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Phytochemical and Chromatographic Studies of the Polar Extracts of the Leaves of *Cassia alata* Linn.

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Abstract

The dried leaves of *cassia alata* Linn were pulverized and extracted successively with hexane, ethylacetate, methanol and water in that order. Phytochemical screening of the methanol extract and water extract (polar extracts) showed that both extracts contained anthraquinones, flavonoids and saponins. Methanol extract, however, contained terpenes in addition to those mentioned above. Thin layer chromatography gave three fractions for each of the extracts. The Rf values for methanol extract fractions Me1, Me2, Me3 and water extract fractions We1, We2, We3 were 0.402, 0.646, 0.744and 0.333, 0.583, 0.833 respectively. Crude extracts as well as the purified fractions were tested on five bacteria (*Escherichia coli, Salmonella typhi, Streptococcus pneumonia, Yesinia enterocolitica and Shiggela sonnei*) and two fungi pathogens (*Microsporum audouinii and Trichophyton mentagrophyte*). Methanol fraction Me3, accounted for the antifungal activity while water fractions We2 and We3 accounted for the antibacterial activity.

Key Words: *Cassia alata*, antibacterial activity, anthraquinone, purgative.

1. Introduction

Cassia alata is one of the 18,000 species of leguminosae family. In Nigeria, it is referred to by the Yoruba speaking people as 'asuwon'. Its common names are wild senna, candle bush, ringworm cassia etc. This plant, a 6-25 feet tall, perennial shrub, has erect waxy yellow spikes that resemble fat candles before they blossom open. The large leaves are bilateral-symmetrical opposed and fold together at night. The fruit is a pod, whose length ranges from 6-12inches with dry and hard covering. The seeds are small and square, and do not attract wildlife (Hutchinson et. al., 1976).

The leaves are reported to be sudoforic, diuretic and purgative, being used in the same manner as senna. The leaves in decoction are used in Thailand for the treatment of constipation and skin diseases (de Padua *et. al.*, 1999; Farnsworth and Bunyapraphatsara, 1992). As a result of the traditional uses in Thailand, *Cassia alata* has been approved as a laxative drug in the Thai Herbal pharmacopoeia 1998 and the Thai National list of essential drug 1999 (subcommittee on the establishment of the Thai herbal pharmacopoeia, 1998).

In Nigeria, Guatemala and Malaysia, the leaves of *Cassia alata* are used to treat various skin diseases (Carceres *et. al*., 1991; Ibrahim and Osman, 1995; Gill and Akinwumi, 1986; Ogunti *et. al*., 1991; Benjamin and Lamikanra, 1981); also against stomach disorders in Nigeria, Gabon, Malaysia and Cameroon (Ibrahim and Osman, 1995; Bhat *et. al*.,1990; Akendengue and Louis, 1994; Ahmad and Holdsworth, 1995; Noumi and Yomi, 2001).

The efficiency of herbal medicines depends on the plant raw material quality, which is usually related to the content of the active compounds (Thaweephol *et. al*., 1993). Hydroxyanthracene derivatives were found to be the active constituents in this plant (Elujoba *et. al*., 1989). There are also reports that the hydroxyl anthracene derivatives are more concentrated in the younger and mature leaves rather than in the older leaves (Panichayupakaranant and Intaraksa, 2003).

2. Materials and Methods

2.1 Plant materials:- Experts at the Federal College of Forestry, Jos-Nigeria, carried out the identification of the plant after the leaves were obtained from Bauchi, 430km North East of Abuja-Nigeria, The leaves were air dried at

room temperature and pulverized in a mortar. The powder was sieved with a 1.5mm plastic sieve. The powder was stored in plastic bags at room temperature and was used for the present study within months.

