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Antibacterial activity of the active Component of *Cassia alata* (Linn) Leaves

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Abstract: Fresh leaves of *Cassia alata* Linn were extracted using different solvents first with water, then methanol then n-hexane and lastly with acetone. On comparison between methanol extract and extracts of other solvents it was revealed that methanol gave the maximum extraction, this was evident during the chromatographic separation as it gave large number of fractions. Chromatographic method was used to separate the different components of the extract. The crude extract as well as the chromatographic fractions were tested for antibacterial activity using disc diffusion method. Among the fractions, only one component show antibacterial activity on *Staphylococcus aureus*. The active chromatographic fraction revealed the presence of steroids.

KEYWORDS: Antibacterial, *Cassia alata*, *Staphylococcus aureus*

INTRODUCTION

Wild senna (*Cassia alata* Linn) was native to Ghana and Brazil, but it is now widely distributed in the Americas and all over Africa, Nigeria (northern and western part of the country) inclusive (Kumar, 1984). Different parts and constituents of the plant were reported to exhibit several therapeutic properties, such as antibacterial, antifungal, antimicrobial and analgesic. The leaves of this plant are used in the treatment of ringworm. The plant is traditionally acclaimed to be effective in treating skin infections in man (Igoli *et al.,* 2005) and animals.

C. alata leaf is also credited for the treatment of haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes (Abo *et al.,* 1998; Adjanahoun *et al.,* 1991; Kochar, 1981). The seed is used as antihelminthic, the roots are used against uterus disorder, and the crushed leaves are used for skin infections (Herman *et al.,* 1978). All parts of this plant were reported to have one or more medicinal action especially antimicrobial activities, (Makinde *et al.,* 2007).

Staphylococcus aureus is said to be responsible for pimples and boils and it is the common cause of infection in wounds (Robert, 1988). Although the organism is still susceptible to some common antibiotics such as ampiclox, there is still the need to find alternative drugs before it develop resistance to the current ones. Fortunately, extracts of *C. alata* leaves demonstrated strong activity against the organism and thus, contained potential alternative remedy to the menace of the organism. It is therefore the aim of this paper to isolate and identify the active component responsible for the antibacterial activity against *S. aureus*, from the leaves of this plant with the view of producing an effective substitute to the current antibiotics.

MATERIALS AND METHODS

Collection and Treatment of Samples: Fresh leaves of *C. alata* were obtained from the botanical garden Usmanu Danfodiyo University, Sokoto and identified at Botany Unit, Department of Biological Sciences of the same institution.

Extraction procedure: Water, methanol, n-hexane and acetone were used for the extraction. Fifty grams (50g) of the sample were weighed and boiled in 150cm^3 of each solvent for 5 minutes. The content was then corked and allowed to soak for 48 hours. The extract was decanted and kept under fan for 24 hours to concentrate the liquid extract.

Separation of the extract constituents: Column chromatographic method was used to separate the methanol extracts on silica gel and methanol as eluting solvent. 25cm long and 5mm internal diameter column was used, the column was dried packed with the silica gel and wetted with methanol. The sample was then introduced into the column and eluted under

gravity with methanol. Six fractions were collected and labelled k_1, K_2, k_3, k_4, k_5 and k_6 in the order of which they were collected.

Antibacterial sensitivity test: The test organisms used were clinically isolates of *S. aureus* obtained from Medical Microbiology Department, Usmanu Danfodiyo University Teaching Hospital, Sokoto.

Paper disc diffusion method was used (Makinde *et al.*, 2007) and the dried extracts and fractions 1mg/ml, 2mg/ml, 3mg/ml and 4mg/ml loaded paper discs (10mm) for each of the components were placed on the solidified and inoculated agar medium and incubated at 37°C for 24 hours (Omoregbe *et al.*, 1997).

Phytochemical analyses: Chemical tests were carried out on the active component for the qualitative determination of phytochemical constituents as described by Harborne, (1973), Trease and Evans (1989) and Sofowora (1993).

RESULTS AND DISCUSSION

Table 1 gives the result of the yield from each of the solvent in order to determine the most suitable of the solvents. On yield comparison, methanol gave the highest percentage yield of 5.98% which is more than double that of water and more than four times that of n-hexane and acetone. The high yield of methanol extract suggested that most of the phytochemicals were extracted there in, thus it was chosen for chromatographic studies.

