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Screening of Siam Weed (*Chromolaena Odorata*) and African Custard Apple (*Annona Senegalensis*) for Nematicidal Activity

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Abstract
Siam weed (*Chromolaena odorata*) and closely related species of *Annona senegalensis* had been investigated for nematicidal activity for the control of nematodes. This study was carried out to provide some information on the phytochemical constituents in these plants for nematode management.
Chromolaena odorata leaves and roots, *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 was carried out in the Department of Pharmacognosy, University of Ibadan.
The IR revealed that functional groups were alcohols, alkenes, carbonyl, carboxylic acids and phenols. The phytochemicals were tannins (46.2 ± 17.9 mgg-1), saponins (81.5 ± 76.8 mgg-1), alkaloids (12.6 ± 0.7 mgg-1), flavonoids (5.3 ± 2.6 mgg-1), phenols (43.7 ± 1.6 mgg-1), cardenolides and anthraquinones.
Saponins were of the highest concentrations, followed by tannins, phenols.The phytochemicals identified have been reported to be nematicidal in activity.
Keywords: constituents, dry powders, functional groups, nematode management, phytochemicals.

1. Introduction

Pest control approaches in the developed and some developing countries had relied heavily on the application of chemical pesticides. These chemicals have attendant problems of being scarce and expensive, with hazardous effects on target and non-target organisms and pollutant effects on the environment (W.H.O., 2008). The banning of methyl bromide and non-fumigant pesticides (W.H.O., 2008) has led to the search for alternative control measures. Some plants are able to kill or repel pests, disrupt their life-cycle or discourage them from feeding (Guerena, 2006).

The Siam weed (*Chromolaena odorata* L., R. M. King and Robinson) belongs to the family Asteraceae, which grows throughout West Africa. The leaves have glandular dots that emit a strong insecticidal smell (Akobundu and Agyakwa, 1998). The African Custard Apple (*Annona senegalensis* Pers.) belongs to the family Annonaceae which is primarily distributed in tropical and subtropical regions of the world. In West Africa, it is represented by 124 species in 24 genera. Oil cells are present in the tissues of all parts. The fruit is an aggregate of berries (Gill, 1988). The Siam weed (*C. odorata*) has been reported to possess nematicidal activity against plant-parasitic nematodes (Adekunle and Fawole, 2003; Adegbite and Adesiyan, 2005). *Annona senegalensis* was reported to be an effective anthelminthic on livestock (Abdu *et al*., 2000). Other species such as *A. muricata* and *A. squamosa* were reported to possess nematicidal activity (Salawu, 1992; Abid *et al*., 1997). There is paucity of information on the chemical constituents of these plants. Fatoki and Fawole (2000) reported that earlier studies showed that the water extracts and milled samples of Siam weed leaf and root were effective in controlling *Meloidogyne incognita* but the nematicidal ingredients remained unknown. This study was carried out to provide some information on the chemical constituents of these plants for nematode management.

1.1 Materials and Methods

Collection of plant parts

Leaves and roots of *C. odorata* were collected from the Crop Garden of the Department of Crop Protection and Environmental Biology, University of Ibadan. The leaves and bark of *A. senegalensis* were collected from the Ajobe village of Otukpo district of Otukpo LGA of Benue State.

Preparation of samples

The collected plant parts, were air-dried in a room at room temperature for a period of two months on polythene sheets spread on tables. The air-dried plant parts were milled into powdery form with a waring blender. The samples of the milled plant parts were taken to the Central Laboratory, University of Ibadan for Infrared (IR) Analysis and also taken to the Department of Pharmacognosy, University of Ibadan to identify the active

chemical ingredients.

Infrared (IR) spectrum and phytochemical analyses of Siam weed and African Custard Apple plant parts
Dry powders of Siam weed leaves and roots; and African custard apple leaves and bark were used for the

analysis. This was carried out with standard procedures in the Multi-Disciplinary Central Research Laboratory of the University of Ibadan, Ibadan. Dry powders of Siam weed leaves and roots; and African custard apple leaves and bark were used for the analysis. 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 a transparent disc using a compressor. The disc was then scanned in a Fourier Infra red Transform (FITR) 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 Phytochemical Research Laboratory, Department of Chemistry, University of Ibadan, Ibadan.

The phytochemical analysis of the plant samples was carried out in the Pharmacognosy Department of the University of Ibadan, Ibadan using standard procedures to identify the constituents as described by Trease and Evans (1989) and Sofowora (1993) to test for tannins, saponins, flavonoids and alkaloids, anthraquinones (Borntrager test), cardenolides test (Keller-Killiamis test or Kedde's test) (Harborne, 1973; Chhabro *et al*., 1984).

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.

Determination of tannin content

Tannin content of samples was determined according to the method of Padamaja (1989). Samples 0.1g 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 0.2g 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.

Total saponin determination.

Total saponins (SP) were determined by the method as described by Makkar *et al*. (2007). Plant samples 0.5g 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 60^0C 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.

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.

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 ± 2^0C. 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 g-1 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.

Determination of total flavonoid content (TFC)
Total flavonoid content was determined by the aluminum chloride method (Kale *et al*., 2010). 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). 300µl 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 300µl 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).

