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Full Length Research Paper

Determination of infochemicals and the phytochemical screening of the foliage and stem-bark of Senna siamea (lam.) in Yola, Adamawa State

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Alkaloids, anthraquinonnes, cardiac glycosides, flavonoids, phlobatannin, polyphenols, saponins, steroids, tannins and terpenoids were ten groups of infochemicals studied in the foliage and stem-bark of Senna siamea (Lam.), a plantation species grown in Yola. The experimental design was the Randomized Complete Block Design (RCBD) with four replications. Results obtained from the experiments indicated that all the ten groups of infochemicals were present in this plant species. It was further observed that there were significant difference between the tree parts of foliage and stem-bark for four infochemicals (alkaloid, flavonoid, saponins, and total phenols) determined ($p > 0.05$). Generally, there were more amounts of total phenols (17.49 ± 0.17) than that of the other infochemicals. However, alkaloids, flavonoids and saponins did not differ significantly at $p < 0.05$ with mean values of 0.06 ± 0.01, 0.06 ± 0.01, and 0.19 ± 0.06 g/g respectively. The results implied that the species had potentials in pharmaceutical, agrochemical and in other allied industries.

Key Words: Infochemicals, tradomedicine, allelopathy, phytotoxicity.

INTRODUCTION

The search for natural products to cure diseases represents an area of great interest in which plants have been the most important source. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these plants bioactive chemical constituents (That is, phytochemicals or infochemicals) are alkaloids; tannins, flavonoids, and phenolic compounds (Hill, 1952; Okwu, 2001).

The knowledge about these infochemicals, including the discovery of several new organic substances is of importance to many disciplines. These include: Botany: utilization of the chemical and biosynthetic knowledge for studying systematics and evolution, as an aid in botanical classification; Ecology: studies on the structural variation of secondary metabolites in space could convey to the discovery of adaptive mechanisms and coevolution of organisms in their ecosystems and to the knowledge of defense, pollination and dispersion strategies of plant species; Pharmacology: chemical diversity of phytochemicals represents an endless source of new drugs and the pharmacological investigation of phytomedicines and accelerate the development of screening techniques; Biotechnology: phytochemical analysis furnishes the background for the selection of species for micropropagation and for monitoring infochemicals produced by cell cultures; etc (Marjorie, 1999; Raimundo, 1999; Akenga et al., 2005; Edeoga et al*.,* 2005). Despite the widely achieved importance of Infochemicals from plants, only very few tropical species have been screened (Edeoga et al*.,* 2005). This is notwithstanding the facts that in many typical African societies like Nigeria, plant extracts are traditionally administered to patients as drugs (Marjorie, 1999; Akenga et al., 2005; Edeoga et al., 2005). For instance, the infusion of the floral parts of *Senna siamea* is one of such patronized for the treatment of typhoid fever in Nigeria. The fact that these plants, whose extracts are administered to patients as drugs, do not pass through a standard pharmacological screening is a major source of concern to many scientists. In addi-

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tion, the upsurge in the drive for tradomedicine could result in increased deforestation along with the resultant adverse effects. To this end, this study attempts to explore the infochemicals present in *S. siamea* through pharmacological means. Thus, the objectives of this study are: to pharmacologically screen the foliage and stem-bark of *S. siamea*, to quantify some of the identified infochemicals, and to compare the concentrations of such bioactive compounds in the foliage and stem-bark of the species.

S. siamea wood is used for furniture, poles, small timber, and fuel wood. It is hard, with specific gravity of 0.6 - 0.8. The whitish sapwood and the dark brown to nearly black heartwood with stripes of dark and light are for charcoal production due to its highly calorific value of 4500 - 4600 Kcal/kg. The wood produces a lot of smoke and is used in intercropping systems, windbreaks, and shelterbelts. It is also used as a shade tree in cocoa, coffee, and tea plantations (F/FRED, 1994). The tree produces an extensive root system in the upper layer of the soil and, in intercropping systems, can aggressively compete for nutrients and water (Gutteridge, 1997). The leaves and seeds can be eaten by ruminants (Sahni, 1981) but are toxic to non ruminants such as pigs and poultry. The young leaves and flowers are used in curry dishes. The species is also used for the production of honey and tannins. *S. siamea* is hardly used in afforestation in the Sahel, but in the Sudanian ecozone (Burkill, 1995).

MATERIALS AND METHODS

Sample collection and preparation

Fresh, undamaged and matured foliages (about 1 kg) were collected from several parts of the inner most canopies of the mean trees as well as stem-bark for the species studied in four replications. These samples were obtained from plantations in Sangere village near the Federal university of Technology main campus in Yola.

