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Phytochemical and antimicrobial studies on some Nigerian medicinal plants

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Abstract
Medicinal plants are employed in traditional medicine for diverse uses such as in the treatment or management of disease conditions like malaria, diabetes, sickle-cell anaemia, tumors, inflammations, cardiac troubles, mental disorders and especially microbial infections. There is an ever growing need to subject these ethnomedicinal uses to scientific scrutiny. Hence, the phytochemical and antimicrobial studies on some plants found in the Nigeria were undertaken. The phytochemical screening revealed that all the twenty plants tested negative for anthraquinones while only *Acalypha indica* gave a positive reaction for cyanogenic glycosides. However, each plant either demonstrated the presence or absence of alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids. All but the extract of *A. indica* strongly inhibited the growths of *B. subtilis* and *S. aureus*. Also, extracts of *Pycnanthus angolensis*, *Ageratum conyzoides*, *Rinorea dentata*, *Tridax procumbens* and *Viscum albium* gave very weak antibacterial activities against *E. coli* while most of the plant extracts were generally inactive against *K. pneumoniae*, *Ps. aeruginosa*, *S. typhi* and *S. dysentriae*. Surprisingly, more than ten of the plant extracts tested demonstrated some anti-candidal activity notably amongst which are *Bryophyllum pinnatum*, *Carica papaya* and *Centrosema pulmieri*. Cardiac glycosides, terpenes, flavonoids and tannins had been reported to have demonstrated antimicrobial activities in previous studies. The results of the antimicrobial tests have lent some scientific credence to some of uses of these plants in the treatment or management of infections, especially those of microbial origin.

Keywords: Phytochemical; Antimicrobial; Extracts; Antibacterial; Anticandidal

INTRODUCTION

Medicinal plants are defined as any plants which contain substances that can be used for therapeutic purposes in one or more of its organs or substances which are precursors for the synthesis of useful drugs (Sofowora, 1982). Furthermore, a plant becomes a medicinal plant only when its biological activity has been scientifically established (Elujoba, 1987). Plants are a great source of medicines, especially in traditional medicine (Bako *et al*., 2005) and it has been estimated that about 90 % of the population in developing countries rely on the use of medicinal plants to meet their primary health care (WHO, 2002). The use of medicinal plants has contributed immensely to the treatment or management of disease conditions such as HIV/AIDS, malaria, diabetes, sickle-cell anaemia, mental disorders (Elujoba *et al*., 2005) and microbial infections (Okigbo *et al*., 2005). Their use is

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safer, more affordable and offer profound therapeutic benefits than the synthetic alternatives (UNESCO, 1998; Iwu *et al*., 1999). Chemical and biological investigations of plants which started about two centuries ago (Roja and Rao, 2000) routinely involve the use of extracts, infusions and decoctions of leaves, stem, bark, roots and seeds (Ogbulie *et al*., 2004). Phytochemicals are by-products of primary metabolic processes which are otherwise called secondary metabolites (Richter, 1978). These chemical compounds serve as defence against herbivory and pathogenic attacks. They also serve as growth regulators, modulators of gene expression in signal transduction and ecophysiology of plants (Bako *et al*., 2005; Kafmann *et al*., 1999; Briskin, 2000). These chemical principles vary in distribution within the plant parts and also amongst species. This variation is influenced by cultivation period, season of collection and plant-to-plant variability (Bako *et al*., 2005; Nalawade and Tsay, 2004). Consequently, it is important that phytochemical screening of plants be done and also subject the numerous uses claimed in folkloric medicine to scientific scrutiny. Therefore, this present study was carried out by screening some medicinal plants found in the Nigerian floral environment for the presence of secondary metabolites and also bioprospecting them for antimicrobial activity.