2.2 Extraction

A 200g sample of the powdered leaves was wrapped in a filter paper and carefully placed into the porous thimble. The thimble was then inserted into the inner tube of the sohxlet extractor. The loaded thimble was then fitted to a 500cm^3 round-bottomed flask containing hexane to boil over a heating mantle until the vapour passed up through the tube into the condenser. The condensed solvent extracted the plant material in the thimble into the flask. The process was allowed to run for 4hours. At the expiration of 4hours, when it was evident that there was no longer extraction by the solvent, the process was stopped. The wrapped sample was removed from the thimble and dried before returning it back into the thimble. This process was repeated using ethylacetate at 30-40^{o}C for 12hours, methanol at 50-60^{o}C for 8hours and water at 60-70^{o}C for 12hours in that order. Each extract was concentrated *in vacuo* with the aid of a rotary evaporator, and the respective extract concentrates were kept in desiccators to dry for atleast 3 days before further tests were carried out with them.

2.3 Phytochemical Screening

The extracts were examined for the presence of cardiac glycosides, saponins, anthraquinones, tannins, terpenes, steroids, alkaloids and flavonoids.

2.3.1 Test for cardiac glycosides

Keller Killiani test

A small quantity (0.5g) of the extract was dissolved in a small quantity of glacial acetic acid containing one drop of iron (iii) chloride solution. This was then under laid with concentrated tetraoxosulphate (VI) acid. No brown ring was observed at the interface between the glacial acetic acid and the tetraoxosulphate (VI) acid. This indicated the absence of a deoxy sugar characteristic of cardenolides (Trease and Evans, 1989).

2.3.2 Test for saponins (Frothing test)

Small portions of each of the plant extracts were shaken vigorously with 10.0cm^3 of distilled water in a test tube. The test tubes were placed in a water bath. Frothing which persisted on warming was taken as evidence for the presence of saponins.

2.3.3 Test for anthraquinones: Borntrager's test: 0.5g each of the plant extract was shaken with 2.0cm^3 of benzene and filtered. 4.0cm^3 of 10% ammonia solution was added to the filtrate. The resultant mixture was shaken and the presence of a red colour in the ammonia solution (lower layer) phase was taken for the presence of free anthraquinones.

2.3.4 Test for steroids and Terpenes

A solution of each of the extracts was made by dissolving 0.5g of the extract in 2.0cm^3 of chloroform. The solution was subjected to Liebermann-Burchard test where a bluish-green or blue colour was taken for the presence of steroids while a pink colour was taken for the presence of terpenes.

2.3.4.1 Liebermann-Burchard test

To the solution of the plant extract in chloroform, 3.0cm^3 of acetic anhydride was added, mixed gently and allowed to cool in ice. This was followed by the careful addition of a few drops of concentrated H_2SO_4. A pink colour was observed.

2.3.5 Test for flavonoids

2.3.5.1 Lead acetate test: - 0.5g of the extract was dissolved in 5cm^3 of distilled water and 1cm3 of 10% lead acetate solution was added to it. The appearance of a yellow precipitate was considered a positive test for flavonoid.

2.3.5.2 Iron (ii) chloride test: two drops of iron (ii) chloride were added to a solution of the plant extract made by dissolving 0.5g of the crude extract in water. A dark colouration was taken for the presence of phenolic compounds.

2.3.6 Test for tannins:- about 0.5g of each of the plant extract was boiled with 10cm^3 of distilled water for 5minutes. The solution was filtered and the filtrate obtained. To 2.0cm^3 of the cooled filtrate, a few drops of ferric chloride were added. The absence of a blue-black, green, or blue-green precipitate was taken for the absence of tannins.

2.3.7 Test for alkaloid:- 0.5g of each extract was dissolved in 5cm^3 of 2M HCl and the resultant solution was warmed on a steam bath for 5min. The solution was filtered. 1cm^3 of the filtrate was treated with a few drops of Mayer's reagent. A second 1cm3 was treated with Dragendorff's reagent. The absence of a creamy white precipitate with Mayer's reagent and orange-red colour or turbidity with Dragendorff's reagent indicated a preliminary evidence for the absence of alkaloids.

