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Full Length Research Paper

Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn

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Phytochemical screening of the leaves and roots of *Cassia alata* (Linn) revealed the presence of some bioactive components, which have been linked to antimicrobial properties. The effects of water, methanol and chloroform extracts on some pathogenic *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Proteus mirabilis* showed that the plant parts can be used to treat infections caused by these bacteria. *S. aureus, S. pyogenes* and *P. mirabilis* were more susceptible, while *E. coli* and *P. aeruginosa* were less sensitive. The effectiveness of the crude extracts were enhanced at elevated temperatures and at near neutrality pH values, which attests to its use in traditional medicine to treat skin, urinary tract and gastrointestinal infections. The aqueous extract was less effective than the organic solvents, thus suggesting the inability of the traditional medicine practitioners to exhaustively extract all the bioactive components with water. The MICs and MBCs of the extracts against the test bacteria were high and correlate with sensitivity test results. The effectiveness of the extracts were less than the conventional antibiotic, metronidazole.

Key words: Antimicrobial bioactive, pathogenic, phytochemical, traditional medicine.

INTRODUCTION

The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide (Falodun et al., 2006). Many investigations are being conducted on medicinal plants based on information supplied by the local populations with the object of finding out phytochemical constituents for application in the prevention and treatment of infectious diseases and other diseases of non-microbial etiology. Several studies have been conducted to provide scientific basis for the efficacy of plants in herbal medicines. The development of resistance to most of the available antimicrobial agents and the high costs of treatments consequent upon this resistance, has necessitated the search for new, safe, efficient and cost effective ways for the management of infectious conditions. Akinpelu and Onakaya (2006) have warned that unless concerted efforts are made to acquire new agents, very soon the population of bacteria developing resistance will not match the arsenal to fight. The rising interests in products of natural origin in the developed economics led to the extraction and development of several drugs and chemotherapeutic agents from plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Extracts of higher plants have served as good sources of antibiotics against various bacterial and fungal pathogens (Falodun et al., 2006). Plant based antimicrobial compounds have great therapeutic potential as they can serve the purpose without any side effects often associated with synthetic drugs and also little chance of development of resistance. The common view in the society and the medical community is that plant based products are healthier, safer, and more reliable than synthetic products (Benli et al., 2008), even though safety and efficacy data are available for only a few number of plant materials. Plants act generally to stimulate and supplement the bodies' healing forces, they are the natural foods of human beings (Ajayi et al. 2008). The clinical success of quinine and quinidine isolated from the Cinchona tree bark and recently artemisinin from *Artemisia annua* in the chemotherapy of malaria have rejuvenated interests in higher plants as potential sources of novel drugs (Igoli et al., 2005).

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Only few microorganisms cause majority of infections in both the community and the hospital environments. Most of these pathogens do not have any fastidious growth requirements, and can survive in the dry and wet conditions in and outside the hospital environment. In addition, a good proportion of these pathogens have several virulent factors including the formation of biofilms on colonized surfaces (Donlan, 2001; Chandra et al., 2001; Beaudin et al., 2002; Sherer et al. , 2003; Oteo et al., 2005; Branger et al., 2005). Also large proportions of these pathogens are known to be resistant to most of the available antimicrobial agents (Lark, 2001; Lesch et al., 2001).

The medicinal usefulness of the plant *Cassia alata* (Linn) has been the object of many chemical and pharmacological studies. *C. alata* is an ornamental shrub or tree growing up to 12 m high and widely available in the tropics, in the grasslands and around towns and villages throughout West Africa. This tree, apart of its uses as sources of firewood and timber, has very important applications in folkloric medicine (Rai, 1987). In Ghana and Ivory Coast, decoctions of the leaves and roots are used to treat diarrhea, dysentery and other gastrointestinal problems. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases (Dalziel, 1937). The decoction of the stembark and roots are used to treat urinary tract infections, bronchitis and asthma (Rao et al., 1973) . In the northern part of Nigeria, particularly in Adamawa and Taraba States, the root, stem and leaves are used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhoeal diseases, gastrointestinal and upper respiratory tract infections. Earlier reports in the scientific literature indicated that some leaves and roots of *C. alata* (Linn) can be used as a remedy for boils, wound, eye, urinary and gastrointestinal tract infections, diarrhoea and scarlet fever (Benjamin and Lamikanra, 1981) . Recent reports have credited the use of *C. alata* in the successful treatment of haemorroids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes (Makinde et al., 2007). Several studies have been done to provide scientific basis for the efficacy of plants in phytomedicine. This study seeks to ascertain the usefulness of *C. alata* in the treatment of infectious conditions caused by common pathogens. It involves the screening of the leaves and roots of *C. alata* (Linn) for the presence of bioactive components and evaluation of their anti-bacterial activities against some pathogenic bacteria for possible development of new drugs for the prevention and treatment of infections.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves and roots of *C. alata* were collected from the outskirts of Girei town, Girei Local Government Area of Adamawa State. This was subsequently authenticated and identified in the Department of Biological Sciences, School of Pure and Applied Sciences, Federal University of Technology, Yola, Nigeria.

