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PHYTOCHEMICAL, QUANTITATIVE AND IN-VITRO ANTIMICROBIAL ANALYSIS OF *Cassia alata* Linn LEAVES

[1]Salihu, S.O., [1]Osahon, M., [1]Jacob, J.O. and [2]Maji, J.E.
[1] Department of Chemistry, Federal University of Technology, Minna. Nigeria
[2] Department of Chemistry, University of Abuja, Abuja. Nigeria.

ABSTRACT
The leaves of *Cassia alata* Linn, were extracted and the extracts were subjected to phytochemical and antimicrobial analysis. Quantitative analysis of some phytochemicals was also done on the sample. The various extracts revealed the presence of constituents such as alkaloids, anthrquinones, flavonoids, saponins, carbohydrates, tannins, sterols and terpenes. Compared to hexane and chloroform extracts the methanol extract showed higher level of the constituents. Sensitivity and MIC analysis of the extracts against *S. typhi, S. aureua, S. dysenteriae, E. Coli, P. aeruginosa and C. albicans*, revealed that the methanol extract was more active. None of the extracts was sensitive against the fungus *C. albicans.* The MIC gave a lowest concentration of 200μgml^{-1} of the samples against most organisms and a highest concentration of 20,000μgml^{-1} against *E. coli and S. aureus.* TLC showed a higher number of constituents in the chloroform extract. Quantitative analysis of three constituents revealed that C. *alata* leaves contain 14.4% flavonoids.

KEYWORDS: *Cassia alata*, Phytochemicals, MIC, Quantitative, In-vitro

INTRODUCTION

Thousands of indigenous plants have been used world-wide in forms of poultices, topical, infusions, tisanes macerates etc, dating to prehistory. Human disease management in Nigerian history also provides evidence of the relationship between plants and medicine (Raghavendra *et al.*, 2006; Ayandele and Adebiyi, 2007; Dauda *et al.*, 2011).

Research has shown that collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products known as secondary metabolites (Fatope *et al.*, 2001).

Secondary metabolites can be used as precursors for the manufacture of new or synthetic drugs and to help in the elucidation of the physiological mechanisms in drug development or testing. For example, medicinal plants with anti-inflammatory activity are widely employed in the traditional treatment of several disorders. The inflammatory response involves a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair (Vane and Bolting, 1995; Dauda *et al.*, 2011)

Some important groups of these phytochemicals include: alkaloids, glycosides, steroids, flavonoids, fats, phenols, resins, saponins, tannins and terpenes. (Finar, 1986).

Medicinal plants represent a rich source from which antimicrobial agents may be obtained. These plants are generally of local origin. Herbalists use them without sufficient scientific knowledge, often relying on past experiences and observations orally passed on, or in recent times, written (Bennerman *et al.*, 1986). They have been used successively as laxatives, anti-malarial, analgesics, anti-inflammatory drugs etc. (Dauda *et al.*, 2011)

The use of plant and animal parts in medicine has since been widely documented in the records of ancient China, India and Egypt. This practice was based on series of "trial and error", and not substantiated by scientific means then. Over the years however, these methods have produced results of proven efficacies alongside conventional modern medicine (Dauda *et al.*, 2011). In recent times, herbal medicines have become an integral part of the Primary Health Care system of many nations (Fajimi and Taiwo, 2000; Dauda *et al.*, 2011).

There are about 400 species of Cassia around the world. Many of these species are used in folk medicine, as anti-fungal, anti-bacterial, and laxatives (Samy and Ignacimuthu, 2000)

Cassia alata, commonly known as Wild senna, Candle bush, Empress candle plant, Ringworm plant and Candle stick is called Nelki, Okpo (Igbo), Gunkoroko (Nupe), Asunwon (Yoruba).

It is a beautiful flowering shrub of about 1-2 meters in height. It produces pretty yellow flowers in a column that resembles yellow candle sticks, earning its common name "candle stick" or "candle bush". It is native to the Amazon Rainforest and can be found in Peru, Brazil, French Guiana, Suriname, Venezuela, Colombia and Nigeria. Due to its beauty, it has been cultivated around the world as an ornamental plant and has naturalized in many tropical regions in the world. The trunk or branches grow generally upright with no thorns, and are easily damaged.

The leaves are found to contain saponins. In addition, it also consist s of chrysophanic acid which is a fungicide used to treat infections like ringworm, scabies and eczema, (Puntac, 2012).

Since phytochemicals are produced by plants by up-regulation and down-regulation of biochemical pathways in response to the environment, Mallika, *et al.*, (2007), it is important to assess the phytochemical properties and microbial activities of herbs in all regions. This will encourage collaboration between stake holders globally in the collective fight against microbial infections in addition to providing data base for communities. This informed the importance of this research.

