Phytochemical And Antimicrobial Properties Of The Genus Pericopsis Thwaites (Papilionaceae) In Nigeria

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Abstract: This present study examined the phytochemical and Antimicrobial properties of the two occurring Nigerian species of Pericopsis (Allopatric taxa): Pericopsis elata and Pericopsis laxiflora. The phytochemical content and antimicrobial properties of the leaves and stem bark of both species were examined using standard techniques. Antimicrobial activity of the stem bark and leaf of the two species were confirmed using the agar diffusion method and the minimum inhibitory conc. test (MIC) against four standard organism viz: Shigella dysentriae, Staphylococcus aureus, Candida valida and Pennicillum atrovenetum. The powdered leaves and stem bark were screened and results were properly recorded as observed. Extracts of the leaves and stem bark of the two species were also analyzed quantitatively for the following phytochemicals: Tannin, Alkaloid, Flavonoid, Terpenoid, Saponin and Phenols. Folin ciocalteu reagent was used to determine the total Phenolic content. Preliminary phytochemical screening showed that both plants had almost all phytochemical screened for. Quantitative analysis of bioactive components revealed that total phenols are also found in the extracts expressed as mg of GAE per gram. Results showed that the saponin content was the highest with about 47.13% shown in the stem bark extract of P. elata. This is followed by alkaloid with 39.40% in the leaf of P. laxiflora and 36.86% of flavonoid shown in the stem-bark of P.elata. Antimicrobial activity exhibited a concentration dependent profile as the extract exhibited antimicrobial activity against all the test microorganisms. The test organisms were most susceptible at concentration of 200 and 100mg/ml respectively and most resistant at low concentration. This study, however, supports the fact that Nigerian species of genus pericopsis contain important compounds which may be useful in medicine, it also suggests that further research should be conducted into the plant as a whole.

Keywords: Phytochemical properties, Antimicrobial properties, Pericopsis elata, Pericopsis laxiflora, Powdered leaves, Stem bark, Microorganisms, Phytochemicals

INTRODUCTION

Biologically active compounds from natural sources have always been in great interest for scientists working on infectious diseases (Burkill, 2000, Roja et al., 2000, Sofowora 1982 and Perumal and Ignacimuthu, 2000). As a matter of fact, it has been estimated that about 25% of all prescribed medicines today are substances derived from plants (Egwaikhide and Gimba 2007 Zheng and Wang 2001). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996, Rojas et al. 1992). Presently, there is a growing interest in phyto-medicinal research. Emphasis so far are directed towards a systematic search for useful bioactive compounds in medicinal plants as a rational approach in drug research (Adaramola et al. 2012). Furthermore, increasing reliance of the of medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutic from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). The genus Pericopsis Thwaites belongs to the papilionaceae, Tribe: Sophoreae (Soladoye and Lewis, 2003). The Plant List includes 6 scientific plant names of species rank for the genus Pericopsis. Of these, 4 are accepted species names: three in Africa and one in Asia (Michael, 2006). The two species found in Nigeria are Pericopsis elata (Harms) Meeuwen and Pericopsis laxiflora (Benth.) Meeuwen. P.elata is a timber-producing species native to countries of west and central Africa occurring in the Guinean Equatorial forests. It has a disjunct distribution with several isolated sub-populations occurring in Cote d'Ivoire and Ghana; Central African Republic (CAR), eastern Cameroon and Congo; and the Democratic Republic of Congo (DRC). Details of the ecology of the species remain poorly known. It is currently classified as Endangered by IUCN (CITES, 2003). The species occurs in dryer parts of moist semi-deciduous forests with annual rainfall of 1000 – 1500 mm (Swaine and Whitmore 1988). The tree is mainly known under the local names of afrormosia (DRC, Congo, trade name most commonly used), assamela (Cameroon, Côte d'Ivoire) and kokrodua (Ghana). Howland (1979) and Dahms (1999) have cited other local names including ole and oleo pardo (Congo), bohalala and ole (DRC), ejen and obang (Cameroon), mohole (Ghana), and ayin, aneran and elo uta (Nigeria). Also commercialized as African teak or gold teak (because of the colour of its dry heartwood), this high commercial value timber species is restricted to moist semi-deciduous African forests (West and Central Africa). P. elata is valued for the high quality of its wood, and its exploitation started more than 50 years ago, mainly in Ghana and Côte d'Ivoire (Dickson et al., 2005). The tree also offers good resistance against termites and its wood density is quite high. Its wood is considered to be an excellent substitute for teak (Tectona grandis L.f.) in all respects (Kukachka, 1960). The principal industrial uses are flooring, furniture, window/door frames, decorative veneer and shipbuilding (Kukachka, 1960). They are also commonly used in African traditional medicine as the pulped bark is applied after scarifications for localized pain in Congo (Burkill, 1994). P. laxiflora (Benth.) Van Meeuwen (Leguminosae) is used in Côte d'Ivoire to the traditional treatment of many ailments such as: headache, stomach ulcers, stomach aches, upset stomach, gastritis, enteritis, heart pain, abdominal pain (Ake-Assi L., 1988). In Nigeria, it is used as an antiulcer ancestral area Benoue (Ekpendu, 2003). Harms (1913) had earlier stated that the main differences between P. elata (forest tree) and P. laxiflora (savanna tree/shrub) lay in the size of the leaves and of the rachi, with additional differences to be found in some traits of fertile branches (the former species presenting more pubescence). Adeniji and Ariwaodo (2012) had further stated that polygonal cell shape, stomata distribution as well as the presence and absence of

