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Phytochemistry and Antimicrobial Activity of the Leaf of *cassia alata* LINN.

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ABSTRACT

The leaves of cassia alata Linn were successively extracted with n-hexane and ethylacetate using the soxhlet method. Preliminary phytochemical screening of the extracts revealed the presence of free anthraquinones, flavonoids, steroids and saponins. The n-hexane crude extract exhibited some anti – bacterial activity against Yersinnia enterocolitica, streptococcus pneumonia and salmonella typhi. Anti–fungal activities against Microsporum audouini and Trichophyton meritagrophyta were also exhibited. A synergic test of n–hexane and ethylacetate extracts showed an improved sensitivity against shigella sormei and strep. pneumonia. A confirmatory phytochemical analysis performed on the most mobile tlc isolate (R_f 0.94) from the n-hexane extract revealed the presence of steroidal saponin. This was found to be active against strep. pneumonia.

Key words: Cassia alata, Phytochemistry,Steroids and Antimicrobial activity

1.0 Introduction

A number of plant have been reported in literature to possess antibacterial and antifungal properties. Among the plants with such activities is cassia, alata Linn. It is one of the `18,000 species of leguminosae family. This plant is a small perennial shrub, which grows rapidly in full sun on a wide range of soils from a height of 3m to 4.5m with a straight, wooden stem (Giron, 1991). The large leaves are bilateral – symmetrical opposed and fold together at night. The fruit is a pod with a woody brown shell about 120cm long and hard to touch.

Cassia alata is a common plant in Japan, Indonisia, Mexico, Ghana, Trinidad and Nigeria (Giron, 1991). The leaves have been reported to be sudorific, diuretic and purgative. According to Bhat, Eterjere, and Okdipo (1990), the leaves have been used for the treatment of constipation, ringworm and other skin diseases. The leaves in decoction are also used to treat bronchitis and asthma. The use of the leaves of the plant as anti venom and as an abortificient are also known (Bhat, et,al 1990).

The present work was therefore designed to do the following:

i. identify the phytochemicals present in the non-polar n-hexane and ethylacetate extracts of the leaves of cassia alata Linn.
ii. Confirm the antibacterial and antifungal activity of the non-polar extracts of the leaves of the plant.
iii. Purify the crude extracts in order to identify the principles responsible for the activity.

2.0 Experimental

2.1 Collection and Preparation of Plant Materials

The fresh leaves of the plant cassia alata Linn, were collected from their plants on the field in Bauchi, 430km North – East of Abuja, Nigeria. identification and authentication of the plant were carried out by experts in the herbarium section of the biological sciences programme of ATBU, Bauchi.

The leaves were then air-dried at room temperature, ground into powder and sieved with a 1.5mm plastic sieve.

2.2 Extraction

200g of the powdered leaves of *cassia alata* Linn was successively extracted with soxhlet extractor using each of the following solvents: n-Hexane (30 – 40^0C) and ethylacetate (30–40^0C) in that order. The extraction was carried out at near the boiling point of the extracting solvent. Each extract was concentrated in-vacuo with the aid of a rotary evaporator. The respective extract concentrates were kept in desiccators to dry for at least 3 days before further tests were carried out on them.

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the crude n-hexane and ethylacetate extracts. Known standard procedures for phytochemical screening were adopted to test for the presence of cardiac glycosides, glycosides, saponins, anthraquinones, tannins, terpenes, steroids alkaloids and flavonoids (sofowora, 1993). These same phytochemicals were tested for in the tlc fraction obtained from the n-hexane extract.

2.4 Chromatographic Purification of n-hexane Crude Extract.

To purity the n-hexane extract, various fractions were spotted on a silica tlc plates and developed using various solvent mixtures. It was observed that a 17:3 chloroform/n-hexane mixture gave the best resolution. This solvent mixture was used to resolve these fractions. The most mobile yellow band (R_f: 0.94) on a preparative tlc (Braithwaite and Smith, 1985) plate was isolated with hexane as a yellow oil. This was further dried several days in a desiccator.

2.5 Antimicrobial Studies

Pure clinical isolates of steptoccocus pneumonia, salmonella typhy, E.coli, yersinia enterocolitica, shigella sormei, microsporum audouinii and trichophyton mentagrophyte were obtained from the National Veterinary Research Institute, (NVRI) Vom, near Jos in Plateau State. The bacteria were grown on nutrient agar slants in an incubator at 37^0C for 24 hours while the fungi were sub-cultured on the slant sabourand dextrose agar and incubated at 25^0C for 48 hours. Growth of microorganisms in the broth was indicated by turbidity. The turbidity produced was adjusted to

match 0.5 Mcfarland standards (10^8 cfu/ml), which was further adjusted to 10^5 cfu/ml and 10^3 cfu/ml for bacteria and fungi respectively.

The cork and bore diffusion method was used to carry out the antimicrobial screening. Solutions of the crude extracts were prepared for the n-hexane and ethylacetate extracts. 20mg/ml of each was prepared.

Meanwhile, inoculation of the prepared plates with the organisms was done using wire – loop to transfer strands of the organisms into the plates followed by a cross streaking with the same wire loop to achieve uniform spread of the microbes on the plates. These were done for both the nutrient agar (NA) and sabourand dextrose agar (SDA) plates. After about 30 minutes, wells were punched on the plates using a sterile cork borer of 5mm diameter.

