ANTIDIARRHOEA AND PHYTOCHEMICAL EVALUATION OF THE LEAF EXTRACT OF *ACALYPHA MARGINATA* SPRENG (EUPHORBIACEAE)

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ABSTRACT

The leaves of *Acalypha marginata* are used ethnomedically across Africa without scientific basis. Determination of its phytochemical constituents, antimicrobial activity and evaluation of its effects on the gastrointestinal tract (GIT) will provide supportive scientific evidence in favour of its continuous usage. Chemical and chromatographic tests were employed in phytochemical investigations. Inhibitory activity against clinical strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* and *Streptococcus faecalis* were compared with Gentamycin. Our report includes minimum inhibitory concentration (MIC) against the tested organisms. The effect of the methanol extract on the motility of the GIT in mice using the charcoal plug method and castor oil induced diarrhoea in rats was evaluated. Phytochemical studies revealed the presence of alkaloids, tannins and saponins. The crude ethanol extract and fractions inhibited the growth of *E. coli, P. aeruginosa, S. aureus* and *K. pneumonia* to varying extents. The degree of transition exhibited by the charcoal meal was dose-dependent and greater than that for Atropine. In the castor oil induced diarrhoea test, all the doses showed anti-spasmodic effects. Methanol extracts of *Acalypha marginata* have demonstrated antimicrobial activity against clinical strains of selected microorganisms. The plant showed potential for application in the treatment of diarrhoea, thereby justifying its usage ethnomedically.

Keywords: *Acalypha marginata*, antidiarrhoeal, antimicrobial, phytochemical.

INTRODUCTION

The Euphorbiaceae family also known as the Spurge family is composed mostly of monoecious herbs (Kaey, 1989), shrubs and trees (Michael and Chve, 1994), sometimes succulent and cactus-like, comprising one of the largest families of plants with about 300 genera and 7500 species that are further characterized by the frequent occurrence of milky sap (Kaey, 1989).

Acalypha marginata is a shrub, monoecious and widely grown as a garden ornament (www.botany.com), and considered by some authorities to be a variant of *Acalypha wilkesiana*. The antimicrobial activity of the genus *Acalypha* has been well established (Adesina *et al*., 1980; Adesina *et al*., 2000; Alade and Irobi, 1993; Mahran *et al*., 1993). In traditional medicine, the leaves of *A. marginata* are eaten as vegetables in the management of hypertension.

Medicinal plants are commonly used in treating and preventing specific ailments and diseases, and are generally considered to play a beneficial role in healthcare. They are already important to the global economy. Demand for medicinal plants is steadily increasing not only in developing countries but also in the industrialized nations (Srivastera *et al*., 1996). World Health Organisation (WHO) estimates that approximately 80 % of the developing world's population meets their Primary Healthcare needs through traditional medicine (Baurmman, 1982). Within the last few decades, many plants have been screened for their biological and pharmacological properties by researchers. Efforts are continually being made to examine the merits of traditional medicine in the light of modern science with a view to adopting effectively beneficial medical practice and discouraging harmful ones (Sofowora, 1986).

In this study, the phytochemical and biological activities of the crude extract of *Acalypha marginata* were evaluated with particular interest in its antidiarrhoea and antibacterial effects. The effects of the methanol extract on the gastric emptying time and on the gastroin-

testinal motility were also assessed.

MATERIALS AND METHODS

Preparation of plant extract: The leaves of *Acalypha marginata* Spreng (Euphorbiaceae) were collected in Ugbowo area of Benin City, Edo State, Nigeria. The plants were authenticated by the curator at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where voucher specimens were deposited. The fresh leaves were air–dried for 72 h and powdered using an electric mill.

Organisms: The organisms used were clinical isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* and *Streptococcus faecalis* obtained from patients in the University of Benin Teaching Hospital, Benin City.

Animals: Swiss albino mice of both sexes (24. 63 $\pm$ 1. 52 g) and male Wistar rats (210 $\pm$ 13. 48 g) were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. All the animals were kept under standard environmental conditions and were handled according to international protocol for use of animals in experiments (National Institute of Health, 2002). They were fed with standard pellets and tap water *ad libitum*. Ethical approval for the study was obtained from the College of Medicine, University of Benin Animal Ethics Committee (ADM/F. 22 A/VOL. VIII/349).

Phytochemical studies: Screening for secondary plant metabolites was carried out according to previously described methods (Brain and Turner, 1975; Ciulei, 1981; Evans, 2006; Harborne, 1992). These include chemical tests for alkaloids, tannins, saponin, anthracene, cardiac glycosides and cyanogenetic glycosides. Thin-layer chromatography of the methanol extract containing 10 % H_2SO_4, shaken with chloroform and free alkaloids precipitated by the addition of excess ammonia and extracted with chloroform, on silica gel–G, activated by heating at 110 ^{0}C for 30 minutes was developed with the solvent system Methanol : chloroform (7: 3). Chromatoplates were viewed under the UV light and sprayed with Dragendorff's spray reagent and R_f values calculated. Paper chromatography of the methanol extract, using ascending method on Whatman No. 3 was developed with the solvent system n-butanol: water: acetic acid (4: 1: 5), examined both in daylight and under UV light, sprayed with Ferric chloride until colours developed and R_f values calculated.

Antimicrobial screening: Powdered leaf material (2kg) of *Acalypha marginata* Spreng was extracted with methanol at room temperature. After 48 h, the extract was clarified by filtration and evaporated to dryness *in vacuo*. Residues were collected and evaluated against the six test organisms at 100 mg/mL in DMSO. The well diffusion method was used for the antimicrobial screening against the six test organisms, following standard procedures with nutrient agar as medium.