trichomes are useful characters which can be employed in the delimitation of the taxa. Despite the significant progress recorded by different research teams, so much remains to be done on medicinal plants, including their activities in connection with multi-resistant microorganisms. This study is aimed at evaluating the antimicrobial activities of the leaves and stem bark extracts of the two occurring Nigeria species of Pericopsis as well as identifying and quantifying the percentage of crude phytochemical constituents present in these plants. Results from this study will provide justification, or otherwise, for the usage of extracts from the plant as traditional remedies against various gram + ve and gram – ve pathogens and its potentials in traditional herbal medicine. It can also serve as basis of comparison between the two plants.

MATERIALS AND METHOD

COLLECTION AND IDENTIFICATION OF PLANT

The leaves and stem-bark of the plants were collected within the premises of Forestry Research Institute of Nigeria Ibadan, Oyo State. The voucher specimens were then deposited at the Forest Herbarium Ibadan (FHI) Oyo state, Nigeria listed in Holmgreen et.al 1990.

TEST ORGANISMS

Clinical isolates (maintained on Nutrient and Sabouraud dextrose Agar) of Shigella dysentriae, Stapphylococcus aureus, Candida valida and Penicillium atrovenetum used in this study were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria

PREPARATION OF PLANT EXTRACT

Ethanolic extract of the leaf and stem bark of the plant was extracted according to the method described by Okogun (2000) with slight modifications. 50 g of the plant samples were air-dried, ground into powder using an electric blender (National MX4911V, Matsushita electronics). The blended material was transferred into a beaker and 10 ml of 95% ethanol was added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation on a rotary shaker. Extraction was allowed to proceed for 48 h. The mixture was decanted and the solvent was removed by evaporation at room temperature (28 ± 2°C) to obtain the extract.

PHYTOCHEMICAL SCREENING OF EXTRACT

The phytochemical components of the leaves and stem bark of extracts were screened by using standard procedures as described by Harborne 1998, Sofowora 1993, Marcano and Hasenawa, 1991 and Trease and Evans, 1989. Dragendorffs reagents were used to test for alkaloids, ferric chloride for tannins, while Benedict's solution was used to test for saponins.

QUANTITATIVE DETERMINATION OF THE CHEMICAL CONSTITUENCY

Tannin determination: Determination of total tannins: 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van Burden, 1981)

Alkaloid determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid determination by the method of Bohm and Kocipai- Abyazan (1994): 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Terpenoids determination : 1g of the sample was weighed into 10ml Petroleum Ether. It was then allowed to extract for 15min, filtered and the Absorbance read at wavelength of 420nm.

Saponin determination by the method of Obadoni and Ochuko (2001): The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water-bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Determination of total phenolics: The amount of total phenolics in the extracts was determined using Folin-Ciocalteu procedure (Singleton et al., 1999) with slight modifications. Samples (2.5ml in triplicates) were introduced into test- tubes; 0.5ml of Folin-Ciocalteu's reagent was added. The content in the tubes were mixed and allowed to stand for 30 minutes. Absorption was measured at 765nm. The total phenolic content was expressed as Gallic acid equivalent in mg/g dry material.

ANTIMICROBIAL SCREENING

Determination Of The Antimicrobial Sensitivity Test Of The Leaves And Stem Bark Extracts Against Selected Bacteria And Fungi Isolate

The antimicrobial activity of the leaves and stem bark extracts P. elata and P. laxiflora was determined using the agar well diffusion method (for bacteria) and pour plate method (for fungi) by the procedure of Oyetayo et al., (2009) with slight modifications. The medium employed was diagnostic sensitivity agar. Nutrient agar and Sabouraud Dextrose Agar was inoculated with the given microorganisms by spreading the microorganisms inoculums on the media. Wells (8mm diameter) were punched in the agar and filled with plant extracts. Controls wells filled with 500 mg/ml of standard antibiotic solution, Gentamycin (10 ug/ml) and Tioconazole (0.5 mg/ml) , were run as parallel positive controls in the same plate. (Valsaraj et al., 1997). The plates were incubated at 37^0C for 24 hrs for bacteria and at 26^0C for 48 hours for fungi. After the incubation period, the zone of inhibition was measured in millimeters. All the tests were carried out in triplicate and their means recorded.

