. They were pulverized into fine powder using pestle and motor (stainless steel) and stored in screw capped containers. The distilled water, methanol, ethyl acetate and hexane extracts were prepared by soaking 100 g of the powdered plant materials of each extractant at room temperature for 2 days. The extracts were filtered separately through whatman filter paper no. 42 and concentrated using rotary evaporator (Heidoph 4001 efficient), warmed on water bath at 70°C for the hexane extract and temperature of 50°C for distilled water, ethyl acetate and methanol extracts, to obtain semi solid products [18].

Phytochemical analysis of the samples

All the samples were subjected to preliminary phytochemical screening as described by Knowles and Watkins, Swain, Fapohunda, Soladoye and Chukwuma. The quantitative phytochemical analysis of the leaves of the 6 plant species studied was carried out by employing standard conventional protocols outlined by Sofowora, Trease and Evans. The phytochemical constituents screened included; cyanogenic glycosides, oxalate,

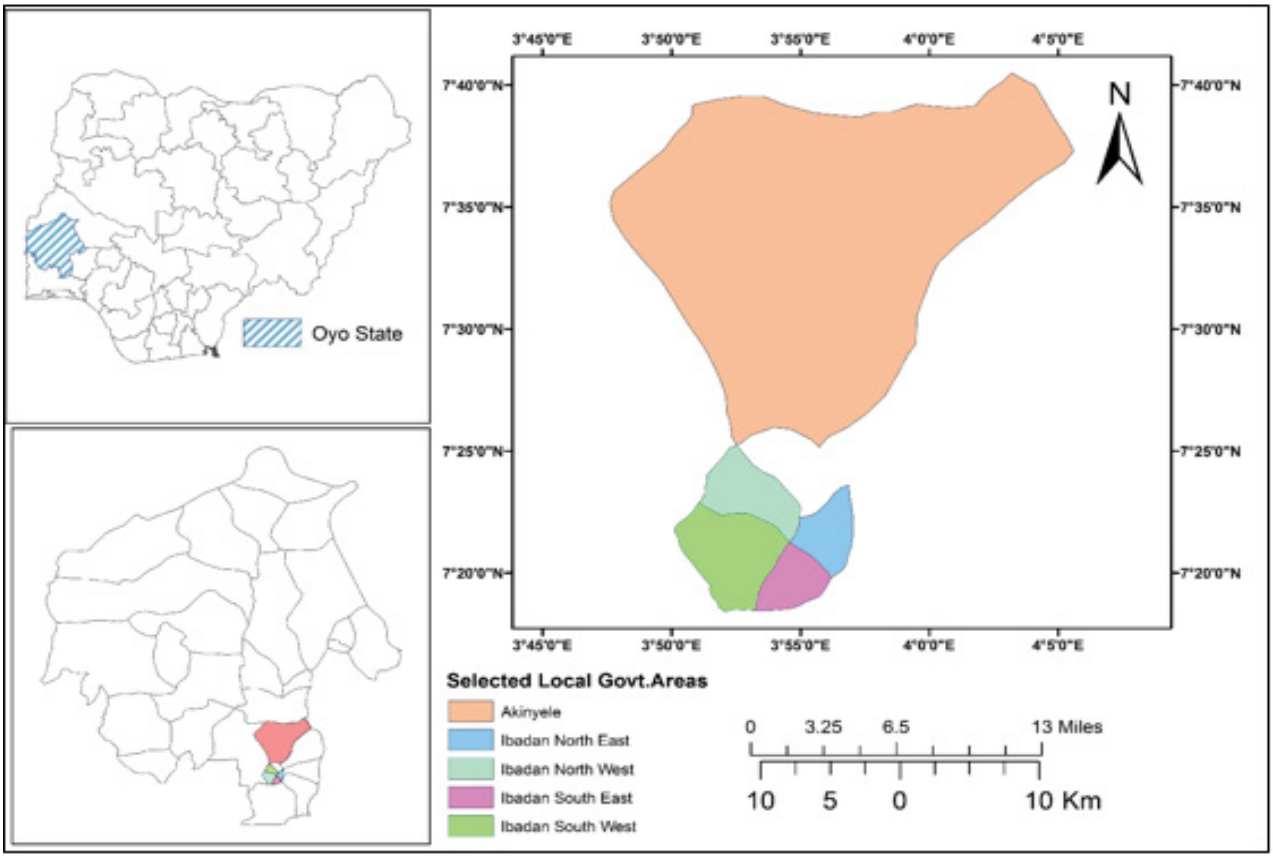

Figure 1 Map of the study area.