The minimum inhibitory concentration (MIC), the lowest concentration of a compound that inhibits growth of a microorganism, was determined by the standard two–fold dilution technique using nutrient broth medium (Washington, 1985).

Intestinal transit test: The mice used for this test were starved for 24 h prior to the experiment but allowed access to water. They were divided into 7 groups of 5 each. Mice in groups A, B, C and D were administered with 100, 200, 300 and 400 mg/kg extract, the fifth group 5ml/kg normal saline (vehicle for the reconstitution of the extract), sixth group 2.5 mg/kg Atropine sulphate (Adrich. Chem. Co. UK) all by the intraperitoneal route. The seventh group received 0.5 mg/kg Carbachol. After 30 minutes, they were fed through the gastric tube with 0.5 ml of freshly prepared charcoal meal (10 % charcoal in acacia gum). The mice were sacrificed 20 minutes later by cervical dislocation and gastrointestinal tract removed. The distance travelled by the charcoal meal from the pylorus was measured and expressed as the percentage of the total length of the small intestine extending from the gastropyloric to the ileocaecal junction (Onwukaeme and Anuforo, 1993). The intestinal motility was derived from the equation:

$$\% \text{ motility} = \frac{\text{Distance traveled by the meal}}{\text{Total length of small intestine}} \times 100$$

Castor oil induced diarrhea: Rats for this

test were first observed for any wet faeces/ droppings (their somewhat rounded or irregular shape, soft consistency and the presence of a brown stain surrounding each faeces on the filter paper). They were easily distinguished from the normal dry faeces which were elongated, regular in shape, hard and did not stain the filter paper. Those that produced wet droppings were not used for the test.

Diarrhoea was induced by oral administration of castor oil (0.5 ml rat $^{-1}$) to overnight fasted rats. *Acalypha marginata* extract (100 - 400 mg/kg) or vehicle (normal saline) or reference (loperamide) were given intraperitoneally 60 minutes to the rats before cathartic administration of the castor oil.

Two hours after dosing with castor oil, the individual rat cages were inspected for the presence of wet faeces, their absence was recorded as a positive result, indicating protection from diarrhoea at that time (Izzo *et al*., 1992).

RESULTS

Phytochemical screening of the leaves of *Acalypha marginata* revealed the presence of alkaloids, tannins and saponins (Table 1). Chromatographic fingerprints were established using Thin-layer and Paper Chromatographic techniques (Table 2) detecting with Dragendorff and Ferric chloride for the presence of alkaloids and phenolic compounds respectively.

The *in vitro* antimicrobial screening (Table 3) showed the susceptibility against Gram-negative and positive organisms. The methanol extracts of *A. marginata* exhibited inhibition all the Gram- negative organisms, except *Proteus mirabilis* which was also not susceptible to the positive control. Gentamycin, however exhibited inhibition against the two Gram -positive organisms used for the test. The extract only exhibited activity against *Staphylococcus aureus*. The results of the Minimum Inhibitory Concentration (MIC) are presented in Table 4.

The methanol extract of *A. marginata* had a dose-dependent decrease in intestinal motility of the treated mice (Table 5). The results for *A. marginata* were more pronounced when compared to atropine sulphate (positive control). In the castor oil induced diarrhoea (Table 6), doses of the methanol extracts of *A. marginata* as well as Loperamide did not produce any wet faeces.

DISCUSSION

Phytochemical screening of the leaves of *Acalypha marginata* for secondary plant metabolites revealed the presence of alkaloids, tannins and saponins. Typical alkaloids derived from plant sources are basic in nature. They contain one or more nitrogen atoms (usually in a heterocyclic ring) and often have marked pharmacological effects when administered to man and other animals, thus their presence is of particular interest (Evans, 2006). Tannins being astringent generally have antidiarrhoeal properties while the anthraquinones (which were absent in *Acalypha marginata*) have purgative properties (Onwukaeme and Udoh, 2000). Plants containing alkaloids and/or tannins have been known to possess antidiarrhoeal activities, amongst which are *Ageratum conyzoides* and *Vernomia cinerea* (Obasi *et al.,* 1990). It could therefore be suggested that the secondary plant metabolites present in *A. marginata* are responsible for the biological activities observed.

Most diarrhoeas are known to be infective in origin and there is therefore a role for anti-infective agents in the management of such diarrhoeas (Obasi *et al.,* 1990). Among other possible causative microorganisms implicated in diarrhoea, the role of enteroaggregative *Escherichia coli* has been reported (Abere and Agoreyo, 2006). The results obtained when the methanol extract was subjected to antibacterial test indicated that *A. marginata* compared favourably with Gentamycin for the organisms employed.

Another common observable feature in diarrhoea cases is hypermotility of the gut. Reduction of this motility has been achieved by inclusion of anti-spasmodic agents in preparations intended for use in the treatment of diarrhoea. The effects of graded doses of the methanol extract of *A. marginata* on intestinal transit using freshly prepared charcoal meal was examined using atropine and carbachol as reference drugs (both have known effects on the GI tract). The extract had a dose-dependent decrease in intestinal motility of the rats so treated. Atropine sulphate that was

used as a reference drug reduced the motility probably by its antimuscarinic effects on the intestinal tract. In the castor oil induced diarrhoea experiment, the extracts exhibited the same characteristics as the reference, Loperamide by non-production of wet faeces. Inhibition of the intestinal motility and non-production wet faeces could be useful actions in the treatment of diarrhoea.

CONCLUSION

On the basis of the overall results obtained from our investigations, Acalypha marginata Spreng possesses an antidiarrhoeal property which lends credence to its use in ethnomedicine.

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