1.1.1 Results:
Table 1: Functional chemical groups of *Chromolaena odorata* leaf and root, and *Annona senegalensis* leaf and bark identified by Infrared Analysis.

	Functional Groups								
Plant part	Alcohol	Amine	Alkane	Carbonyl	Unsaturated/aromatic	Alkene	COOH	Phenol	Metal (Fe)
C.o leaf	+	+	+	+	+	+	+	+	-
C.o root	+	+	+	-	+	-	-	+	+
A.s leaf	+	+	+	-	+	+	-	+	+
A.s bark	+	+	+	-	+	-	-	+	-

Key + = indicates presence, - = 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	Alkaloids	Cardenolides	Anthraquinones	Saponins	Tannins	Flavonoids
C.o leaf	+	+	+	+	+	+
C.o root	+	+	+	+	+	+
A.s leaf	+	+	-	+	+	+
A.s bark	+	+	+	+	+	+

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

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)
C. odorata root	14.3	14.5	1.5	34.8	11.5
C. odorata leaf	38.6	41.0	7.7	331.7	12.2
A. senegalensis leaf	31.0	31.7	11.5	101.3	12.0
A. senegalensis bark	91.2	93.6	0.5	156.2	14.7

Infrared and Phytochemical tests

The functional groups identified by the IR analysis (Table 1) showed that *Chromolaena odorata* leaf contained alcohols, amides, alkanes, alkenes, carbonyl, unsaturated/aromatic, double bonds, carboxyl and phenol groups. *Chromolaena odorata* root contained alcohols, alkanes, unsaturated/aromatic double bonds, phenolic and metallic groups. *Annona senegalensis* leaf contained alcohols, alkanes, unsaturated/aromatic, double bonds, phenol and metallic groups. *Annona senegalensis* bark contained alcohols, alkenes, alkanes, unsaturated/aromatic double bonds and phenol groups. 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, *C. odorata* root contained alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins. *Annona senegalensis* leaf contained alkaloids, phenols, flavonoids, saponins, cardenolides and tannins while *A. 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 3), *C. 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. *C. 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. *A. 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 *A. 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.

1.1.2 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). 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). The effects of single phytochemical or their mixtures on the physiological processes in nematodes include disruption of membrane permeability, ion uptake, electron transport, alteration of enzymatic activity and cell division (Anaya, 2006).

Plant extracts contain phenolic compounds such as benzoic acid, p-coumaric acid and caffeic acid have been demonstrated to possess nematicidal activity against plant-parasitic nematodes (Shaukat *et al*., 2004; Rotimi and Moens, 2005). Phenols 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). Phenols are involved in causing tolerance of cells against the invasion and the development of nematodes (Tiyagi and Alam, 1995; Siji *et al*., 2010). Plant extracts which contain some water-soluble compounds, probably phenolics have the potential to cause suppression of nematode population densities (Shaukat *et al*., 2002). 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 (Fatoki and Fawole, 2000) and toxic to plant-parasitic nematodes. The alkaloids present in *Chromolaena odorata* have shown nematostatic and nematicidal effects on plant-parasitic nematodes (Thoden *et al*., 2009). The 1,2-dehydropyrrolizidine alkaloids, a class of metabolities are well-known as feeding deterrents against herbivores and are toxic for a wide range of non-adapted animals (Narberhaus *et al*., 2005; Thoden *et al*., 2009) have potential for nematode management. Alkaloids act as protease inhibitors (Wen *et al*., 2013). Anthraquinones are aromatic compounds that occur naturally in certain plants such as senna pods, aloe and rhubard and fungi and insects. Anthraquinones have antibacterial, antiviral, antifungal and antiprotozal 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 antifeeding 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 cellwalls, also disrupting microbial membranes (Ciocan and Bara, 2007).

Tannins are polyphenols that are toxic to small mammals (Fatoki and Fawole, 2000). Tannins act as a defense mechanism in plants against pathogens and herbivores (Kumbasli *et al*., 2011). Tannins 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 heamolysis, 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 (Armah *et al*., 1999; 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). Phenols, alcohols, ketones, acids, aldehydes and hydrocarbons have been reported to be nematicidal in activities (Choi *et al*., 2007)

The two plants, *Chromolaena odorata* and *Annona senegalensis* contain functional and phtyochemical groups that are reported to be nematicidal in activity as they interfere with the enzyme protein structure of the nematodes (Katooli *et al*., 2010). Saponins were of highest concentrations, followed by tannins, total phenols and alkaloids in these plants. The plants have been found to be ovicidal and larvicidal on root-knot nematodes. Thus botanicals obtained from these plants could be used against plant-parasitic nematodes as well as other pests and pathogens of agricultural crops as the phytochemicals are toxic to other pests. Nigeria is rich in flora life and as such, investigations into the chemical constituents of the plants could provide useful data on the availability of botanicals for their usage in pest management. Therefore, the identification and formulation of analogues through basic information by studies such as this one would go a long way in the management of pests and diseases in agriculture. The applications of information on phytochemicals may reduce the use of convectional synthetic herbicides, fungicides, nematicides and insecticides thereby less disruptions of the ecosystem. These could lead to increased agricultural production resulting in providing food security and reducing poverty in developing nations.

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