EXPERIMENTAL

Collection of plant materials. The plants used in this study are *Acalypha ciliata, Acalypha hispida, Acalypha indica, Acalypha wilkesiana* (Red variety), *Acalypha wilkesiana* (Golden-yellow variety), *Acalypha wilkesiana* (Lace variety), *Ageratum conyzoides, Anthocleista djalonensis, Bryophyllum pinnatum, Calotropis procera, Carica papaya, Centrosema pulmieri, Cyathula prostrata, Dacryodes edulis, Garcinia kola, Nymphaea odorata, Pycnanthus angolensis, Rinorea dentata, Tridax procumbens* and *Viscum albium.* The different plants were simultaneously collected around March, 2012 from forests, open fields, abandoned and cultivated farms in the Uyo Local Government Area of Akwa Ibom State, Nigeria. The plants were identified in their fresh states by Okon Abia-William, a taxonomist in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria while authentication was done by comparison with herbarium samples at the National Institute of Horticulture (NIHORT) and Forestry Research Institute of Nigeria (FRIN) both in Ibadan, Oyo State, Nigeria. Afterwards, voucher specimens labelled No H102 to No H121 were prepared and deposited in the Herbarium Unit, Faculty of Pharmacy.

Extraction and processing of plant materials. Fresh seeds were peeled and pulverized in a wooden mortal using a pestle to obtain the ground powder. However, other organs such as the leaves, stem and roots were separately oven-dried (40 ^{0}C) and then ground into coarse powders on an electric mill (Gallenkamp, UK). The resultant powders were then extracted with cold 96 % ethanol at room temperature (27± 2 ^{0}C) for 72 h. The obtained filtrates were also separately evaporated to dryness *in vacuo* on a rotary evaporator (Buchi CH-920, Laboratorium Technic, Flawk/SG, Switzerland). The resultant dried extracts were then stored in appropriately labelled amber bottles in a refrigerator at -4 ^{0}C prior to the tests.

Phytochemical screening. The dried crude extract of each plant was separately investigated for secondary metabolites (alkaloids, saponins, tannins, cardiac glycosides, terpenes, anthraquinones, flavonoids and cyanogenic glycosides) according to the laid down procedures (Watt and Brandwijk, 1962; Shoppee, 1964; Stahl, 1965; Robinson, 1967; Gibbs, 1974; Brain and Turner, 1975; Nahrstedt *et al.*, 1982;

Akerele, 1984; Harborne, 1984, Harborne, 1988;, Moffat, 1986; Sofowora, 1998; Oladimeji, 1997; Evans, 2009; Oladimeji *et al.*, 2012).

Antimicrobial sensitivity test. The micro-organisms used in this study, namely, *Bacillus subtilis* (NCTC 8853), *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Klebsiella pneumoniae* (NCTC 6750), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (NCTC 8236), *Shigella dysentriae* (NCTC 8112) and *Candida albicans* (NCYC 6) were clinically isolated from specimens of diarrheal stool, abscesses, necrotizing fascitis, osteomyelitis, urine, wounds and vaginal swabs. They were collected in sterile bottles, identified, typed by convectional biochemical tests (Gibson and Khoury, 1986; Murray *et al*., 1995) and then refrigerated at 0-5 ^{0}C at the Pharmaceutical Microbiology and Parasitology Unit, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria prior to the antimicrobial screening. The hole-in-plate agar diffusion method was used. The inoculum of each micro-organism was introduced into each Petri-dish (Pyrex, England). Cylindrical plugs were removed from the agar plates by means of a sterile cork-borer (Pyrex, England) to produce wells with diameter of approximately 5 millimetres. The wells were equidistant from each other and the edge of the plate (Washington, 1995; NCCLS, 2003). A concentration of 20 mg mL^{-1}for each plant extract was introduced into the wells. Also, different concentrations of 10 μg mL^{-1} of ampiclox (Fidson, Nigeria), 1 mg mL^{-1} of nystatin (Emzor, Nigeria) and 100 % methanol were introduced into separate wells as positive and negative controls respectively (Oladimeji, 1997; Oladimeji *et al*., 2012; Adesina *et al*., 2000). The experiments were carried out in triplicates. The plates were left at room temperature for 2 h to allow for diffusion. The plates were then incubated at 37 ^{0} C for 24 h. Zones of inhibition were measured in millimetres (mm).

