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Evaluation of the phytochemical constituents of leaf and root bark of *Calotropis procera* and *Parquetina nigrescens*: A potential bioinsecticide of insect pests

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Abstract
The study investigated the phytochemical constituents of leaf and root bark of *Calotropis procera* and *Parquetna nigrescens*. This was with a view to determining quantitatively the phytochemical constituents of leaf and root bark of *C. procera* and *P. nigrescens* for their potential use as bio-insecticide of insect pests. Total phenolic, flavonoid, condensed tannins, alkaloid and saponin content of leaf and root bark of *C. procera* and *P. nigrescens* were quantified. The quantity of saponin and alkaloid were the highest, followed by phenol while the least contents were the condensed tannins and flavonoid. The highest alkaloid content (60.40±0.02 mg/g) was in the ethanolic extract of *P. nigrescens* leaf, followed by methanolic extract of *P. nigrescens* leaf (39.50±0.01 mg/g) and the lowest alkaloid content of 16.50±0.02 mg/g was recorded with methanolic extract of *P. nigrescens* root bark.

Keywords: phytochemicals, phenolic, flavonoid, condensed tannins, alkaloid and Saponin

Introduction
Calotropis procera (Gentianale: Apocynaceae) is morphologically an erect plant, much branched and perennial shrub that grows to a height of 5.4 m. Milky latex is common with all the plant parts. Leaves are green coloured with fine cottony pubescent hair. Seeds are broadly ovate, acute, flattened, minutely tomentose, brown coloured and silky [1].
Parquetina is a monotypic genus with *P. nigrescens* being the only species. It is commonly found in secondary forests and around villages in Senegal and Nigeria [2]. According to Okwu (3), phytochemicals are chemical compounds formed during the plants' normal metabolic processes. These chemicals are often referred to as "Secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids [3].
The presence of certain chemicals in plants prevents insects from feeding on them and this leads to starvation of the insects and, in some cases, eventual mortality [4]. Phytochemical such as tannins possess amazing stringent properties and they bind to proline rich proteins and interfere with the protein synthesis [5]. Plant such as *Ocimum viride* exhibited strong repellency towards *Tribolium casteneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil, *Sitophilus orzyae* (Linnaeus) (Coleoptera: Curculionidae) and treatment of rice grain with leaf extract of *Ocimum viride* resulted in less than 25 % survival of the two insects after 10 days [6]. This study determined the phytochemical constituents of leaf and root bark of C*alotropis procera* and *Parquetina nigrescens* to provide additional knowledge for their potential use as bioinsecticide of insect pests.

Materials and Methods
Collectons of plant materials and extraction procedure
Leaf and root bark of *Calotropis procera* (Apple of Sodom) and *Parquetina nigrescens* (Ewe ogbo) (Gentianale: Apocynaceae) were used for this study. The leaf and root bark of *C. procera* and *P. nigrescens* were collected from Iyara village in Kogi State, Nigeria.
The leaf and root bark were rinsed with water, dried in the oven at 40^{o}C for 48 hours, ground to a powder with an electrical blender (USHA MG 2053 N) and stored in the refrigerator at the temperature of 4^{0} C in sealed plastic bags prior to extraction. Thirty grams (30 g) each of the powder of leaves and root bark of *C. procera* and *P. nigrescens* were soaked in 300 ml of ethanol and methanol (99.8%) separately in flasks for 6 hours, stirring intermittently with a sterile glass rod, then, filtered through Whatman No.4 filter paper. The solvents were removed in a water bath at 79^{0} C for ethanol and 66^{0}C for methanol to obtain a semi solid extract.

Determination of total phenolic content (TPC)
Folin-Ciocalteu reagent was used to determine the total phenolic content of the crude extracts [7]. Gallic acid was used as a reference standard (20-100 µg/mL) for plotting calibration curve. Two grammes (2 g) of each plant sample were weighed into 20 mL of solvent mixture (of ratio 80: 20 pure acetone: 0.2 % formic acid) in a flask and this was allowed to stand for 2 minutes. The mixture was filtered through Whatman No.4 filter paper. A volume of 2 mL of the plant extract was pipette into a test tube and mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 1 mL of sodium carbonate solution (7.5 %, w/v). The reaction mixture was kept in the dark at room temperature (20-25^{0}C) for 30 min with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured by using a double beam UV-VIS spectrophotometer (UV Analyst-CT 8200) at wavelength of 765 nm. A calibration curve using gallic acid standard of 0.02 mg, 0.04 mg, 0.06 mg, 0.08 mg and 0.1 mg of gallic acid per mL of methanol

was used to determine the total phenolic content of samples, and the results were expressed as mg gallic acid equivalents (GAE), per g of each sample.

