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**PHYTOCHEMICAL CONSTITUENTS OF SIAM WEED
(*CHROMOLAENA ODORATA*) AND AFRICAN CUSTARD
APPLE (*ANNONA SENEGALENSIS*)**

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ABSTRACT

Siam weed (*Chromolaena odorata*) and closely related species of *Annona senegalensis* such as *Annona muricata*, *Annona squamosa* had been investigated for pesticidal activity for the control of plant-parasitic nematodes and other pests on crops. This study was carried out to provide some information on the phytochemical constituents in these plants. Siam weed (*Chromolaena odorata*) leaves and roots, African Custard Apple (*Annona senegalensis*) leaves and bark collected, air-dried and ground into powder were taken to The Central Laboratory, University of Ibadan for Infrared (IR) Analysis. Phytochemical analysis of the plant samples was carried out with standard methods in the Department of Pharmacognosy, University of Ibadan. The concentrations of some phytochemicals were determined. Results of IR revealed the functional groups in *Chromolaena odorata* and *Annona senegalensis* were alcohols, amides, alkenes, carbonyl, unsaturated/aromatic, carboxyl, metals and phenols. The chemical constituents were alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins. *Chromolaena odorata* leaf contained total phenols 38.69 mg/g, tannins 41.09 mg/g, flavonoids 7.74 mg/g, saponins 331.76 mg/g, alkaloids 12.25mg/g while *Chromolaena odorata* root contained total phenols 14.33 mg/g, tannins 14.50 mg/g, flavonoids 1.54 mg/g, saponins 34.84 mg/g, alkaloids 11.58 mg/g. *Annona senegalensis* leaf contained total phenols 31.07 mg/g, tannins 31.73 mg/g, flavonoids 11.55 mg/g, saponins 103.32mg/g, alkaloids 12.02 mg/g and *Annona senegalensis* bark contained total phenols 91.27 mg/g, tannins 97.67 mg/g, flavonoids 0.51 mg/g, saponins 156.28 mg/g, alkaloids 14.74 mg/g. The pesticidal activity of these plants might be attributed to the phytochemicals contained.

Keywords: Siam Weed, African Custard Apple, Infrared (IR), Functional Groups, Phytochemicals

INTRODUCTION

Chemical control has been the central pivot in the management of pest and diseases of crops throughout the world. With the banning of pesticides due to human health and environmental pollution implications associated with the traditional synthetic nematicides (W.H.O., 2008), led to the search for naturally occurring toxicants in plants, which are apparently effective, cheaper available and environment-friendly arose (Khan *et al.*, 2008). Phytochemicals are secondary metabolites produced by the plant and are available in plant residues and extracts when applied to soil as botanicals (Ntalli and Menkissoglu-Spiroudi, 2011).

The phytochemicals in green manures and crop residues opened a new area for synthesis of plant-based nematicides or botanical pesticides (Tsai, 2000). The botanicals used in this study were Siam weed (*Chromolaena odorata*), Family-Asteraceae and African custard apple (*Annona senegalensis*), Family-Annonaceae (Gill, 1988; Odugbemi and Akinsulire, 2006). *Chromolaena odorata* extracts have been reported as being nematicidal (Adekunle and Fawole, 2003; Thoden *et al.*, 2009). *Annona senegalensis* was reported as an effective anthelmintic on livestock (Abdu *et al.*, 2000), *Annona muricata* and *Annona squamosa* were reported to be nematicidal (Salawu, 1992; Abid *et al.*, 1997). This study was aimed at reporting the chemical constituents found in *Chromolaena odorata* and *Annona senegalensis* for management of crop pests and disease-causing agents.

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MATERIALS AND METHODS

Collection and Preparation of Botanicals

The *Chromolaena odorata* leaves and roots were collected from the Crop Garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria and *Annona senegalensis* bark and leaves were collected from Otukpo village in Benue State, Nigeria. The collected plant parts were air-dried for three months at room temperature. The dried plant parts were milled to powders with grinding machines and stored in plastic bags. Infra-red analysis was carried out in the Multi-Disciplinary Central Research Laboratory University of Ibadan, Ibadan with standard procedures. Determination of tannin content, total saponin content, total alkaloid content, total flavonoid content, and total phenolic content were carried out with standard procedures.