RESULTS AND DISCUSSION

Processing of plant materials. The plants used in this present study were identified, authenticated and collected observing basic guidelines of plant collection. Also, the rules governing extraction and phytochemical screening of extracts were strictly adhered to, thus preventing any changes to the chemical composition of the crude extracts (Odebiyi and Sofowora, 1978; Odebiyi and Sofowora, 1979).

Phytochemical screening. The phytochemical investigations revealed that all the plants tested negative for anthraquinones while the screening for cyanogenic glycosides showed that only *Acalypha indica* gave a positive reaction as presented in Table 1. However, each plant either demonstrated the presence or absence of alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids (Table 1). Generally, it was observed that the extracts contained between moderate and abundant amounts of the secondary metabolites. Phytochemical compounds are by-products of primary metabolic processes and are used by plants for protection and repair purposes (Bako *et al*., 2005; Richter, 1978). Interestingly, phytochemicals which are otherwise referred to as secondary metabolites have demonstrated in several previous studies (Adesina *et al*., 2000; Hillar *et al*., 1990; Rios *et al*., 1990; Lamikanra *et al*., 1990; Burapadaja and Bunchoo, 1995; Harouna *et al*., 1995; Aiyelaagbe *et al*., 1998; Adewunmi *et al*., 1998; Ibewuike *et al*., 1998) to be responsible for the cure or management of many ailments caused by microbes and different kinds of disease conditions in the ethno-medicine of plants. Alkaloids were found present in eight of the twenty plants tested with the formation of orange precipitate. Previous studies on some of the plants by authors confirm these findings. A study had earlier reported that *A. wilkesiana* contains alkaloids (Akinyemi *et al*., 2006)

while two other ones had obtained licopsannine from *Ageratum conyzoides* (Vyas and Mulchen, 1984; Horie *et al*., 1993). Alkaloids are revered as the most pharmacologically active of the secondary metabolites and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastro-intestinal tract, uterus, therapies of malignant diseases and malaria (Evans, 2009). Furthermore alkaloids have been ranked among the most efficient and therapeutically significant plant substances (Okwu, 2005). Over 6,000 alkaloids are known and prominent amongst them are nicotine, cocaine, morphine, quinine, solasodine and codeine (Harborne, 1973; Stary, 1998; Okwu and Okwu, 2007). Also, these compounds have marked physiological effects on animals (Edeoga and Eriata, 2001) and many of them are nitrogenous compounds widely used as therapeutic agents in the management of cancers (Chabner and Howitz, 1990). Twelve plants tested positive for saponins in this study. Saponins are inferred to be present in a plant sample if frothing or foams are observed. These two reports had earlier indicated the presence of these compounds in *A. wilkesiana* (Akinyemi *et al*., 2006; Alade and Irobi, 1993). This group of phytochemicals are glycosides of tri-terpenes and steroids having hypotensive and cardiac properties which have been detected in over seventy families in the plant kingdom (Basu and Rastogi, 1967, Olaleye, 2007). These compounds lower cholesterol levels (Cheeke, 1971; Malinor *et al*., 1977) and act as expectorants and emulsifying agents (Edeoga *et al*., 2006), possess anti-diabetic and anticancer properties (Evans, 2009). Furthermore, saponins are a major component of antifungal secondary metabolites (Onwuliri and Wonang, 2003). Sixteen of the plant samples tested positive for tannins with the characteristic formation of greenish-black colouration. These compounds are well known for their antimicrobial properties which suggest that they may be of great use in the treatment of venereal diseases. In addition, tannins have soothing relief, help to regenerate the skin, act as anti-inflammatory and diuretic agents (Okwu and Okwu, 2007) . Also, they are used in wound healing, employed in treating neoplastic cells (Duke and Wain, 1981) and possess astringent properties (Egunyomi *et al*., 2009) . A previous study had reported the presence of corilagin and gerannin from the leaves of *A. wilkesiana* and *A. hispida* (Adesina *et al*., 2000). All but one of the plant samples gave the characteristic positive test (appearance of brown interface) for cardiac glycosides. This group of secondary metabolites are cardio-active and possess hypotensive properties (Brain *et al*., 1985). These inherent activities reside in the aglycone portions of their sugar attachments. Furthermore, cardiac glycosides increase renal flow (diuretic) and act on smooth muscles of the vascular system (Olaleye, 2007). Fifteen plants tested positive for terpenes. These phytochemical compounds have anti-hepatotoxic properties, hence are able to prevent liver damage. Also, they possess antimicrobial and antiseptic activities. Previous studies had obtained β-amyrin from *Rinorea dentata* and two terpenoidal oils from *Cyathula prostrate* (Oforah *et al*., 1999; Oladimeji and Usifoh, 2013). Twelve of the twenty plants investigated were found to contain flavonoids. These compounds are 15-C compounds which are generally distributed in plant kingdom (Harborne, 1988). Flavonoids possess antimicrobial, anti-tumour, cardiatonic, anti-oxidant, anti-carcinogenic and anti-radical properties (Salisbury and Ross, 1992; Hertog *et al*., 1993; Kandaswani *et al*., 1994; Nakayoma and Yamada, 1995; Mankanda *et al*., 2006). Agecony and hexamethoxyl flavones had been isolated from *Ageratum conyzoides* (Vyas and Mulchen, 1984; Horie *et al*., 1993). Also, these three previous