Estimation of total flavonoid content (TFC) by aluminum chloride colorimetric method

Total flavonoid content in crude plant extract was determined by the procedure of [8]. One gramme (1 g) of well blended plant sample was weighed into a flask containing 10 mL of 80% methanol. This was allowed to stand for 2 hours with intermittent shaking and filtered through Whatman No.4 filter paper to collect the extract. An aliquot of 500 µl of plant extract, 2 ml of distilled water and 150 µl of 5% sodium nitrate were added. After 5 min, 150 µl of 10% aluminium chloride were added. A total of 2 mL of 1M sodium hydroxide was added after 1 min and followed by 1.2 mL of distilled water. The test tubes were incubated at 20°C for 30 minutes to complete the reaction. The absorbance of the reaction mixture was measured at 415 nm with double beam UV-VIS spectrophotometer against blank. A typical blank solution contains all reagents except aluminium chloride which was replaced by the same amount of distilled water. A yellow color indicated the presence of flavonoids. A calibration curve using quercetin standard of 0.02 mg, 0.04 mg, 0.06 mg, 0.08 mg and 0.1 mg of quercetin per mL of methanol was used to determine the total flavonoid content of samples, and the results were expressed as mg quercetin equivalents (QE), per g of each sample.

Determination of total condensed tannins (TCT).

Condensed tannin (proanthocyanidin) was determined according to the method of Sun *et al.* [9] One gramme (1 g) of well blended plant sample was weighed into a flask containing 25 mL of 70% acetone and 30% water. This was left for 5 hours and filtered through Whatman No.4 filter paper to collect the extract.

To 1mL of extract, 5 mL of vanillin hydrochloric reagent (equal volumes of 8% hydrochloric acid in methanol and 4% vanillin in methanol) were added. The mixture was allowed to stand for 15 min, and absorbance was measured at 500 nm against vanillin hydrochloric reagent as a blank. A calibration curve using catechin standard of 0.02mg, 0.04mg, 0.06mg, 0.08mg and 0.1 mg of catechin per mL of methanol was used to determine the total condensed tannin content of samples, and the results were expressed as mg catechin equivalents (CE), per g of each sample.

Determination of total alkaloid content (TAC).

The alkaloid content was determined gravimetrically [10]. Two grammes of each of the sample were put into flask containing 40 mL of 10% acetic acid solution in ethanol. The mixture was properly shaken and then allowed to stand for about 4 h before it was filtered. The filtrate was evaporated to one quarter of its original volume on heating mantle. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper to enable determination of the weight of the alkaloid was used to filter off the precipitate and it was then washed with 1 % ammonium hydroxide solution. The filter paper containing the precipitate was dried in the oven at 60^{o}C for 30 minutes, put in a desiccator to prevent moisture absorption and this was then reweighed until a constant weight was obtained. The weight of the alkaloid was then determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed as shown below.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weigth of sample}}$$

Where: W1 = weight of filter paper, W2 = weight of filter paper + alkaloid precipitate.

Determination of total saponin content (TSC).

The method of Obadoni and Ochuko [11] was used. Two grammes of each sample were placed in a conical flask containing 30 mL of 20 % aqueous ethanol and heated in a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue washed three times with 20 % aqueous ethanol. The extract was reduced to 5 mL in a water bath at 90°C. 5 mL of petroleum ether were added to the concentrated extract in a 250 mL separating funnel. This was agitated vigorously and the aqueous layer was recovered while the ether layer was discarded. A volume of 5 mL of butanol were added to the aqueous layer and the butanol layer was recovered, this was evaporated to dryness in the oven in a known weight Petridish at 45^{0}C. The residue weight was recorded as saponin content and expressed as a percentage of the sample weight.

Data analysis

Data obtained was subjected to analysis of variance (ANOVA) procedure of Minitab 16.1 [12]. Tukey's Test at P = 0.05 was used to compare means.

Results and Discussion

Table 1: Phytochemical constituents of ethanolic and methanolic extracts of leaf and root bark of *Calotropis procera* (Cp) and *Parquetina nigrescens* (Pn)

Plant materials Concentration of Phytochemicals (mg/g)					
	Phenol	**Alkaloid**	**Flavonoid**	**Saponin**	**Tannin**
Cp leaf methanolic extract	19.30±0.10^{b}	30.10±0.00^{d}	10.20±0.1^{c}	73.50±0.01^{d}	6.45±0.01^{b}
Cp root methanolic extract	15.85±0.01^{e}	20.50±0.01^{g}	1.85±0.01^{g}	132.30±0.01^{a}	4.25±0.01^{d}
Cp leaf ethanolic extract	22.01±0.01^{a}	35.50±0.01^{c}	12.02±0.01^{a}	92.30±0.01^{c}	7.08±0.01^{a}
Cp root ethanolic extract	16.20±0.20^{d}	28.00±0.10^{e}	2.10±0.10^{g}	124.00±0.1^{b}	5.01±0.02^{c}
Pn leaf methanolic extract	14.75±0.02^{f}	39.50±0.01^{b}	9.45±0.02^{d}	5.50±0.01^{h}	0.30±0.01^{g}
Pn root methanolic extract	8.55±0.01^{h}	16.50±0.02^{h}	3.35±0.01^{f}	30.00±0.1^{f}	0.09±0.01^{h}
Pn leaf ethanolic extract	17.03±0.01^{c}	60.40±0.02^{a}	11.31±0.01^{b}	27.00±0.01^{g}	0.91±0.00^{e}
Pn root ethanolic extract	10.45±0.01^{g}	21.90±0.00^{f}	4.30±0.10^{e}	55.00±0.1^{e}	0.70±0.10^{f}