Preparations of plant materials

The freshly collected leaves and roots were air-dried under shade at room temperature for 7 - 14 days. Upon drying, the plant materials were pounded separately using mortar and pestle into smaller particles and then subjected grounded to fine powder using an electric blender (Kenwood) . The powdered samples were stored in airtight containers and kept at room temperature until required.

Test organisms

The test organisms used were clinical isolates of five bacteria obtained with the help of the hospital staff from the 750-bed referral Federal Medical Center, Yola. These organisms which were submitted as clinical specimens to the Microbiology Laboratory of the hospital were collected in peptone water. They included *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Preliminary identification of the bacteria was carried out in the hospital following the methods described by Cheesbrough (2002). Biochemical tests to confirm the identity of the organisms were performed at the Microbiology Laboratory of the Federal University of Technology, Yola as described by Cheesbrough (2002). The organisms were stored at 2 - 8^{o}C until required. Purity of the organisms was checked at regular intervals (Elmahmood and Amey, 2007).

Extraction and phytochemical screening of bioactive constituents

The method described by Odebiyi and Sofowora (1978) was used. The air -dried powdered plant samples (10.0 g of each) was soaked in 100 ml each of distilled water, methanol and chloroform contained in separate 500 ml sterile conical flasks. The flasks were covered with cotton wool plug and then wrapped with aluminium foil and shaken vigorously at 3 h intervals for 48 h at room temperature. The crude extract was then filtered using muslin cloth and then Whatman no. 1 filter paper. The filtrate was evaporated to dryness and the dried substance stored in airtight bottles until required.

Preliminary screening was carried out on leaves and roots of *Cassia alata* for the presence of the bioactive components saponins, alkaloids, phenol, tannins, flavonoids, cardiac glycosides anthraquinones and carbohydrates as described by Elmahmood and Amey (2007).

Antibacterial susceptibility assays

The standardization of culture was carried out as described by Bakar and Thomsberg (1983) and National Committee for Clinical Standard (NCCLS, 1993) . Briefly, an 18 h culture of the test organism was suspended in a sterile universal bottle containing nutrient broth. Normal saline was added gradually to it so as to compare the turbidity to that of 0.5 McFarland standard corresponding to approximately 10^8 cells/ml. This was then diluted to produce10^6 cells/ml that was used in the experiments. To carry out the antibacterial susceptibility test, the method described by Emeruwa (1982) was used. One milliliter (1ml) of test organism (10^6 cells/ml) was inoculated into Petri plates (90 mm diameter), then 19 ml molten Mueller Hinton agar (MHA) at 45^{o}C added, and the plates shaken gently for even mixing of the contents. The agar was allowed to solidify on a flat bench. Wells (6 mm diameter and 4 mm deep) were punched in the agar with the aid of a sterile cork borer. Each of the plant powders was reconstituted by dissolving 200 mg

Table 1. Phytochemical constituent of *Cassia alerta* (Linns).

Phytoconstituent	Leave extract	Root extract
Alkaloids	+	+
Carbohydrates	+	+
Tannins	+	+
Saponins	+	+
Phenols	+	+
Flavonoids	+	+
Anthraquinones	+	+
Cardiac glycosides	+	+

of each in 1 ml distilled water and then pippetted (0.5 ml) into holes bored from the agar. 0.5 ml of each of the pure solvents (methanol and chloroform) was used as negative controls, and 0.5 ml of 50 mg/ml solution of metronidazole antibiotic was used as positive control. The plates were left on a flat bench for 1 h to dry, before incubating at 37^{O}C for 18 h. Antibacterial activity was evaluated by measuring the diameters of zones of growth inhibition in triplicates and the mean of three results taken for both the test and control organisms.

Effects of pH and temperature on activity

This was performed using the method of Emeruwa (1982). 200.0 mg of dried powdered sample was dissolved in 1.0 ml solvent (methanol), then either 1N HCl or 1N NaOH was added drop-wisely on to the extracts in different sets of test tubes adjusted to various range of pH (3 - 8. 0.5 ml) and allowed to soak for 1 h. After 1 h of acid-base treatment, the extract was again neutralized to pH 7 using either 1N HCl or 1N NaOH as the case may be. 0.5 ml of extract so was then introduced into wells bored on nutrient agar plates containing a culture of the organism previously adjusted to 10^{6}cells/ml. Solutions of the untreated methanol extracts and water (neutral pH) were used as negative controls while metronidazole (0.5 ml, 50 mg/ml) was used as positive control. The plates were incubated at 37^{O}C for 24 h and the zones of inhibition produced were measured.