2.4 Invitro antimicrobial test using the Agar Diffusion Method.

2.4.1. Standardization of inoculums:- seven microorganisms: *Sreptoccocus pneumonia, Salmonela typhi, Yersinia enterocolitica, Shigela sonnei, Microsporum auduinii and Trichophyton meritagrophyte* were sub cultured to Nutrient Agar(NA) slants using a wire loop (done aseptically)and incubated for 24hours at 37^{o}C for bacteria and 48hours at 25^{o}C for fungi. Growth of microorganisms in broth was indicated by turbidity. The turbidity produced was adjusted to match 0.5 McFarland standard (10^{8}cfu/ml), which was further adjusted to 10^5 cfu/ml and 10^3 cfu/ml for bacteria and fungi respectively.

2.4.2 Preparation and storage of media:- clean petri dishes sterilized in autoclave were allowed to equilibrate at 48-50^{o}C before use. They were labeled, indicating the test organisms and type of extracts. Nutrient Agar was used for bacteria and Saboraud Dextrose Agar (SDA) for fungi.

2.4.3 Inoculation of the plates and application of the extracts

To inoculate the plate, one drop of the adjusted sub-cultured nutrient broth was applied to the surface of the nutrient agar and saboraud dextrose agar and evened to cover the surface of the agars with microbes. One microbe was inoculated to one plate making a total of seven plates for seven microorganisms. After 30mins, four wells were punched on the plate using a sterile cork borer of 5mm diameter, 2 for the water and methanol extracts, 1 for negative control and 1 for positive control. A 0.1ml of the extract from each solvent (equivalent to 20mg of the extract) was dropped into each appropriately labeled well. Into the remaining two wells, distilled water and tetracycline of the same concentration as the extracts were introduced to serve as negative and positive controls respectively for the bacteria. For the remaining two wells in the fungi plates, distilled water and climaxol of the same concentration as the extracts were introduced to serve as negative and positive controls respectively. The inoculated plates were left on the table for 1 hour to allow for proper diffusion. Agar plates were incubated aerobically at 37^{o}C while the saboraud dextrose agar, containing *microsporun audoinii and Trichophyton mentagrophyte*, was incubated for 48hours at 25^{o}C. Zones of inhibition produced after incubation was measured by linear measurement of diameter.

2.5 Thin Layer Chromatography

2.5.1 Spotting the plates: Commercially prepared Tlc plates of adsorbent made of silica gel G was used. The solid test sample of 10mg was placed in a watch glass and dissolved with a few drops of methylene chloride. A small capillary pipette was filled by dipping the pulled end into the solution to be examined. The pipette was emptied to

the thin-layer plate at a point about 1cm from the bottom. The spotting was repeated. It was, however, ensured that the solvent was allowed to evaporate between applications.

2.5.2 Developing (running) Tlc plates

A solvent system of methanol:chloroform (7:3v/v) was best for the separation of water extract while ethylacetate:chloroform(4:1v/v) was best for the separation of methanol extract. The same solvent systems that gave neat separation with qualitative Thin Layer Chromatography were used for the preparative thin layer chromatography.

3. Results and Discussion

Preliminary phytochemical screening (Table 1) showed that the crude polar extracts contained saponins, flavonoids, anthraquinones and terpenes. These classes of compounds have been reported to possess a number of pharmacological properties which include antimicrobial and anti-fungal activities (Ogunti *et. al*.; 1991 Ibrahim and Osman, 1995; Bhat *et. al.*,1990; Akendengue and Louis, 1994;).

Table 1: Results of phytochemical screening of the polar extracts

Extracts	**Test**						
	Saponin	Alkaloid	Tannin	Anthraquinone	Flavonoid	Cardiac glycoside	terpene
Methanol	+	-	-	+	+	-	+
Water	+	-	-	+	+	-	-

NOTE: A positive result is indicated by (+), while a negative result is indicated by (-).

Table2: invitro antimicrobial activity of the water extract of *C. alata* leaf.