Infra-Red Test

Powders of Siam weed leaf and root; African Custard Apple leaf and bark were prepared by milling the air-dried plants. Potassium bromide (KBr) disc was prepared by mixing 0.8 mg of each of the samples with 80 mg of KBr and compressing the whole into transparent disc using a compressor. The disc was then scanned in a Fourier Infrared Transform (FIR) spectrometer (Perkin Elmer Spectrum BX11). The IR spectrum was printed out with the aid of machine printer (William, 1987).

The identification of the functional groups was carried out in the Department of Chemistry, University of Ibadan, Ibadan.

Phytochemical Tests

The phytochemical analysis of the plant samples was carried out in the Pharmacognosy Department of the University of Ibadan, Ibadan using standard procedures.

Test for Tannins

To two ml of each of the ethanol extracts was added 5 drops of ferrous chloride solution. If a dirty green precipitate was observed, it indicated the presence of tannins. If otherwise, it indicated the absence of tannins (Trease and Evans 1989).

Determination of Tannin Content

Tannin content of samples was determined according to the method of Padamaja (1989). Samples 0.1 g each of the test plants were extracted with five ml of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes.

The mixture was centrifuged at 3000 rpm for 20 minutes, 0.1 ml of the supernatant was added with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, one ml of 35% sodium carbonate solution and diluted to ten ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured at 760 nm.

Blank was prepared with distilled water instead of the sample. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material. The calibration equation for tannic acid was $Y = 0.069x + 0.0175$ (Regression coefficient = 0.9978).

Test for Saponins

Plant extract of 0.2 g was shaken with five ml of distilled water and then heated to boil. Frothing showed the presence of saponins. If otherwise, it indicated the absence of saponins (Trease and Evans 1989).

Total Saponin Determination

Total saponins (SP) were determined by the method described by Makkar *et al.*, (2007). Plant samples of 0.5 g each were extracted with 25 ml 80% aqueous methanol by shaking on a mechanical shaker for two hours, after which contents of the tube were centrifuged for 10 minutes at 3000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous sulphuric acid were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 minutes. Then the tubes were cooled in ice for four minutes and then left at room temperature. Subsequently, the absorbance was measured in UV/visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

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Test for Alkaloids

Aliquots, two ml each of the ethanol extracts was stirred with five ml of 1% aqueous HCl acid on a steam bath. One ml of the filtrate was treated with 2 drops of Mayer's reagent. The second one ml portion was treated with Wagner's reagent. If creamy white (Mayer) and reddish brown (Wagner) precipitated were observed, it indicated the presence of alkaloids. If otherwise, it indicated the absence of alkaloids (Trease and Evans 1989).

Total Alkaloid Determination

The total alkaloid contents in the sample were measured using 1, 10-phenanthroline method described by Singh *et al.*, (2004). 100 mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000 rpm for 10 minutes. Supernatant obtained was used for the further estimation of total alkaloids. The reaction mixture contained one ml plant extract, one ml of 0.025 M FeCl₃ in 0.5 M HCl and one ml of 0.05 M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of $70 \pm 2^\circ\text{C}$. The absorbance of red coloured complex was measured at 510 nm against blank. Alkaloid contents were estimated and calculated with help of standard curve of quinine (0.1 mg/ml, 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with distilled water). The values were expressed as g/100 of dry weight.

Test for Flavonoids

Aliquot of four ml aqueous NaOH was added to two ml of each ethanol extract. If a yellow precipitate was observed, it indicated the presence of flavonoids in the extract (Trease and Evans 1989).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by the aluminium chloride method (Kale *et al.*, 2010). An aliquot of 0.5 ml of extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminium chloride (10%), 0.1 ml 1 M potassium acetate and 2.8 ml distilled water. The reaction mixture was mixed, allowed to stand for 30 minutes, before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g. The calibration equation for quercetin was $Y = 0.0395x - 0.0055$ (Regression coefficient = 0.9988).

Test for Anthraquinones

The Borntrager test was used; two ml of the test sample was shaken 4 ml of hexane. The upper lipophilic layer was separated and treated with four ml dilute ammonia. If the lower layer changed from violet to pink it indicated the presence of anthraquinones (Harborne, 1973).

Test for Cardenolides

The extracts thoroughly mixed with 20 ml distilled water and kept at room temperature for two hours and four drops of Kedde's reagent added. The suspension was filtered into two separate test tubes (A&B). The appearance of a blue violet colour indicated the presence of cardenolides (Chhabro *et al.*, 1984).