MATERIALS AND METHODS

The leaves of *Cassia alata* were collected at Gidan Kwano village, Niger State, opposite Federal University of Technology, Minna Nigeria. Identification of the plant was done at the Biological Science Department, Federal University of Technology Minna, Nigeria.

The leaves were properly rinsed with tap water and air-dried and pulverized with a sterile electric blender and stored in a clean screw-cap sample bottles. The micronized sample was macerated. Gradient exhaustive extraction process was done using hexane, chloroform and methanol.

Extraction

Ninety grams of micronized sample was soaked in 300ml of hexane in a screw-cap bottle for 24 hours. The extract was filtered using a Whatman No. 42 filter paper and concentrated with a rotary evaporator at 40^0C. Further concentration was done on a water bath at 40^0C after which extract was transferred into a sample bottle pending further analysis. The residue was dried and extracted with chloroform and then with methanol.

Thin Layer Chromatography (TLC) of crude extracts.

Thin layer chromatography of the crude extracts of *C. alata* leaves was carried out using pre-coated glass TLC plates (K5 Silica gel, 150Å, layer thickness 250µm; Whatman USA) in the following solvent systems; Hexane extract: hexane/ethyl acetate (4:1 v/v); Chloroform extract: hexane/ethyl acetate/chloroform (4:1:1 v/v/v); Methanol extract: hexane/ethyl acetate/methanol (4:1:0.5 v/v/v)
R_f (Retardation factor) values for each extract were calculated and recorded thus,
R_f = Distance moved by sample / Distance moved by solvent

Phytochemical screening

Chemical tests were conducted on extracts using standard methods described by Olabiyi, *et al.*, (2008), Banso, (2009) and Kumar, *et al.*, (2009).

Test Organisms

The test organisms used for the antimicrobial analysis include five bacteria and one fungus. The bacteria include *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae* and the fungi *Candida albicans.* Pure isolates of these organisms were obtained from Microbiology laboratory of the Department of Microbiology, Federal University of Technology Minna.

Microbial screening of the crude extracts

The agar diffusion method was used as described by Dauda *et al.*, (2011) and Jimoh *et al.*, (2010). Sterile nutrient agar was prepared and placed in labeled Petri dishes and allowed to gel. Wells were bored into the nutrient agar using a 7 mm sterile cork borer. Each crude extracts was reconstituted by adding 2ml of its mother solvent. 0.2ml of the reconstituted extract was dispensed into each well and allowed to diffuse for 30minutes.

The test organisms were inoculated onto the labelled Petri dishes with a swab stick before incubating at 37°C for 24 hours.

Minimum Inhibitory Concentration (MIC)
Minimum inhibitory concentration was carried out on sensitive extracts. Seven test tubes were used for each organism. Broth dilution method was used.

9ml of nutrient broth was dispensed into each of the seven test tubes used per organism. The test tubes were autoclaved at 121°C for 15 minutes and allowed to cool and properly labeled.

One milliliter of reconstituted extract, prepared by dissolving 2g extract in 10ml solution, was dispensed into the first test tube and shaken. Form the first test tube, 1ml of the mixture was taken and dispensed into the second and shaken. This was repeated for the rest of the test tubes. From the last test tube, 1ml was discarded. This serial dilution was carried out for all viable extracts, giving rise to 20,000, 2,000, 200, 20, 2, 0.2 and 0.02 µg/ml of extracts respectively in the test tubes. The test tubes were then inoculated with test organisms using a sterile wire loop. The seventh test tube was not inoculated and serves as a control. After inoculation, the test tubes were properly sealed and incubated for 24 hours. They were observed for turbidity (growth of organism). The test tube with least concentration which showed no turbidity indicates the MIC.

Quantitative analysis on phytochemical constituents
Total alkaloid, flavonoids and saponins were determined using the method described by Krishnaiah *et al,* 2009.

Determination of alkaloids
Five grams of the plant sample was placed in a 250ml beaker and 200ml of 10% CH_3CO_2H in C_2H_5OH was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH_4OH was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference.

Determination of flavonoids
Ten grams of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference.

Determination of saponins
Twenty grams of plant sample was weighed into a 250ml conical flask. 100 ml of 20% C_2H_5OH was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. It was then filtered with a Whatman No.42 paper. The residue was re-extracted with another 200ml of 20% C_2H_5OH. The combined extract was reduced to 40ml over a water bath at about 90°C. The concentrated extract was then transferred into a 250ml separator funnel and 20ml of $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered while the $(CH_3CH_2)_2O$ layer was discarded. This purification process was repeated.

60ml of n-butanol was added and the combined n-butanol extract was washed twice with 10ml of 5% NaCl. The remaining solution was then heated on a water-bath in a pre-weighed 250ml beaker. After evaporation the residue was dried in a Gallenkamp moisture extraction oven (Size 1) to a constant weight. The % saponin was calculated by difference.