A 0.1ml of the crude extract solution was introduced into each appropriately labeled well. Tetracycline of the same concentration as the extracts was used as control for NA plate containing bacteria while climaxol was used as control for SDA plates containing the fungi. After proper diffusion had taken place the plates were incubated at 37^0C for 24 hours for bacteria and 48 hours at 25^0C for fungi. Zones of inhibition of growth were determined by linear measurement of diameter.

This same procedure was adopted for the pure yellow oil fraction obtained from the preparative tlc studies.

2.6 Test for Synergism

Synergic effect of the extracts of the leaves of cassia alata Linn was tested on the human pathogens used for antimicrobial activity test. This was carried out by measuring equal amounts of the n-hexane and ethylacetate extract and thus preparing a solution of the mixture. The cork and bore diffusion method was repeated for the mixture to test its sensitivity.

3.0 Results

Table 1.0: Phytochemical constituents of the leaf of cassia alata Linn

Metabolites / Extracts	1	2	3	4	5	6	7	8	9
HE	-	++	-	++	++	-	-	-	++
EAE	-	++	-	++	++	-	-	-	++

HE = n-hexane extract. EAE = ethylacetate extract.

l = Alkaloids 2 = Free anthraquinones, 3 = Tannins

4 = Saponins 5 = Steriods 6 = Terpenes

7 = Glycosides 8 = Cardiac glycosides 9 = Flavonoids

Table 2.0: Results of antimicrobial activity test of crude extracts from the leaf of *cassia alata* Linn.

Test Organism	Diameter of Zone of Inhibition (mm)				
	HE	EAE	HE/EAE	TCN	C
Strep pneumonia	20.0	10.0	22.0	13.0	NA
Shigella sormei	6.0	8.0	20.0	13.0	NA
Yersinnia enterocolitica	12.0	10.0	12.0	11.0	NA
Escherichia coli	7.0	10.0	7.0	12.0	NA
Salmonella typhi	12.0	9.0	6.0	15.0	NA
Microsporum audouinii	25.0	22.0	16.0	NA	18.0
Trichophyton meritagrophyta	23.0	22.0	23.0	NA	20.0

HE = n-hexane extract, EAE = Ethylacetate extract.

HE/EAE = a mixture of equal amounts of HE and EAE

TCN = Tetracycline (control against bacteria)

C = Climaxol (control against fungi)

NA = Not applicable.

Table 3.0: sensitivity test of the pure component of n-hexane extract of the leaf of cassia alata Linn.

	Zones of Inhibition (mm)		
Test Organisms	HE	TCN	C
Strep. pneumonia	25.0	17.0	NA
yersinnia enterocolitica	20.0	30.0	NA
Salmonella typhi	0.0	17.0	NA
Microsporum audouinii	29.0	NA	38.0
Trichophyton mentagrophyte	10.0	NA	17.0

HE_f = Preparatory tlc fraction from n-hexane extract.

4.0 Discussion of Results

Table 1.0 shows the phytochemical constituents of the leaves of cassia alata Linn. Anthraquinones, saponins, steroids and flavonoids were present in both solvents used for extraction. On the other hand alkaloids, tannins, terpenes, glycosides and cardiac glycosides were not detected in the solvents.

Anthraquinones according to Mohammed (1994), are known for their purgative (cathartic) property, especially in habitual constipation. Saponins on the other hand are known to possess both antimicrobial and anti-inflamatory properties (Margineanu, Cucu, Grecu & Parvu, 1976; Singh, Ehana & Dhar, 1974). They are also known to serve as precausor of steroidal substance with a wide range of physiological activities. Pharmacologically, some flavonoids have also been found to exhibit antitumour, antibacterial and antifungal properties (Trease and evans, 2002; Finar, 1980). The presence of these phytochemicals may account for the spectrum of activity of the plant.

The n-hexane extract of the leaves of cassia alata Linn exhibited some antibacterial activity against yersinnia enterocolitical streptococcus pneumonia and salmonella typhi.

Also ethylacetate extract demonstrated anti-bacterial activity against strep. pneumonia, yersinnia enterocolitica and E. coli. Both n-hexane and ethylacetate extracts were found to be active against microspurum audouinii and Trichopyhton mentagrophyta. These were the two fungi used for the test (Table 2.0). A synergic test of n-hexane and ethylaceate crude extracts show an improved activity against Shigella sormei and streptococcus pneumonia (Table 2.0). Preparatory tlc carried out on the n-hexane extract resulted in the isolation of a yellow and oily component (R_f 0.94): a confirmatory phytochemical analysis performed on this isolate revealed the presence of steroidal saponin. This was found to be active against strep. Pneumonia (Table 3.0).

The results of the antimicrobial activity of the crude extracts are in agreement with the uses of the extracts of the leaves of *cassia alata* Linn in traditional medicine for the treatment of constipation, sexually transmitted disease (STD), bronchitis and asthma, ring worm and other skin diseases. These results further substantiate the literature reports that extracts of the leaves of *cassia alata* Linn have both antibacterial and anti-fungal properties (Okafor, Eze and Njoku, 2001; Ibrahim & Osman, 1995; somchit, Reezal, Elsha and Mutali, 2003). The leaves of the plant therefore appear to be a potential source of broad spectrum antibiotics.

5.0 Conclusion

In conclusion, the antimicrobial screening gave some justification for the use of the leaves of cassia alata Linn in the traditional medicine. The results have also shown that n-hexane and ethylacetate are good solvent for the extraction of phytochemicals from the leaves of *cassia alata* Linn. spectroscopic studies are in progress to characterize the active principles in the leaves of the plant.

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