Determination of Total Phenolic Content (TPC)

The total phenolic content of samples extracts was determined using the Folin-Ciocalteu method (Chan *et al.*, 2006). An aliquot of 300 μ l of extract was dispensed into test tube (in triplicates), to which was added 1.5 ml of Folin-Ciocalteu reagent (diluted ten times) followed by sodium carbonate solution (7.5w/v). The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 μ l of distilled water instead of sample extract. Total phenolic content (TPC) was expressed as gallic acid equivalent (GAE) in mg/g material. The calibration equation for gallic acid was $Y = 0.0645x - 0.0034$ (Regression coefficient = 0.999).

RESULTS AND DISCUSSION

Results

The functional groups found in the two plants (Table 1) showed that *Chromolaena odorata* leaf contained alcohol, amine, alkane, carbonyl, unsaturated/aromatic, alkene, carboxylic acid and phenol, while *Chromolaena odorata* root contained alcohol, amine, alkane, unsaturated/aromatic, phenol and metals. *Annona senegalensis* leaf contained alcohol, amine, alkane, unsaturated/aromatic, alkene, phenol and

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metals, while *Annona senegalensis* bark contained alcohol, amine, alkane, unsaturated/aromatic and phenol. The active chemical ingredients in the various plants from the phytochemical tests, (Table 2) showed that *Chromolaena odorata* leaf contained alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins, *Chromolaena odorata* root contained alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins. *Annona senegalensis* leaf contained alkaloids, phenols, flavonoids, saponins, cardenolides and tannins while *Annona senegalensis* bark contained alkaloids, phenols, tannins, flavonoids, saponins, cardenolides and anthraquinones. The concentrations of some of the phytochemicals in the test plants are shown (Table 2), *Chromolaena odorata* root contained total phenols 14.3 mg/g, tannins 14.5 mg/g, flavonoids 1.5 mg/g, saponins 34.8 mg/g and alkaloids 11.5 mg/g. *Chromolaena odorata* leaf total phenols 38.6 mg/g, tannins 41.0 mg/g, flavonoids 7.7 mg/g, saponins 331.7 mg/g, alkaloids 12.2 mg/g. *Annona senegalensis* leaf total phenols 31.0 mg/g, tannins 31.7 mg/g, flavonoids 11.5 mg/g, saponins 103.3 mg/g, alkaloids 12.0 mg/g and *Annona senegalensis* bark total phenols 91.2 mg/g, tannins 97.6 mg/g, flavonoids 0.5 mg/g, saponins 156.2 mg/g, alkaloids 14.7 mg/g. Saponins were of the highest concentrations in the plant parts, followed by tannins, total phenols and alkaloids.

Table 1: Functional Chemical Groups of *Chromolaena odorata* Leaf and Root, and *Annona senegalensis* Leaf and Bark Identified by Infrared Analysis

Plant and Part	Alcohol	Amine	Alkane	Carbonyl	Unsaturated /Aromatic	Alkene	COOH	Phenol	Metal (Fe)
<i>C.o</i> Leaf	+	+	+	+	+	+	+	+	-
<i>C.o</i> Root	+	+	+	-	+	-	-	+	+
<i>A.s</i> Leaf	+	+	+	-	+	+	-	+	+
<i>A.s</i> Bark	+	+	+	-	+	-	-	+	-

Key

+ = indicates presence, - indicates absence, *C. o* = *Chromolaena odorata*, *A. s* = *Annona senegalensis*

Table 2: Active Chemical Ingredients in *Chromolaena odorata* Leaf and Root, *Annona senegalensis* Leaf and Bark

Plant Part	Alkaloids	Cardenolides	Anthraquinones	Saponins	Tannins	Flavonoids
<i>C.o</i> Leaf	+	+	+	+	+	+
<i>C.o</i> Root	+	+	+	+	+	+
<i>A.s</i> Leaf	+	+	-	+	+	+
<i>A.s</i> Bark	+	+	+	+	+	+

Key

+ = indicates presence, - indicates absence, *C. o* = *Chromolaena odorata*, *A. s* = *Annona senegalensis*

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Table 3: Concentrations of Some Phytochemicals in *Chromolaena odorata* Leaf and Root, *Annona senegalensis* Leaf and Bark

Plant Part	Total Phenols (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)
<i>C. odorata</i> Root	14.3	14.5	1.5	34.8	11.5
<i>C. odorata</i> Leaf	38.6	41.0	7.7	331.7	12.2
<i>A. Senegalensis</i> Leaf	31.0	31.7	11.5	101.3	12.0
<i>A. Senegalensis</i> Bark	91.2	93.6	0.5	156.2	14.7