Minimum Inhibitory Concentration (MIC) Assay: MIC is defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. It was determined by Agar dilution assay method (Forbes et al., 1998).The MIC values were studied for the microbial strains, being sensitive to the extracts in the agar well diffusion method. The crude hexane extracts were partitioned into acetone, ethanol and pet-ether fractions and the fractions were tested on the organisms. Ethanol fraction was found more effective than the other two. The MICs of the Ethanol fractions of the three samples were thus determined against two bacteria and two fungus : Shigella dysentriae, Staphylococcus aureus, Candida valida and Penicillium atrovenetum respectively. The different organisms were then inoculated on the different plates with different concentrations. The bacteria plates were incubated at 37^0C for 24 hours while that of fungal plates was incubated at 26^0C for 24-48 hours. The first plate in the above series with no sign of visible growth was reported as the minimum inhibitory concentration.

RESULTS AND DISCUSSION

Table 1: Preliminary Qualitative/Phytochemical analysis of leaf and stem bark extract of P. elata and P. laxiflora

	LEAVES		STEM-BARK	
Active principle	P. elata	P. laxiflora	P. elata	P. laxiflora
TANNINS	+	+	+++	++
ALKALOIDS	+	+++	++	+
FLAVONOIDS	++	+	++	+
TERPENOIDS	++	++	+	++
SAPONINS	+++	+	+++	+
PHENOLS	+	++	+	+
CARDIAC GLYCOSIDES	-	+	-	-

+ = Present, - = Absent, +++ = Abundance

In this study, the results of the phytochemical screening show that the extracts from the two plants samples contain tannin, alkaloids, flavonoids, terpenoids, saponin and phenols (Table 1). The leaf and bark extracts of the two plant samples compared favourably. P. elata bark and leaf extract showed more of the phytochemicals as compared with the stem bark and leaf extracts of P. laxiflora . Negative results were recorded for cardiac gylcosides in all the extracts except the leaf extract of P. laxiflora. This was however confirmed in the antimicrobial activity against test organisms. (Table 3). Several plants which are rich in alkaloids, tannins and glycosides have been shown to possess antimicrobial activity against a number of microorganisms. (Adebajo et al. (1983). Saponins are a special class of glycolsides which have soapy characteristic and facilitate the absorption of foods and medicine. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973). It therefore suggests that the medicinal plant used in the present study may have a general antimicrobial activity.

Table 2: Qualitative/ phytochemical evaluation of ethanolic extracts of leaf and stem bark of P.elata and P. laxiflora

	LEAF				STEM-BARK			
Active Principle	P. elata		P. laxiflora		P. elata		P. laxiflora	
Tannins	0.6±0.3	2.12%	1.7±0.9	5.63%	1.2±0.0	3.63%	1.1±0.3	7.80%
Alkaloid	2.8±2.7	9.82%	11.9±2.9	39.40%	3.3±2.3	9.97%	5.3±6.5	37.59%
Flavonoid	10.5±3.9	36.84%	6.9±0.6	22.85%	12.2±4.9	36.86%	4.7±0.7	33.33%
Terpenoid	1.2±0.4	4.21%	1.0±0.4	3.31%	0.7±0.4	2.11%	1.1±0.1	7.80%
Saponin	13.3±7.2	46.66%	8.6±14.2	28.48%	15.6±6.8	47.13%	1.8±1.9	12.77%
Phenols (GAE/100g	0.1±0.0	0.35%	0.1±0.0	0.33%	0.1±0.0	0.30%	0.1±0.0	0.71%

Mean±SD (mg/g); Relative % mean

The quantitative phytochemical estimation present in the two Pericopsis species studied as shown in Table 2 revealed that the leaves and stem-bark are very rich in flavonoids and low in phenols. The presence of tannins confirms its astringent property. Tannins have also shown potential antibacterial and antiviral effects (Akiyama et al. 2001; Lu et al., 2004). The large amounts of saponin in the leaves and stem bark of P. elata makes the plant an important source of detergents, surface active agents used

in industrial applications and also possesses beneficial effects (Shi et al., 2004). The high flavonoid content present in the plant indicates that the plant has a high antioxidant effect. Allan and Miller, (1996) earlier reported that flavonoids have antibacterial, anti-inflammatory, antiallergic, antimultagenic, antiviral, antineoplatic, antithrombotic and vasodilatory activities. The alkaloid content in both samples of P. elata is relatively normal i.e. not too high to make the plant harmful for consumption. Onyeka and Nwambekwe (2007) had earlier reported that alkaloid content of some edible vegetables ranged between 12.8-29.6mg/g (Onyeka et al., 2007). Trease and Evans (2005) have also pointed out that plants containing alkaloids do not feature strongly in herbal medicine because they are extremely toxic, yet, they have always been important in allopathic systems where the dose is strictly controlled and in homoepathy where the dose-rate is so low as to be harmless.