Table 1: Profile of Plants Used for the Study

S.No.	Plant	Family	Common Name	Local Name (Yoruba)	Plant Habit	Part Used
1	Chromolaena odorata (Linn.) R.M. King and H. Rob.	Asteraceae	Siam weed, Christmas Bush, Devil Weed and Triffid Weed	Ewe Akintola, Akintola-t a-ku (Òyọ́)	Perennial Shrub	Leaves
2	*Euphorbia hirta* Linn.	Euphorbiaceae	Asthma Herb, Snake Weed, Pill bearing spurge, Common Spurge	Adidin (Òndó), Bije (Ìjẹ̀bú), Emile (Ilorin), Iroko-Iju (Idoani), Oro-Elewe.	Annual Herb	Leaves
3	*Ficus asperifolia* Miq.	Moraceae	Sandpaper Tree	Epin, Epindo, Upen (Òndó).	Tree	Leaves
4	*Momordica charantia* Linn.	Cucurbitaceae	Bitter Melon, Bitter Gourd	Ejinrin, Ejinrin-aja (Òyọ́), Ejinrin-dudu (Ìwó), Ejinrin-weere (Òndó)	Climbing Herb (tendril-bearing vine)	Leaves
5	*Nicotiana tabacum* Linn.	Solanaceae	Tobacco	Aasa, Ewe-Taba, Kataba.	Shrub	Leaves
6	*Spondias mombin* Linn.	Anacardiaceae	Hog Plum, Yellow mombin	Iyeye, Yeye, Akika-etikan, Okikan (Owo), Ekikan, Olosan (Ìjẹ̀bú).	Tree	Leaves

Phytic acid, polyphenol, alkaloids, trypsin inhibitor and saponins, tannin, phenol and flavonoids.

Phytochemical Screening

Test for phlobatannins

A few drops of 1% hydrochloric acid were added to 1 mL of extract. A reddish-brown precipitate indicates the presence of phlobatannins.

Cyanogenic Glycosides: Knowles and Watkins method in Agricultural chemistry was used; 6 g of each sample was weighed into 250 ml conical flask. Each sample was incubated for another 16 hours at temperature of 38°C. After the extraction, the filtration was done using double layer of hardened filter paper. The distillation was done with Markham distillation in apparatus. Each sample extracted was transferred into a two-necked 600 ml flask connected with a steam penetrator. This was steam distilled with saturated sodium bicarbonate solution contained in 50 ml conical flak for 60 minutes [19-22]. 1ml of starch indicator was added to 20 ml of each distillate and was titrated with 0.2 N of iodine solution. The percentage hydrocyanide was calculated with the formula:

$$\%Hydrocyanide = \frac{Titre \times 0 \times 0.27 \times 100}{1000 \times Weight\ of\ Sample}$$

Oxalate: The extraction was done by weighing 1 g of each sample into 250mls conical flask and soaked with 100 ml of distilled water. These were allowed to stand for 3 hours and each was filtered through a double layer of filter paper, 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm standard solution of oxalic acid were prepared and read on the spectrophotometer at 420 nm for the absorbance [23,24]. The absorbance of filtrate from each sample were also read on the spectronic 20, the percentage oxalate was calculated using the formula:

$$\%\ Oxalate = \frac{Sample\ absorbance \times Average\ gradient\ from\ the\ cure\ for\ Standard \times Dilution\ factor}{100{,}000}$$

Phytic Acid (Phytate): 2 g of each sample was weighed into 250 m.m.l.s. conical flask 100 m.m.l.s. of 2% concentrated hydrochloric acid was used to soak each sample into conical flask for 3 hours. This was filtered through a double layer of hardened filter paper, 50 ml of each filtrate was placed in 250 ml beaker and 107 m.l.s. of distilled water was added in each case to give proper acidity. 10 m.l.s. of 0.3%, Ammonium Thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution which contained 0.00195 g iron per ml. The end point was slightly brownish yellow which persisted for 5 minutes. The percentage Phytic acid was also calculated [25].

Alkaloids: 5 ml of the sample was measured into a 250 ml beaker and 20 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours at 28°C. This was filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and measured. Alkaloid content was calculated and expressed as a percentage of the sample analyzed [26].