Means in the same column with the same alphabets are not significantly different (P<0.05).

The quantitative analysis of the phytochemical constituent of ethanolic and methanolic extracts of *Calotropis procera* and *Parquetina nigrescens* leaf and root bark are shown in the Table 4.1.

The total phenolic compound was highest (22.01±0.01 mg/g) in the ethanolic extract of *C. procera* leaf, followed by methanolic extract of *C. procera* leaf (19.30±0.10 mg/g). The lowest phenolic content was recorded in the methanolic extract of *P. nigrescens* root bark (8.55±0.01 mg/g). The highest alkaloid content (60.40±0.02 mg/g) was in the ethanolic extract of *P. nigrescens* leaf, followed by methanolic extract of *P. nigrescens* leaf (39.50±0.01 mg/g) and the lowest alkaloid content of 16.50±0.02 mg/g was recorded with methanolic extract of *P. nigrescens* root bark. Total flavonoid content ranged from 1.85±0.01 mg/g to 12.02±0.02 mg/g in the methanolic extract of *C. procera* root bark and ethanolic extract of *C. procera* leaf respectively. The quantity of flavonoid content in *P. nigrescens* root bark was relatively higher (4.30±0.10 mg/g, 3.35±0.01 mg/g) than *C. procera* root bark (2.10±0.10 mg/g, 1.85±0.01mg/g) in both ethanolic and methanolic extracts respectively. The highest saponin content (132.30±0.01 mg/g) was recorded in the methanolic extract of *C. procera* root bark, followed by ethanolic extract of *C. procera* root bark (124.00±0.1 mg/g) and lowest saponin content (5.50±0.01 mg/g) in the methanolic extract of *P. nigrescens* leaf. The total saponin contents were higher in *C. procera* root bark than *P. nigrescens* root bark and also comparatively higher in the leaf of *C. procera* than the leaf of *P. nigrescens* in both methanolic and ethanolic extracts respectively. The total tannin content was more in *C. procera* than *P. nigrescens* plant. The highest tannin content (7.08±0.01 mg/g) was in the ethanolic extract of *C. procera* leaf, followed by methanolic extract of *C. procera* leaf (6.45±0.01 mg/g) and the lowest (0.09±0.01 mg/g) was in the methanolic extract of *P. nigrescens* root bark. There was significant difference in the phytochemical constituents in all the extracts of leaf and root bark of *C. procera* and *P. nigrescens* ($P<0.05$).

The quantity of phytochemical constituents varies from plant to plant and from one plant part to another [13, 14]. The relative amount of phytochemical substances from plant extract depends on the solubility of the phytochemical in the solvent used for the extraction [15, 16]. The amount of phytochemical constituents present in ethanolic extracts observed in this study was more than methanolic extract which indicate that the phytochemical constituents of *C. procera* and *P. nigrescens* are more soluble in ethanol than methanol solvent.

The results of this present study on the total phenolic and flavonoid contents in *C. procera* root bark are similar to the findings of Shashank *et al.* [17]. The study of Souhila *et al.* [18] on the total phenolic and flavonoid contents of ten Algerian varieties of *Ficus carica* reported lower contents when compared with the present study. Phytochemical constituents of *C. procera* leaf as reported [13, 19, 20] are similar to the results of this study. The content of saponin and flavonoid present in the leaf of *P. nigrescens* [21] was slightly lower when compared with the result of this work. Alkaloid content of the ethanolic extract of *P. nigrescens* leaf in this study is similar to the report of Sutharsingh *et al.* [22]. The tannin content of leaf and root bark of *Hypochaeris radicata* according to Jamuna *et al.* [23] and that of *P. nigrescens* in this study are similar. Phytochemical constituents of various medicinal plants in Nigeria [24] contain lower contents of alkaloid, flavonoid, tannin and saponin when compared with the results of this study.

Conclusion and Recommendation

In conclusion, the study shows that the leaf and root bark of *C. procera* and *P. nigrescens* contain substantial level of phytochemicals that could be explored to mitigate storage insect pest.

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