studies had reported the presence of flavonoids in *Tridax procumbens*, *Centrosema pulmieri* and *Cyathula prostrata* (Oladimeji *et al*., 2007 Oladimeji, 2012).

Antimicrobial Screening. The plant extracts were screened for antibacterial and antifungal activities using *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumonia*, *Ps. aeruginosa*, *S. typhi*, *S. dysentriae* and *C. albicans* to represent a desirable spectrum of microbes. The extracts were tested at 20 mg mL^{-1}. All the extracts tested strongly inhibited the growths of *B. subtilis* and *S. aureus* except *A. indica* as displayed in Table 2. Interestingly, the plants investigated were generally inactive or weakly active against *E. coli*, *K. pneumoniae*, *Ps. aeruginosa*, *S. typhi* and *S. dysentriae*. The extracts of *Pycnanthus angolensis*, *Ageratum conyzoides*, *Rinorea dentata*, *Tridax procumbens* and *Viscum albium* demonstrated very weak antibacterial activities against *E. coli*. However, most of the plant extracts were generally inactive against *K. pneumoniae*, *Ps. aeruginosa*, *S. typhi* and *S. dysentriae*. This observation was notably demonstrated by the extracts of *Acalypha* species, *Garcinia kola*, *Calotropis procera*, *Nymphaea odorata*, *Anthocleista djalonensis*, *Bryophyllum pinnatum and Cyathula prostrata* as presented in Table 2. Surprisingly, more than half of the plants investigated gave some anti-candidal activity. The extracts were either poorly active or inactive against gram negative organisms because these organisms in general, unlike gram positive organisms, posses a sophisticated three-layered envelope which does not allow permeation of external agents. Also, fungal strains such as *Candida spp*. limit the permeation of substances because of their allomorphic and facultative nature.

Table 1: Phytochemical screening of plant extracts

Plant	ALKA	SAPO	TANN	CARD	TERP	ANTR	FLAV	CYAN
P-1	-	-	+	+	+	-	-	-
P-2	-	++	+ +	+ +	+	-	+	-
P-3	-	-	+	+	+	-	-	+++
P-4	-	++	+ +	+ +	+	-	+	-
P-5	-	-	+	+ +	+	-	-	-
P-6	++	++	++	+	+	-	-	-
P-7	-	-	++	+	++	-	+++	-
P-8	+++	-	-	+++	+	-	+++	-
P-9	++	++	+++	+++	++	-	+	-
P-10	+	++	+	+++	+++	-	+	-
P-11	-	+	+	+	-	-	+	-
P-12	-	++	+++	-	-	-	+++	-
P-13	-	+++	+++	+++	+++	-	++	-
P-14	-	+++	-	+++	-	-	-	-
P-15	+	+++	++	++	++	-	+++	-
P-16	+	+++	+	+	-	-	+++	-
P-17	-	+	-	+	+	-	-	-
P-18	+	-	-	++	+++	-	-	-
P-19	+	-	+++	++	++	-	++	-
P-20	-	+++	+++	+++	-	-	-	-