Yola is located in Adamawa State at the Northern Guinea Savannah region of Nigeria between latitude 9° 14' and longitude 12° 38' E at an altitude of 158.5 m above sea level (Kowal and Knobe, 1972). It has an annual average minimum temperature of 15.2°C while the maximum is 39°C. The minimum average annual rainfall is 0.4 mm while the maximum is 475 mm with a total rainfall of 1030 mm per annum. Its relative humidity ranges between 15% during the coldest part of the year and 93% (May - September) (Adebayo, 1999; FUTY, 1999).

The preparation of samples collected from the field was according to method described by Edeoga et al. (2005) (That is,) the foliage and stem-bark collected from the experimental sites were air dried at room temperature. These were then ground into uniform powder using a Thomas Wiley Milling Machine. The samples were then sieved, bottled, labeled, and were used for laboratory analysis.

Chemicals procurement

All reagents were purchased from Sigma Aldrich chemical Co. Uk.

Experimental design and treatments

The experiment was carried out in a Randomized Complete Block Design (RCBD) with four replications. The blocks were the two tree parts (foliage and stem-bark) and the treatments constitute the infochemicals.

Procedure for phytochemical screening

Standard Pharmacological procedures were used to screen for the infochemicals (qualitatively and quantitatively). These phytochemical tests were carried out using aqueous specimens. Qualitatively, alkaloids, flavonoids, and polyphenols were screened according to the procedure outlined by Akenga et al. (2005). Anthraquinones was screened according to Borntrager's test as described by Trease and Evans (1989). Cardiac Glycosides and Saponins were screened according to the methods described by Sofowora (1982). Steroids, Tannins, Terpenoids, Phlobatannins were screened according to the methods described by Edeoga et al. (2005).

Quantitatively, alkaloid was determined using the procedure put forward by Harborne (1973) as described by Edeoga et al. (2005). Briefly, five grammes (5 g) of the powdered sample were weighed into 250 ml beaker. 100 ml of 10% acetic acid in ethanol was then added. The mixture was covered and allowed to stand for 4 h. This was then filtered and the extract concentrated on a water bath to ¼ of the original volume. Thereafter, concentrated ammonium hydroxide added drop wise until precipitation was completed. The solution was then allowed to settle and the precipitate collected, washed with diluted ammonium hydroxide and filtered. The residue that was dried and weighed was alkaloid.

Total phenols were quantified according to the methods described by Edeoga et al. (2005). Briefly, one gramme (1 g) of each plant sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic components for 15 min. Then, 5 ml of the extract pipette into a 50 ml flask, 10 ml of distilled water, 2 ml ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added. The sample was then made up to mark and left to react for 30 min for color development. This mixture was then measured at 505 nm using a spectrophotometer (754 UV- Vis, All Pro- Corporation, U.S.A).

Flavonoids were determined by the methods developed by Boham and Kocipaiabyazan (1994). Briefly, 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No. 42 (125 mm) filter paper. The filtrate was later transferred into crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weight is flavonoids.

Finally, saponins were determined according to the method described by Obadoni and Ochuko (2001). According to this method, 10 g of the powdered sample for each plant species was transferred into a conical flask, and 50 ml of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 h while stirring continuously at 55°C. Thereafter, the mixture was filtered and the residue re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. Then, the concentrate was transferred into a 250 ml separatory funnel. 10 ml Diethyl ether was added to the funnel and the mixture shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

In addition, 30 ml of n–butanol was added. The combined n-butanol extract was washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was then heated in a water bath.

Table 1. Phytochemical screening of *Senna siamea.*

Plant Part	Alkaloids	Anthra-Quinones	Cardiac Glycosi-Des	Flavonoids	Poly-Phenols	Saponins	Steroids	Tannins	Terpe-Noids
Foliage	+	-	+	+	+++	+	++	++	-
Bark	+	+++	+	-	+	+++	-	++	+

- = beyond detectable limits; + = Slight coloration; ++ = Deep coloration; +++ = Very deep coloration.

Table 2. Weight (Mean values in g/g ± SD) of bioactive secondary compounds in *Senna siamea*.

	Alkaloids	Flavonoids	Saponins	Total Phenols
Foliage	0.09 ± 0.01	0.08 ± 0.01	0.23 ± 0.01	10.21 ± 0.25
Stem-Bark	0.03 ± 0.01	0.04 ± 0.01	0.15 ± 0.01	24.77 ± 0.15
Mean	0.06 ± 0.01	0.06 ± 0.01	0.19 ± 0.01	17.49 ± 0.17
LSD	ns	0.03	0.08	0.49

After evaporation, the samples were dried in a hot air oven at 70°C to a constant weight. The weight of samples recorded was the saponin.