For the effect of temperature, the extract was heated to temperatures between 10-100^{O}C in different sets of test tubes in a shaker water bath for 1 h, and then allowed to cool. 0.5 ml of the extract (200 mg/ml) was then introduced into wells bored on nutrient agar plates preseeded with the test organism (10^{6} cells/ml). Solutions of the pure solvents (methanol) were used as negative controls, while the untreated extract and metronidazole (0.5 ml, 50 mg/ml) were used as positive control. This was incubated at 37^{O}C for 24 h and the zones of inhibition produced were measured (Emeruwa, 1982).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration was determined by the macro broth dilution methods (NCCLS, 1993).The crude extracts were diluted to 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/ml respectively in nutrient broth. Duplicate tubes of each dilution were incubated with 1.0 ml (10^{6}Cfu/ml) of test organism and incubated at 37^{O}C for 24 h. Solutions of metronidazole (50mg/ml) were included in each experiment as positive control. The solutions of the pure solvents (methanol and chloroform) were used as negative controls. The MIC was taken as the lowest concentration of extracts that did not permit any visible growth.

For the determination of the MBC, 2 loopfull of culture was taken from each of the broth tubes that showed no growth in the MIC tubes and inoculated onto fresh Mueller Hinton agar (MHA) plates. After incubation for 24 h, the plates were observed for growth. The concentration of the extracts that showed no growth was recorded as the MBC.

RESULTS AND DISCUSSION

Phytochemical screening of the leaves and roots of *C. alata* revealed the presence of alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosides (Table 1), similar to the results presented by Owoyale et al. (2005) and Makinde et al. (2007). Drugs present in plants are known as active principles and these serves to protect the plants themselves against microbial (bacteria, fungi, viruses) attacks as well as predation by pests and animals (Elmahmood and Amey, 2007). The inhibitory activities exhibited by the extracts 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 like alkaloids, tannins, saponins, flavonoids, phenols, glycosides and diterpenes. For example, Mbuh et al (2008) investigated the antibacterial activity of leaf extracts of *Psidinum guajava* (Linn) and reported that the bioactive compounds found in the plant inhibited the growth of *S. aureus, S. typhi, E. coli*, *B. subtilis, Shigella* spp*, Proteus mirabilis* and *Klebsiella pneumoniae* and that the methanol extract was most active. A major contribution of higher plants to both traditional and biomedicine healthcare systems is the limitless capability of the plants to produce a large number of these organic compounds of high structural diversity. The accumulation of these bioactive organic compounds in large proportions in plant cells had, over the last 5 decades attracted the attention of the academic community, and the knowledge so generated had helped in the inclusion of herbal medicines as a vital component of the health care systems, as well as identification of native medicinal plants in indigenous pharmacopeias (Adebolu and Oladimiji, 2005). All the crude extracts inhibited the growth of *E. coli, S. aureus, S. pyogenes, P. mirabilis* and *P. aeruginosa*, though to varying degrees (Table 2). The activity of the plant extracts against both Gram positive and Gram negative bacteria is an indication of the presence of broad spectrum antibiotic compounds or simply metabolic toxins in the plant (Parekh and Chanda, 2007). The activity of also varied between solvents with the methanol extracts demonstrating the highest activity against all the test bacteria. The methanol extracts of the plant parts were more potent than the chloroform extracts, which in turn was more potent than aqueous extract, similar to the reports of Elmahmood and Amey (2007) but contrary to observations of Roy et al. (2006). Its been reported that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity. In a traditional sett-

Table 2. Antibacterial activity of extracts of *Cassia alata* (Linn.)

	Extract (200 mg/ml)/Zone diameter of inhibition (mm)						
	Leaves			Roots			
	Aqueous	Methanol	Chloroform	Aqueous	Methanol	Chloroform	Metronidazole
S. aureus	15	17	16	13	16	12	26
E. coli	11	13	13	11	12	15	21
S. pyogenes	13	15	14	12	14	13	24
P. aerugenosa	8	9	8	7	8	7	13
P. mirabilis	12	14	13	12	13	12	22

Table 3. Effect of temperature (ºC) on the activity of methanol extracts of *Cassia alata* (Linn.).