Key: = Absent; + = Trace ; ++ = Moderately present; +++ = Abundant;
L = Leaf extract; S = Stem extract; R = Root extract; SE = Seed extract;
ALKA =Alkaloids; SAPO = Saponins; TANN = Tannins; CARD = Cardiac glycosides;
TERP = Terpenes; ANTR = Anthraquinones; FLAV = Flavonoids; CYAN = Cyanogenic glycosides
P-1 = *Acalypha ciliata* ; P-2 = *Acalypha hispida*; P-3 = *Acalypha indica*; P-4 = *Acalypha wilkesiana* (Red variety); P-5 = *Acalypha wilkesiana* (Golden-yellow variety); P - 6 = *Acalypha wilkesiana* (Lace variety); P-7 = *Ageratum conyzoides*; P-8 = *Anthocleista djalonensis*; P-9 = *Bryophyllum pinnatum*; P -10 = *Calotropis procera*;

P-11 = *Carica papaya*; P -12 = *Centrosema pulmieri*; P-13 = *Cyathula prostrata*; P-14 = *Dacryodes edulis*; P-15 = *Garcinia kola*; P-16 =*Nymphaea odorata*; P-17 = *Pycnanthus angolensis*; P-18 = *Rinorea dentata*; P-19 = *Tridax procumbens*; P-20 = *Viscum albium;* AMP = Ampicillin; NYS = Nystatin
*The zone recorded is diameter zone of inhibition plus the cup size (5mm).

Table 2: Antimicrobial Activity of Plant Extracts at 20mg/ml in 100% methanol.
* Diameter zone of inhibition (mm) + 5mm

Plant	*B. sub*	*S. aur*	*E. coli*	*K. pneu*	*Ps. aeru*	*S. typ*	*S. dys*	*C. alb*	AMP 10µg/ml	NYS 1mg/ml	% MeOH
P-1	8	10	8	-	5	-	-	5	10	24	5
P-2	9	14	13	-	10	-	-	8	11	18	5
P-3	8	7	7	-	5	-	-	5	11	22	5
P-4	15	16	13	-	11	-	-	9	11	21	5
P-5	14	13	10	-	10	-	-	5	12	22	5
P-6	15	14	12	-	12	-	-	6	10	21	5
P-7	9	10	8	-	10	-	-	5	11	19	5
P-8	14	13	10	12	9	-	-	9	12	19	5
P-9	11	11	11	13	-	12	-	10	11	20	5
P-10	15	16	12	14	-	11	-	9	12	21	5
P-11	17	15	13	13	-	12	-	11	11	23	5
P-12	13	12	10	11	-	9	-	12	11	20	5
P-13	18	17	10	10	10	10	10	10	10	22	5
P-14	18	19	10	16	-	13	-	11	11	21	5
P-15	12	12	10	-	5	-	-	6	10	22	5
P-16	12	13	11	12	-	12	-	10	11	21	5
P-17	16	17	7	10	-	10	-	10	12	22	5
P-18	11	10	7	9	-	-	8	9	11	20	5
P-19	10	9	8	9	9	-	9	8	11	21	5
P-20	14	13	9	8	-	9	-	7	10	19	5

These integral structures differ from cell walls of bacteria but resemble those of higher plants (Brown, 1975). The ethno-medicinal significance of the selected plants for this study corresponds to the pharmacological actions of the phytochemical compounds they contain. Therefore, it is pertinent that further investigations be done on the plant extracts to isolate these compounds. Furthermore, the amounts of these isolated compounds can be quantified. Hence, these isolates can be subjected to various pharmacological processes including antimicrobial screening with the aim of turning them into potent drugs. This has become necessary because of the microbial resistance manifested by common pathogenic micro-organisms to some of the antibiotics currently used in clinical medicine.

Conclusion. The results from this present study indicate a wide diversity in the distribution of secondary metabolites and the recorded antimicrobial activities in the plants tested.

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