Statistical analysis

Data collected were analyzed statistically using the analysis of variance (ANOVA) fixed effects model. The significantly different means were further separated using the Fisher's least significant difference (LSD) at P = 0.05.

RESULTS

Phytochemical screening

Table 1 indicates the result of the phytochemical screening of the foliage and stem-bark of *S. siamea*. The table indicated that anthraquinnones and saponins were highly present (as indicated by the very deep coloration) in the stem-bark of the plant while polyphenols were highly present in the foliage. Flavonoids were observed with slight coloration in the foliage but beyond detectable limits in the stem-bark. In addition, a very deep coloration was observed in the stem-bark of the plant for saponins which is indicative that saponins were highly present. Phlobatannins were beyond detectable limits in both parts of the plant for this species. Tannins were observed as moderately present while alkaloids were slightly present in the plant as indicative of the slight and deep coloration of the test samples.

Quantitative determination of bioactive Secondary Plant Compounds

Table 2 indicates that there was significant differrence in the concentration of the infochemicals in this plant species and between its foliage and bark ($P < 0.05$). A further glance at the results presented in the table 2 indicates that there was more concentration of total phenols (17.49 ± 0.17 g / g) in *S. siamea.* However, saponins, flavonoids and alkaloids did not differ significantly in the species (0.06 ± 0.01 g / g, 0.06 ± 0.01 g / g, and 0.19 ± 0.01 g / g respectively).

DISCUSSION

Results had indicated that phenols, saponins, flavonoids and alkaloids are present in the tested species; consequently, this species could serve as a good source for phenolics, saponins, alkaloids and flavonoids. These bioactive secondary plant compounds are not only useful in cosmetic industries but also in drugs as anti-malarial, anti-diarrhea, anti-inflammatory, anti-germ, anti-tumor, anti-oxidant, vasorelaxation agents, antimicrobial, anti-oxidative damage disease, etc. and other industries such as in nitric Oxide production, manufacture of detergents- especially laboratory detergent as surfactant production of photographic emulsion, cosmetic and shampoos. Since these bioactice compounds are responsible for such actions, it is possible that the plant species could

impact allelopathic effects on other organisms in their ecotype (Omulokoli, et al., 1997; Majorie, 1999; Ray, 1999; Zeev and Bishanu, 2003; Wikipedia, 2006; Michael, 2004; Ray, 2005).

The presence of steroids in this plant makes *S. siamea* useful to many industries. For instance, it should be noted that steroidal compounds are of importance in pharma-ceutical companies due to their relationship with such compounds as sex hormone (Okwu, 2001; AAP, 2006). Therefore, *S. siamea* might be considered by pharmaceutical companies as a good source for the manufacturing of sex hormones. Apart from this, the presence of terpenoids signifies that the plant could be an important source for antimicrobial substance which might help in conditions such as nervous, cardiac, and digestive disorders. It could also serve as a good source for analgesics, garlic acids, pyrogallol, inks and mordant in brewing industries (Marjorie, 1999; Hufford et al., 1993; Batista et al., 1994; Norton, 2000; Funk and Wagnalls New Encyclopedia, 1975). Cardiac glycosides detected in this plant indicated that the plant could be a good source of birds and insects repellants (Funk and Wagnalls New Encyclopedia, 1975).

Taking the plant parts of the *S. siamea* as drugs could be beneficial and / or dangerous. For instance, some alkaloids in plants might be poisonous while others might help in the cure for some diseases. Special example is in curarine, found in the deadly extract curare, which is a powerful muscle relaxant; atropine is used to dilate the pupils of the eyes; and physostigmine is a specific for certain muscular diseases. Narcotic alkaloids used in medicine include morphine and codeine for the relief of pain and cocaine as a local anesthetic (Funk and Wagnals New Encyclopedia, 1975).

Conclusion

The study concludes that all groups of the ten studied infochemicals (alkaloids, anthraquinnones, flavonoids, saponins, phlobatannins, polyphenols, steroids, terpenoids, phenols and tannins) were present in some amount in this species. Thus, *S. siamea* should be considered as a poten-tial source of useful drugs, insecticides and possibly herbicides. It might be very useful as raw materials for other industries such as cosmetics, breweries, and possibly paper and pulping industries. However, if the desired goal is agrisilviculture (agroforestry), agriaquasilviculture or agripasture-silviculture, then these plants species should be avoided for their possible toxicity. Lastly, the possible production of dyes, inks, pesticides and insecticides in commercial quantity should be considered as it will help the society not only economically but also help solve our immediate societal problems of pests and diseases.

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