RESULTS AND DISCUSSION

Phytochemical screening and TLC

The results for phytochemical screening of the crude extracts of *C. alata* revealed the presence of carbohydrates, flavonoids and saponins in all extracts. Tannins, sterols, alkaloids and anthraquinones were not detected in the hexane extract. The tests also revealed that the methanol extract have higher contents of the phytochemicals.
The TLC analysis as shown in Table 2 revealed, from Rf values, that chloroform extract eluted with hexane, ethyl acetate and chloroform (4:1:1 v/v/v) gave 8 spots, hexane extract eluted with hexane and ethyl acetate (4:1

v/v) gave 6 spots. While methanol extract eluted with hexane, ethyl acetate, methanol (4:1:0.5 v/v/v) gave 5 spots. The chloroform extract has the highest number of phytochemicals.

Table 1: Phytochemical screening of crude extracts of *Cassia alata* leaves

Test	Hexane	Chloroform	Methanol
Carbohydrates	+	+++	+++
Tannins	-	+	+++
Saponins	+	+	++
Sterols	-	+	+++
Terpenes	-	++	+++
Flavonoids	+	++	+++
Antraquinones	-	++	+++
Alkaloids	-	++	+++

Key: + = fairly present; ++ = moderately present; +++ = highly present; - = absent

Table 2: R_f values of crude extracts

Hexane	Chloroform	Methanol
0.93	0.93	0.91
0.83	0.80	0.73
0.65	0.66	0.63
0.52	0.49	0.47
0.26	0.38	0.33
0.23	0.24	
	0.19	
	0.07	

Antimicrobial analysis

The sensitivity test showed that the extracts were active against *S. typhi* and *S. dysenteriae.* Only methanol and chloroform extracts showed activity against *S. aureus* and *E. coli.* While only methanol extract was active against *P. aeruginosa.* None of the extracts was active against *C. albicans.* By comparison, methanol extract showed the highest activity against the test organisms.

Table 3: Zone of inhibition (mm) of *C. alata* leaves extracts

Test Organism	Methanol	Chloroform	Hexane	Control
Salmonella typhi	21	20	13	20
Shigella dysenteriae	11	8	13	21
Staphylococcus aureus	12	8	-	22
Escherichia coli	11	8	-	18
Pseudomonas aeruginosa	13	-	-	-
Candida albicans	-	-	-	33

Key: - = absent

The MIC test as shown in Table 4 reveals that the methanol extractranged from 200µg against *S. typhi, S. dysenteriae, S. aureus, and E. coli* to 2,000µg ml-1while P. aeruginosa. The same range was observed in hexane extract against *S. typhi and S. dysenteriae* respectively. While chloroform extract showed MIC of 2,000µg ml^{-1} against *S. typhi and S. dysenteriae*, 20,000µg ml^{-1} against *S. aureus and E. coli.*

Table 4: Minimum Inhibitory Concentration (MIC) (µg ml^{-1}) of *C. alata* leaves extracts.

Test Organism	Methanol	Chloroform	Hexane
Salmonella typhi	200	2,000	200
Shigella dysenteriae	200	2,000	2,000
Staphylococcus aureus	200	20,000	-
Escherichia coli	200	20,000	-
Pseudomonas aeruginosa	2,000	-	-

Key: - = absent

Quantitative analysis

Results for the quantitative analysis carried out on powdered sample of *C. alata* leaves as shown in Table 5, revealed that *C. alata* leaves has flavonoids percentage content of 14.4%, saponins percentage content of 1% and alkaloid percentage content of 6%. Ekwenye and Okorie, (2010) recorded 0.82, 0.54 and 1.28% of flavonoids, saponins and alkaloids respectively from *Tetrapleura tetraptera.* While Krishnaiah *et al.*, (2009) reported a range of 0.24-0.52% alkaloids, 1.1-2.3% saponins and 0.32 - 0.62% flavonoids in various Malaysian herbs. This showed that plants have different concentration of constituents and *Cassia alata* compares favorably.

Table 5: Amount (%) of some phytochemicals in C. *alata* leaves

Phytochemical	%
Flavonoids	14.40
Saponins	1.00
Alkaloids	6.00

CONCLUSION

Antimicrobial screening of Cassia *alata* leaves indicated that the metabolic extract was most active compare to other extracts. None of the extracts was active against the fungus used. Although the chloroform extracted has more constituents, methanol can be a solvent of choice for the extraction of antibacterial against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi,* and *Shigella dysenteriae.* However, none of the solvents is recommended for antifungal extraction against *Candida albicans.* The quantitative analysis indicated a high percentage of flavonoids (14%), the leaves of *Cassia alata* can be a good source of flavonoids.

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Corresponding author
Salihu, S.O.,
Department of Chemistry, Federal University of Technology, Minna. Nigeria
Email: simono.salihu@gmail.com