Saponin (C_{20} H_{20} O_{10}): 2 g of sample was weighed into a 250 ml beaker. 50 m.l.s. of solvent mixture of isobutylakohol and trichloroacetic acid were added and allowed to shake on a UDY shaker for 6 hours to extract the heamagglutinin. The mixture was filtered through a double layer filter paper after colour into a 250 ml conical flask and maintained in a water bath for 2 hours at 80°C. The filtrate was allowed to cool. A set of standard solutions of Heanagglutinin stock solution. The absorbances of the standard solution as well as that of the filtrate were read at 220 nm on a Digital spectrophotometer 21D. % heamagglutinin was calculated using the formula:

$$\%\ Heamagglutinin = \frac{Absorbance\ of\ sample \times Average\ of\ Gradient\ Standards \times Dilution\ factor}{10{,}000}$$

Flavonoids: About 5 g of the sample was boiled in 50 ml of 2M HCl solution for 45 minutes under reflux. It was allowed to cool and then filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl

acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample [27].

5.1.7. Tannins: 0.5 g of finely ground sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with paraffin and placed in a water bath at 75°C-80°C for 1 h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. 1 ml of sample extract was pipetted into 50 ml volumetric flask, 20 ml distilled water, 3.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 25 minutes when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0 ppm-10 ppm were treated similarly as 1 ml of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm [28-30].

Test organism, macroscopic and microscopic identification

Four selected disease-causing fungi species considered **in this study** were; *Aspergillus niger*, *A. tamarii*, *Fusarium oxysporum* and *Penicillium oxalicum*. They were obtained from the Department of Botany and Microbiology, University of Ibadan, Nigeria. The fungal isolates were maintained at 4°C on potato dextrose agar. Identification of fungal species was done on the basis of cultural and morphological characteristics. Macroscopic features like colony colour, texture and margins, as well as microscopic such as size of conidia and conidiophores and their arrangements were examined for species differentiation [30,31].

Agar well diffusion assay and determination of MIC: The modified agar well diffusion method used was described by Rao was employed, while the tube diffusion method for the determination of MICs was described by Donkorand Lin.

Test for antifungal activity

In order to investigate the antifungal activity of the extracts, a micro dilution technique was used. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^7 cfu/ml. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid potato dextrose agar to verify the absence of contamination and to check the validity of the inoculum [32-35].

Inoculum preparation for minimum inhibitory concentration (MIC): Inocula were obtained from an overnight agar culture of the test organism. Inoculum for the MIC test was prepared by taking at least three to five well isolated colonies of the same morphology from agar plate culture. The top of each colony was touched with a sterile loop and the loop was transferred into a tube containing 5 ml of normal saline and then vortexed. The broth culture was incubated at 37°C and monitored for approximately 4 hours until it reached the turbidity of 0.5 McFarland standard (1.5×10^8 cfu/ml).

RESULTS

The result in **Table 2** shows that the plant species did not produce significant ($p>0.05$) effects on *Aspergillus. niger*, *A. tamarii*, *Fusarium. oxysporum* and *Pennicillium. oxalicum* while the extracts had highly inhibitory effects on *F. oxysporum* and *P. oxalicum* except *A. niger*.

The results in **Table 3** shows that the effects of *Chromolaena odorata* on *Aspergillus niger* was significantly ($p<0.05$) higher than the other plant species but not significantly different from them. *Nicotiana tabacum* had higher inhibitory effect on *A. tamarii* and *F. oxysporum* but different from other plant species (**Table 3**). The effects of *C. odorata* and *Euphorbia hirta* as well as *Momordica charantia* on *A. tamarii* are significantly different from one another. Also, *C. odorata*, *E. hirta*, *Ficus asperifolia* and *M. charantia* produced similar inhibitory effect on *F. oxysporum* while plants *C. odorata* and *M. charantia* as well as *F. asperifolia* and *N. tabacum* had similar effect on *P. oxalicum* (**Table 3**).

The results in **Table 4** shows that distilled water was significantly ($p<0.05$) active against *A. niger* (75.56 mm) and *P. oxalicum* (53.89 mm) compared to other extracts. Though, extracts of methanol and ethyl acetate exhibited similar inhibitory effects on *A. niger*. The inhibitory effect of methanol extract on *A. tamarii* and *F. oxysporum* were significantly higher at 52.78 mm and 42.67 mm respectively. The effects of distilled water and ethyl acetate extracts on *A. tamarii* were not significantly different from each other. Moreover, hexane extract was more active against *A. niger* and *A. tamarii* at 48.89 mm and 33.67 mm respectively.

