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SPhytochemical and Cytototoxic Screening of the Leaves of *Cassia nigricans* Vahl

[1]R.G. Ayo and [2]J.O. Amupitan
[1]Samaru College of Agriculture, Division of Agricultural Colleges,
Ahmadu Bello University, Zaria, Nigeria
[2]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

Abstract: Phytochemical tests of the extracts of the leaves of *Cassia nigricans* Vahl revealed the presence of carbohydrates (reducing and combined sugar, starch), glycosides (anthraquinone, cardiac and saponin types); flavonoids, alkaloids, steroids and triterpenenes. Brine shrimp lethality bioassay of the petroleum ether, ethyl acetate, methanol and chloroform extracts of the leaves showed that the extracts were highly cytotoxic. The results of Finney Computer Programme calculations of LC_{50} (lower-upper limits) of the extracts in the solvents were 149.57 (66.86-243.75), 42.77 (11.80-72.94), 26.91 (3.09-52.64), 11.064(0.052-40.39), respectively at 95% confidence intervals and 3 degrees of freedom. The extracts of the leaves of *Cassia nigricans* contain biologically active compounds, which may be of potential use in the therapy and prophylactics of plant, livestock and human diseases.

Key words: *Cassia nigricans, Leguminosae*, leaves, phytochemical, cytotoxicity

INTRODUCTION

Cassia species (Family: *Leguminosae-Ceasalpinoideae*) are well known for their laxative and purgative properties and are also used in the treatment of skin diseases (Dalziel, 1956; Irvine, 1961). The laxative effect is mainly due to the presence of hydroxyanthraquinone derivatives. The commonly used species are *Cassia angustifolia, C. acutifolia, C. fistula, C. sieberiana, C. occidentalis, C. spectabilis, C. tora, C. alata* and *C. podocarpa* (Ajim, 1986; Kinjo *et al*., 1994; Abo *et al*., 1999). Of the thirty-three species growing in Nigeria, only a few have been investigated for their medicinal uses as claimed by traditional healers (Elouioba *et al*., 1999; Abo *et al*., 2000). The comprehensive phytochemical and biological evaluations of the different morphological parts of the numerous local *Cassia* species are scanty (Abo *et al*., 1999). Some *Cassia* species are toxic and may result in death when used in overdose, some species contain hydrocyanic acid (HCN), which is very toxic to man and animals even in very small dose. Most species also contain anthraquinones which are also toxic in overdose (Lewis and Elvin-Lewis, 1977). For example, *Cassia occidentalis* used as purgative, diuretic and anticonvulsant has toxic effect in man. It can damage the kidneys and liver and cause muscle degeneration (Nwude, 1986). It was also found that prolonged administration of *C. tora* can cause severe clinical signs and pathological lesions and death in mice (Ambali *et al*., 2005).

Cassia species such as *C. reticulata, C. alata, C. fistula, C. angustifolia, C. acutifolia, C. podocarpa, C. spectabilis, C. obtustifolia* are known to contain sennosides, rhein emodin, aloe-emodin, 1,3,8-trihydroxy-2-methyl anthraquinone (Kinjo *et al*., 1994; Abegaz *et al*., 1996; Yang *et al*., 2003). *Cassia alata* contains azulene, guiane, 1,5,7- trihydroxy-3-methyl anthraquinone (Kitanaka and Takido, 1995). Di-(2-ethyl)hexylphthalate has been isolated from the leaves of *C. auriculata* and the seeds are rich in oleic and linoleic acids (Balogan and Fetuga, 1985; Nageshwara and Mahesh, 2000).

Cassia nigricans Vahl is among the thirty-three *Cassia* species growing in Nigeria. It is a woody annual herb or undershrub with pubescent stems. It is widespread in tropical Africa in cultivated grounds, old clearings, by the roadsides and open grassy areas. The roots and leaves are widely used in traditional medicine in Senegal and Guinea and are considered as anti-periodic and substitute for quinine. A root infusion is given as a vermifuge in some parts of West Africa. The pulverised leaves are appetizers and febrifuge. The leaf infusion is also used for treating sore throat (Dalziel, 1956; Irvine, 1961). Recent studies revealed that the aqueous extract of the leaves possesses significant anti-ulcerogenic properties. In northern Nigeria, it is used locally for treating stomach ache, skin diseases and yellow fever (Akan *et al*., 1998; Nwafor and Okwuasaba, 2001).

Corresponding Author: R.G. Ayo, Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Nigeria

MATERIALS AND METHODS

The leaves of *Cassia nigricans* Vahl (Hausa *jiwo tsamiya* or *shuwakan gargari*) were collected from Jama'a village near Ahmadu Bello University Dam, Zaria (11° 10'N, 07° 38'E), Nigeria. The plant was identified with Voucher Specimen Number 613 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Extraction procedure: The leaves were air-dried and powdered. Soxhlet extraction of 250 g of the powdered leaves was carried out using each of the following solvents: petroleum ether (60-80°C), ethyl acetate, chloroform and methanol, respectively. Each extract was concentrated and evaporated to dryness on a rotary evaporator.

Preliminary phytochemical analysis: The preliminary phytochemical screening of *Cassia nigricans* leaves was carried out using standard laboratory procedures (Harbone, 1984; Trease and Evans, 1989; Silva *et al*., 1998). The results are shown in Table 1.