Microoganism	Diameter of zones of inhibition (mm)	Standard control	
		TCN	Climazol
E. coli	0	12	-
S. typhi	8	15	-
S. pneumonia	8	13	-
S. sonnei	8	12	-
Y. enterocolitica	10	11	-
M. audoinii	0	-	18
T. mentagrophyte	0	-	20

TCN=tetracycline (-) =standard control not used

Table3: invitro antimicrobial activity of the methanol extract of *C. alata* leaf.

Microoganism	Diameter of zones of inhibition (mm)	Standard control	
		TCN	Climazol
E. coli	0	12	-
S. typhi	0	15	-
S. pneumonia	7	13	-
S. sonnei	7	13	-
Y. enterocolitica	10	11	-
M. audoinii	15	-	18
T. mentagrophyte	20	-	20

TCN=tetracycline (-) =standard control not used

Table 4: Rf values of components of methanol and water extracts

Fraction	Me1	me2	me3	we1	we2	we3
Rf value	0.402	0.646	0.744	0.333	0.583	0.833

Table 5: Result of antimicrobial test with various fractions of methanol extract of *C. alata*.

Fraction	Zones of inhibition (mm)		
	Y. enterocolitica	*M. audouinii*	*T. mentagrophyte*
Me1	*0*	*0*	0
Me2	20	5	8
Me3	4	20	23
Climazol	-	38	32
TCN	30	-	-

Table 6: Results of antimicrobial test with various fractions of water extract of *C. alata*.

Fraction		Zones of inhibition (mm)		
	Y. enterocolitica	*S. sonnei*	*M. audouinii*	*T. mentagrophyte*
We1	4	*0*	*0*	0
We2	30	32	6	4
We3	33	38	7	5
Climazol	-	-	38	17
TCN	36	20	-	-

Results of the antimicrobial activity of the plant extracts are shown in Tables 2&3. The result showed that water extract was active on all the bacteria except *E. coli* (any extract with zone of inhibition above 5mm diameter was considered active) and inactive on both fungi. The methanol extract showed no activity on *E. coli* and *S. typhi* and decreased activity on *S. pneumonia*, *S. sonnei* and *Y. enterocolitica*. It, however, exhibited pronounced activity on the fungi, *M. audoinii* and *T. mentagrophyte*. This may suggest that the active antibacterial principle in water extract is only partially soluble in methanol while the antifungal principle is highly soluble in it. That these crude extracts greatly inhibited the growth of *S. sonnei* and *Y. enterocolitica* (bacteria reputed for causing diarrhea and dysentery), as well as *M. audoinii* and *T. mentagrophyte* (which cause skin diseases), their use by the local people to treat such diseases is justified.

The thin layer chromatography of both methanol and water extracts gave three spots each; me1, me2, me3 and we1, we2, we3 respectively. Their Rf values are represented in Table 4.

The results of the antimicrobial tests performed on the fractions of the methanol extract showed that the fraction me3 greatly inhibited the growth of *microsporum audoinii* and *trichophyton mentagrophyte* as compared with that of a standard drug, climazol (Table 5). This agrees with the reports of Carceres et al. (1991); Ibrahim and Osman (1995); Gill and Akinwumi (1986) and Ogunti et al. (1991) which account for the use of Cassia alata leaves to treat ringworn, eczema and other skin diseases.

The fractions, we2 and we3, from the water extract (Table 6) showed remarkable activity comparable to that of a standard drug Tetracycline on both *Y. enterocolitica* and *S. sonnei,* bacteria reputed for their ability to cause diarrhea, dysentery and other diseases in humans as reported by Bhat et al (1990); Akendengue and Luois (1994).

4. Conclusion

In conclusion, the results of this investigation revealed that polar extracts of the leaves of *C. alata* contain pharmacologically active substance(s) with antibacterial and antifungal properties. This provides the rationale for the use of the leaves of *C. alata* as an antibacterial and antifungal drug by traditional medical practitioners. Research is ongoing to better purify methanol fraction, Me3 and water fractions Me2 and Me3 in order to determine their chemical structures.

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