Table 1: Percentage yield of the extracts

Solvent	% yield
Water	5.98
Methanol	2.65
n-Hexane	1.33
Acetone	1.23

Since methanol was the solvent for extraction, it was expected that all components present in this extract will remain soluble in the methanol as long as the compounds remained as they are (no secondary reaction e.g. hydrolysis). Therefore chromatographic separation was carried out using methanol as the sole eluent. Table 2 summarizes the result of the chromatographic separations of the methanol extract.

Table 2: Chromatographic fractions of *Casia alata* methanolic leaves extracts.

Component	Colour
K_1	Deep brown
K_2	Yellow
K_3	Colourless
K_4	Greenish brown
K_5	Green
K_6	Yellow

The activity of the component also varied between solvents with the methanol extracts demonstrating the highest activity against the test bacteria (Table 3). The methanol extracts of the plant parts were more potent than the water extracts, which in turn was more potent than n-hexane and acetone extracts, similar to the reports of Elmahmood and Amey (2007) but contrary to observations of Roy *et al.* (2006). It's been reported that different phyto-constituents have different degrees of solubility in different types of solvents depending on their polarity. In a traditional setting water is largely solvent used to prepare these concoctions (Elmahmood and Amey, 2007). The higher activity demonstrated by organic solvents in this work is therefore an indication that less of the bioactive components are extracted when water is used as a solvent.

Table 3: Results of the antibacterial activity of *Cassia alata* leave extracts

Extracts	Zones of inhibition (mm)			
	1mg/ml	2mg/ml	3mg/ml	4mg/ml
Water	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	2mm
n-Hexane	-ve	-ve	-ve	-ve
Acetone	-ve	-ve	-ve	-ve

The chromatographic separation gave six fractions which were collected based on colour differences. Each of these fractions was subjected to antibacterial sensitivity test with a view to finding the fraction that harbours the active ingredient. The antibacterial screening was carried out on only one organism (*S. aureus*) as a follow up of Faruk and Adebote (2003) which reported that the plant has highest inhibitory on the *S. aureus*.

Table 4: Antibacterial activity test of the methanolic chromatographic fraction

Component	Zones of inhibition (mm)			
	1mg/ ml	2mg/ ml	3mg/ ml	4mg/ ml
K_1	-ve	-ve	-ve	-ve
K_2	-ve	-ve	-ve	1mm
K_3	-ve	-ve	1mm	2mm
K_4	-ve	1mm	2mm	2mm
K_5	4mm	5mm	6mm	8mm
K_6	-ve	-ve	1mm	2mm

When the sensitivity test was carried out on the bulk extract minimum inhibition zone was witnessed, but after the chromatographic separation, one of the components indicated significant activity of 8mm inhibition zone at 4mg/ml concentration (Table 4). This is evident that, though the active component is present, but its activity is more pronounced when isolated from the bulk extract. Also increase in the concentration of the extract resulted in the corresponding increase in the inhibition on the test organism. The inhibitory activities exhibited by the extract tends to agree with the reports of Levin et al. (1979) and Elmahmood et al. (2008), all of whom linked antimicrobial properties of plants to the presence of bioactive secondary metabolites.

Table 5: Phytochemical screening of the active chromatographic fraction

Tests	**Results**
Saponins	-ve
Steroids	+ve
Tannins	-ve
Alkaloids	-ve
Anthraquinones	-ve

-ve = Absent +ve = Present

The phytochemical studies of the active component revealed that the leaves extract contained steroids only (Table 5). Further more the positive result found with the component (steroid) is not surprising, as variety of important steroids are found in plants and they serve many purposes such as antibacterial activity, (Rao, Shivakumar & Parthasarathi, 1996).

CONCLUSION & RECOMMENDATIONS

From the result obtained in this work, it could be concluded though the bulk extract does not give the maximum zone of inhibition, but upon separation of the components, the active component (found to be steroid) give maximum zone of inhibition, and hence strongly active against the test organism. Therefore, further work should be carried out on the steroidal compound present in the leaves of the plant. Also a research should be advance in order to determine the type of steroid present and the possible way or mechanism by which it acts on the *S. aureus,* aiming at producing a cure for the skin and other diseases caused by this organism.

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