SENSITIVITY TESTS

Table 3: *Result of antimicrobial activity of crude ethanolic extract of leaf and stem-bark P.elata and P. laxiflora showing Diameter of inhibition Zones (Mean± standard deviation) at varying concentration (200-50mg/ml)*

	LEAF						STEM-BARK						Control
Org	P. elata			P. laxiflora			P.elata			P. laxiflora			
	200	100	50	200	100	50	200	100	50	200	100	50	
S.D	30±3.74	27.75±3.86	10±2.83	11.75±1.7	0	0	0	0	0	27.75±4.03	10.5±1.73	0	0
S.A	31.25±0.95	12.5±3.41	0	11.75±2.06	0	0	8.75±2.21	7.75±1.7	0	12.5±3.41	0	0	13.5±3.69
C.V	30.5±2.58	9±2.58	0	0	0	0	10.25±4.03	9.25±2.75	0	10.25±1.7	10.75±2.98	0	6.25±1.25
P.A	9±2.58	0	0	11±1.15	0	0	11.75±1.7	7±1.15	0	12±1.63	0	0	6.25±1.25

S.D= Shigella dysentriae, S.A= Staphylococus aureus, C.V= Candida valida, P.A= Penicillium atrovenetum. Gentamycin 10ug/ml (Bacteria), -Tioconazole 0.5mg/ml (Fungi). Control: Ethanol Solvent at 100mg/ml. Values represents mean ± S.D, n=3. Figures are mean Diameter of zone of inhibition in mm, 0 = no inhibition, Diameter of cork borer= 8mm

Table 4: *Mean diameter of Minimum inhibitory concentration (MIC) (mg/ml) of ethanolic fractions of P.elata and P. laxiflora on selected microorganisms at varying concentration (200-50mg/ml)*

	LEAF						STEM-BARK					
Org.	P. elata			P. laxiflora			P. elata			P. laxiflora		
	200	100	50	200	100	50	200	100	50	200	100	50
S.D	0.5	0	0	8	0	0	0.6	0	0	0.8	0	0
S.A	0.7	0	0	0.9	0.4	0	0	0	0	0.5	0	0
C.V	0	0	0	0.9	0.4	0	15	1.8	0	0.5	0	0
P.A	0	0	0	5	0	0	10	0	0	1	0	0

S.D= Shigella dysentriae, S.A= Staphylococus aureus, C.V= Candida valida, P.A= Penicillium atrovenetum. Gentamycin 10ug/ml (Bacteria), -Tioconazole 0.5mg/ml (Fungi).

The active principles identified in this study exhibited antimicrobial activity against all the test organisms (Table 2). Several plants, which are rich in alkaloids, tannins and glycolsides, have been shown to possess antimicrobial activity against a number of microorganisms. For example, Adebajo et al. (1983) investigated the antimicrobial activity of leaf extract of Eugenia uniflora and reported that tannins, glycosides and alkaloids were detected and that the ethyl acetate and methanolic leaf extract of the plant were active against E. coli, P. vulgaris, K. pneumoniae and Aspergillus niger. Saponins are a special class of glycolsides which have soapy characteristic and facilitate the absorption of foods and medicine. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973). It was recorded that an increase in the concentration of the extract yielded higher activity as shown by the diameter of zone of inhibition (Table 2). The fact that organisms may need higher concentrations of extracts to inhibit or kill them may be due to their cell wall components. Antimicrobial agents with a low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC. (Banso, 2009). This is reflected in Table 4 as almost all the plant extract exhibited low MIC value.

CONCLUSION

Ethanolic leaf and stem bark extract of P. elata and P. laxiflora produced antimicrobial activity against Shigella dysentriae, Staphylococus aureus, Candida valida and Penicillium atrovenetum. Though P. elata has showed more antimicrobial activity against the test microrganism, the stem-bark of P. laxiflora however showed a potent bactericidal and fungicidal effect on the test organisms. This results thus supports that of Ouattara et al., 2013. The extract contains the active principles- flavonoid, saponins, alkaloids, tannins, terpenoids and phenols. In view of the results obtained in the present work, this plants could be used as phytomedicine to combat diseases. To this end, it would be interesting to undertake studies of toxicity of the extracts which are found to be active and to consider the development of improved traditional medicines (ITM) after purification. The results obtained might then be considered sufficient for further studies on the isolation and

identification of the active principles and to evaluate the possible synergism among extract components for their antioxidant and anti microbial activity.

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