Discussion

Phytochemicals are plant metabolites or their products that are released into the environment through volatilization, exudation from roots, leaching from plants or plant residues, and decomposition of residues. These are effective in small quantities and decompose quickly resulting in lower exposure and fewer pollution problems than the convectional pesticides (Kokalis-Burelle and Rodriguez-Kabana, 2006). Phytochemicals in the plant products may control nematodes by direct killing, preventing penetration by causing paralyzation, causing the loss of host-finding ability, repulsion or by unknown mechanisms (Tsai, 2000). The modes of action of phytochemicals on plant-parasitic nematodes may include inducers of resistance, antifeedant, repellent, deterrent, growth disruption, juvenile toxicant and ovicidal properties (Kokalis-Burelle and Rodriguez-Kabana, 2006).

Plant extracts contain phenolic compound, which alter root attractiveness to nematodes and resistance of the plant to nematode development and infestation was correlated to the phenols level in the roots by delaying the formation of giant cells and poor nutrition of larvae (Stirling, 1991). Phenolic compounds act as constitutive protection agents against the invading organism, function as signal and plant defense molecules, involved in resistance to biotic and abiotic stress (Joachim *et al.*, 2007). Alkaloids are complex compounds found occurring naturally in plants, insoluble aqueous hydroxide but soluble in aqueous hydrochloric acid, toxic to insects and plant-parasitic nematodes (Fatoki and Fawole, 2000). The alkaloids present in *Chromolaena odorata* have shown nematostatic and nematicidal effects on plant-parasitic nematodes (Thoden *et al.*, 2009). Protease inhibition is suggested as the mode of action by alkaloids (Wen *et al.*, 2013). Several important drugs are protease inhibitors (Berg *et al.*, 2002). Anthraquinones have antibacterial, antiviral, antifungal and antiprotozoal activity that are related to their inhibitory action on the enzymes necessary for the microorganisms, act as pre-infection toxins that are consistently present in the soil, released by the plants, inhibiting soil microorganisms (Abu-Darwish and Ateyyat, 2008). Cardenolides are steroids found in plants in form of cardenolide glycosides, toxic and arresting the heart. Some plants and animals species use cardenolides as defense mechanisms (Anon, 2010). Flavonoids are a class of phenolic compounds that have anti feeding and attracting deterrent properties, thus are toxic to insects, fungi, bacteria, nematodes and weeds (Carlsen and Fomsgaard, 2008). They are synthesized by plants in response to microbial infection, and their activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with microbial cell walls, also disrupting microbial membranes (Ciocan and Bara, 2007). Tannins are polyphenols that are toxic to small mammals (Fatoki and Fawole, 2000) and act as a defense mechanism in plants against pathogens and herbivores, induce changes in the morphology of pathogens through action on cell membranes by destabilization of cytoplasmic and plasma membranes, inhibition of extracellular microbial enzymes and metabolism and substrate deprivation required for microbial growth (Kumbasli *et al.*, 2011).

Saponins are plant secondary metabolites which cause hemolysis, with inhibitory effects on DNA, RNA and proteins in mammals (Fatoki and Fawole, 2000; Ibrahim and Srour, 2013). These compounds are reported to cause reduction in membrane integrity of cells by the formation of transmembrane pores

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(Bernards *et al.*, 2006). Saponins may act as host chemical defenses leading to resistance in the plants (Bernards *et al.*, 2006), they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which may be the most probable reason for larval death (Ghayal *et al.*, 2010). The nematicidal activity of saponins could be attributed to their ability to inhibit cholesterol accumulation in egg and/or larva (Ibrahim and Srour, 2013).

Phytochemicals are active compounds or precursors of active compounds that can be applied to soil as organic amendments or refined and developed as biopesticide compounds, such compounds as breakdown products are active against nematodes and other pests like insects, fungi and bacteria (Kokalis-Burelle and Rodriguez-Kabana, 2006). Plant and microbial compounds are continuously analyzed as potential sources of herbicides, pesticides and pharmaceuticals because they provide a diversity of carbon skeletons (Anaya, 2006).

Conclusion

The two plants, *Chromolaena odorata* and *Annona senegalensis* contained phytochemical groups that are reported to be pesticidal in activity. Saponins were of the highest amounts followed by tannins, total phenols, alkaloids and flavonoids in these two botanicals.

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