Brine shrimp lethality bioassay (Cytotoxicity): The Brine Shrimp Lethality (BSL) bioassay was carried out, each on petroleum ether, ethyl acetate, chloroform and methanol extracts using standard procedure (Meyer *et al*., 1982; Melaughlin, 1991). The LC_{50} values were calculated using the Finney Computer Program at 95% confidence intervals and 3 degrees of freedom (Finney, 1971).

Table 1: Phytochemical screening of extracts of the leaves of *Cassia nigricans* vahl

Constituents	CNLE	CNPE	CNEA	CNCH	CNME
Carbohydrate					
General test for carbohydrate	+	+	+	+	+
Monosaccharides	+	–	–	–	+
Free reducing sugars	+	–	–	–	+
Combined reducing sugars	+	–	+	+	+
Pentoses	–	–	–	–	–
Ketoses/fructose	–	–	–	–	–
Starch	+	–	–	–	+
Glycosides					
General test for glycosides	+	+	+	+	+
Anthraquinones	+	+	+	+	+
Saponin	+	–	–	+	+
Cardiac	+	+	+	+	+
Flavanoids	+	+	+	+	+
Cyanogenetic	–	–	–	–	–
Anthraquinones glycosides					
Free anthraquinones	+	+	+	+	+
Combined anthraquinones	+	+	+	+	+
Cardiac glycosides					
Cardenolides	+	+	+	+	+
Steroidal aglycone	+	+	+	+	+
Triterpenes	+	–	+	+	+
Alkaloids					
Tropane alkaloids	–	–	–	–	–
Tannins	–	–	–	–	–

Key: + = Present, – = Absent, CNLE = *Cassia nigricans* leaves, CNPE = Petroleum ether extract, CNET = Ethyl acetate extract, CNCH = Chloroform extract, CNME = Methanol extract

RESULTS AND DISCUSSION

The methanol extracted the most polar and higher component (6.48%) of the leaves of *Cassia nigricans* Vahl. The weights and percentage yields of each extract are shown in Table 2. The results of phytochemical screening of the leaves of *Cassia nigricans* are shown in Table 1. The general test for carbohydrates was positive and the specific tests for free and combined reducing sugars as well as starch were positive. The general tests with the specific tests for saponin, cardiac, anthracene glycosides were also positive. Further tests on the anthracene derivative showed that it is an easily reducible form as the iron (III) chloride tests gave the expected cherry red colouration. Tests for the cyanogenic glycosides and tannins gave negative results.

Since the leaves contain anthraquinone and gave negative results for the presence of tannin and hydrocyanic acid, they can be used as substitute for quinine and, like any other species of *Cassia*, as purgative, laxative and for treating fever and skin diseases.

Table 2: Weight and percentage yield of extracts from 250 g of the leaves of *Cassia nigricans* vahl

Solvent	Weight of extract	% Yield
Petroleum ether (60-80°C)	9.10	3.64
Ethyl acetate	10.17	4.18
Chloroform	2.97	1.19
Methanol	16.20	6.48

Table 3: Brine shrimp lethality bioassay of chloroform extract of the leaves of *Cassia nigricans* vahl

Concentration (μg cm^{-3})	1000	500	250	125	62.5
Number of shrimps per test sample	30	30	30	30	30
Number of survivors	0	3	4	5	7
Number of deaths	30	27	26	25	23
Percentage mortality	100	90	86.7	83.3	76.7

LC_{50} = 11.0639 (0.0052 - 40.39)

Table 4: Brine shrimp lethality bioassay of methanol extract of the leaves of *Cassia nigricans* vahl

Concentration(μg cm^{-3})	1000	500	250	125	62.5
Number of shrimps per test sample	30	30	30	30	30
Number of survivors	0	0	2	5	8
Number of deaths	30	30	28	25	22
Percentage mortality	100	100	93.3	83.3	73.3

LC_{50} = 26.91 (3.09-52.64)

Table 5: Brine shrimp lethality bioassay of ethyl acetate extract of the leaves of *Cassia nigricans* vahl

Concentration(μg cm^{-3})	1000	500	250	125	62.5
Number of shrimps per test sample	30	30	30	30	30
Number of survivors	0	2	4	7	12
Number of deaths	30	28	26	23	18
Percentage mortality	100	93.3	86.7	76.7	60

LC_{50} = 42.77 (11.80-72.94)

Table 6: Brine shrimp lethality bioassay of petroleum ether extract of the leaves of *Cassia nigricans* vahl

Concentration (μg cm^{-3})	1000	500	250	125	62.5
Number of shrimps per test sample	30	30	30	30	30
Number of survivors	6	10	12	15	20
Number of deaths	24	20	18	15	10
Percentage mortality	80	66.7	60	50	33.3

LC_{50} = 149.57 (66.86-243.75)

The BSL bioassay was selected for screening the leaves of the plant because it is a convenient general bioassay, indicative of cytotoxic and other pharmacological effects. The results of the BSL bioassay are summarised in Tables 3-6. The extracts of the leaves were moderately cytotoxic at high concentrations. The chloroform extract showed the strongest cytotoxic activity (LC_{50} = 11.064), followed by methanol extract (LC_{50} = 26.91). The petroleum ether extract was least cytotoxic (LC_{50} = 149.57). Thus, all the extracts were found to be cytotoxic. It seems promising to attempt to isolate and identify the constituents of the leaves of the plants since all the extracts showed strong cytotoxic activity.

CONCLUSION

The extracts of the leaves of *Cassia nigricans* contain biologically active compounds, including carbohydrates, reducing sugars, anthracene, flavonoids, saponin and cardiac glycosides as well as alkaloids, which may be of potential use in the therapy and prophylactics of plant, livestock and human diseases.

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