The correlation coefficient result in **Table 5** shows the relationship that exists among the plant species, plant extracts and the fungal isolates. *Aspergillus tamarii* and *Fusarium oxysporum* are positively associated with *A. niger* at r = 0.62 and 0.62; $p < 0.05$ respectively. Similarly, *F. oxysporum* and *P. oxalicum* are positive

Table 2: Mean square effect of Plant species and Extracts on Fungi.

Sources of Variation	df	*A. niger*	*A. tamarii*	*F. oxysporum*	*P. oxalicum*
Plant species	5	368.75^{ns}	174.00^{ns}	85.25^{ns}	43.13^{ns}
Extracts	3	1137.04^{ns}	639.74	480.19	1258.44
Error	15	439.12	54.74	36.02	23.4
Total	24				
Corrected Total	23				
ns: Significant p value					

Table 3: Inhibitory Effect of Plant species on fungi isolates.

Plant species	*A. niger*	*A. tamarii*	*F. oxysporum*	*P. oxalicum*
Chromolaena odorata	80.00^{a}	44.50^{bcd}	35.00^{b}	44.25^{a}
Euphorbia hirta	52.50^{a}	42.50^{bcd}	31.25^{b}	44.00^{a}
Ficus asperifolia	52.50^{a}	37.50^{d}	31.25^{b}	43.75^{a}
Momordica charantia	52.50^{a}	40.00^{cd}	33.75^{b}	44.25^{a}
Nicotiana tabacum	67.50^{a}	57.50^{a}	45.00^{a}	43.75^{a}
Spondias mombin	65.00^{a}	52.50^{ab}	37.00^{ab}	35.00^{b}
a, bcd, b, ab, cd, d: Significant p value				

Table 4: Inhibitory Effect of Extracts on Fungi.

Extracts	*A. niger*	*A. tamarii*	*F. oxysporum*	*P. oxalicum*
Distilled water	75.56^{a}	50.00^{a}	38.89^{ab}	53.89^{a}
Methanol	64.44^{ab}	52.78^{a}	42.67^{a}	48.33^{b}
Ethyl acetate	57.78^{ab}	45.56^{a}	34.78^{b}	38.89^{c}
Hexane	48.89^{b}	33.67^{b}	25.67^{c}	26.89^{d}
a, ab, b, c, d: Significant p value				

Table 5: Correlation Coefficient the Plant Extracts and the Fungal Isolates.

Plant Fungal	Plant species	Plant species	A. niger	A. tamarii	F.oxysporum
Plant Extracts	0.00^{ns}				
A. niger	0.01^{ns}	-0.45^{ns}			
A. tamarii	0.18^{ns}	-0.56*	0.62**		
F. oxysporum	0.16^{ns}	-0.58*	0.62**	0.92**	
P. oxalicum	0.14^{ns}	-0.89**	0.42^{ns}	0.51*	0.60**
ns, *: Significant p value					

and significantly related with *A. tamarii* at r = 0.92 and 0.51. Though, *P. oxalicum* and *F. oxysporum* are positively correlated with each other at r = 0.60. On the other hand, plant extracts are negative and significantly correlated with *A. tamarii* (r = -0.56), *F. oxysporum* (r = -0.58) and *P. oxalicum* (r = -0.89) at p< 0.05 (**Table 5**).

DISCUSSION

The inhibitory activities of the extracts of plant on *Aspergillus tamarii*, *Fusarium oxysporum* and *Pennicillium oxalicum* compared to unextracted leaves of the plant species could be due to the efficacy of bioactive compounds in accordance with the observation made by Olawuyi *et al*. (2010) and Jonathan *et al*. (2011). The extracts of plant possess antifungal activities against *A. tamarii*, *F. oxysporum* and *P. oxalicum* causing some plant infections.

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Conclussion and Recommendation

Phytochemical constituuents present includes tannin, phenol, saponins, alkaloids, phylate, oxalate, cyanogenic glycosides, trypsin inhibitor and flavonoids. It is therefore recommended that *Euphorbia hirta*, *Ficus asperifolia*, *Momordica charantia*, *Nicotiana tabacum*, *Spondias mombin* and *Chromolaena odorata* can be used as source for antibiotics substances for possible treatment of ailments like malaria, asthma, diabetes, fibroids, hypertension, epilepsy, fever and some mycotic infections.

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