Bacteria	Antibacterial activity (mm)/Temperature (ºC)									
	Leaf extracts					Root extracts				
	30*	10	50	70	100	30*	10	50	70	100
S. aureus	17	17	18	20	20	16	16	18	18	20
E. coli	13	13	15	18	19	12	12	15	17	19
S. pyogenes	15	15	18	21	20	14	14	17	17	19
P. aerugenosa	9	9	12	15	18	8	8	12	14	16
P. mirabilis	14	14	16	18	21	13	13	14	16	18

*untreated (normal) pH of extract

Table 4. Effect of pH on the activity of methanol extracts of *Cassia alata* (Linn.).

Bacteria	Antibacterial activity/pH									
	Leaf extracts					Root extracts				
	4*	3	5	7	8	4*	3	5	7	8
S. aureus	17	17	19	18	13	16	16	18	15	11
E. coli	13	13	15	14	8	12	12	14	11	8
S. pyogenes	15	15	17	16	10	14	16	16	12	8
P. aerugenosa	9	9	13	8	6	8	8	12	10	6
P. mirabilis	14	14	17	14	10	13	13	15	15	12

*untreated (normal) pH of extract

ing 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 principles are extracted when water is used as a solvent. The antibacterial activity of the extracts was enhanced by the increase in temperature (80 - 100°C), and this supports the boiling of the plant parts in herbal medicine (Table 3) Traditionally, the leaves and roots are boiled before being taken orally to treat various stomach and other gastrointestinal tract disorders. Treatment of plant extracts to high temperature could inactivate volatile compounds, but could also increase the release of active components and free radicals. The activity of the crude extracts of *Cassia alata* is optimal under slightly acidic pH but diminished as pH was adjusted to alkalinity (Table 4). Activity at acidic pH is indication of acid stability, while diminished activity at alkaline pH is an indication of lability to alkaline conditions. Practitioners of traditional medicine usually add some additives like *kanwa* (potash) which is a basic salt, this therefore means that from the result of this work this practice might not favour high efficacy of the plant in local practice. This also explains why the plants have to be taken for longer periods before any curative effect is noticed by the patient. The MIC of the leaf extracts ranged from 50 - 300 mg/ml, while the MBC ranged from 100 - 350 mg/ml. Similarly, for the root extracts, the range of the MIC was 100 - 350 mg/ml and the MBC 200 - 400 mg/ml (Table 5). The effects of the crude leaf extract correlates with the reports that microorganisms vary widely in their degree of susceptibility to agents (Emeruwa, 1982). High MIC and MBC values are indication of low activity while

Table 5. Minimum inhibitory concentration (MIC) and (minimum bactericidal concentration) MBC values of methanol extracts of *Cassia alata* (Linn.)

Bacteria		Leave extracts						Root extracts					
	MIC/MBC*	MIC			MBC			MIC			MBC		
	Metro	Aq	Met	Chlo	Aq	Met	Chlo	Aq	Met	Chlo	Aq	Met	Chlo
S. aureus	50	150	50	100	250	100	200	200	100	250	300	200	250
E. coli	100	200	150	250	300	200	250	250	150	250	300	250	300
S. pyogenes	10	150	100	150	200	150	200	150	150	150	250	200	200
P. aerugenosa	100	300	200	250	400	300	350	350	250	250	350	300	400
P. mirabilis	25	200	100	150	250	200	250	200	200	150	300	200	250

*Control; Metro = metronidazole; Aq = aqueous extracts; Met = methanol extracts; Chlo = chloroform extracts

while low MIC and MBC are indication of high activity. In this study, *P. aeruginosa* and *E. coli* had higher MIC and MBC values, thus suggesting lower susceptibility to the efficacy of the crude extracts and slightly lower values for *S. aureus, S. pyogenes* and *P. mirabilis* thus suggesting higher activity against the corresponding organisms. In all of the experiments conducted, water used as controls did not show any appreciable activity. Also, the standard anti-biotic, metronidazole consistently displayed superior po-tency when compared with the crude extracts. This may be attributed to the fact that metronidazole as a conven-tional antibiotic is a refined and purified product, while extracts of herbal medicines are a mixture of various plant constituents some of whom can interfere with anti-microbial activity and are subject to degradation and decomposition on storage (Elmahmood and Amey, 2007).

Conclusion

The study has confirmed that crude extracts of *C. alata* (Linn) possessed reasonable activity against some bac-teria and if adjusted to suitable conditions of temperature and pH and further purified can be used to treat urinary tract and gastrointestinal tract disorders, provided the infections are caused by susceptible bacteria. The effect of this plant against a wider range of bacteria and fungi, and toxicological studies of the extracts is recommended.

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