

Short Communication

Evaluation of the anti-edematogenic activity of the aqueous extract of *Leea guineensis*

Falodun A.*, Okunrobo L. O. and Agbo L. O.

Department of Pharmaceutical Chemistry, Faculty of Pharmacy University of Benin, Benin city. Nigeria

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Leea guineensis leaf is a medicinal herb used in folk medicine for the management of inflammatory disease conditions such as in splenomegaly in children. Because of this use, the plant was evaluated for anti-inflammatory properties. The phytochemical studies revealed the presence of saponins and glycosides as the secondary metabolites. Using the carrageenan-induced paw oedema, there was a significant ($P < 0.001$) reduction in edema. The study also revealed a dose dependent anti-edematogenic activity. These preliminary investigations seem to support its use by herbalists to treat inflammatory disease condition such as splenomegaly.

Key words: Carrageenan, *Leea guineensis*, edematogenic activity.

INTRODUCTION

Herbal medicine has been recognized by the World Health Organization (WHO) as an important component of primary health care and as such efforts are being made to harness its therapeutic potential with that of orthodox medicine (WHO, 1995). The use of plants or plant parts as source of medicine to treat different ailments and disease conditions is common to all tribes in the world. For example in 1996 alone, six of the top 20 pharmaceutical drugs sold were natural products and more than 50% of this top 20 were linked directly to natural product research (Buss, 1999).

Leea guineensis is an evergreen shrub belonging to the family Ampelidaceae. It blooms throughout the year and is found mostly in tropical forest zones like in Southern Nigeria especially around Benin City. The aqueous extract of the leaves is used locally by herbalists for the treatment of enlarged spleen in children.

Preliminary work in our laboratory has shown that it has no antibacterial activity, but may have significant anti-edematogenic effects. The purpose of this work, therefore, was to investigate this latter aspect and perhaps confirm the pharmacological basis for its successful application in the treatment of inflammatory disease con-

ditions by traditional medical practitioners in Nigeria.

MATERIALS AND METHODS

Collection of plant materials

The plant was collected from Iguosa village in Ovia North East Local Government of Edo State around the month of August 2005 with the help of an herbalist. It was identified and authenticated by Dr. B. A. Ayinde a staff of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City Nigeria. A specimen has been preserved in the herbarium of the Department for future reference.

Extraction and chromatography

The fresh leaves were air dried for a period of 2 weeks. They were ground to a fine powder with the aid of a mechanical grinder. 250 g of the powdered plant was extracted with 110 ml of distilled water for 48 h using distilled water at room temperature. The extract was concentrated to dryness with a rotary evaporator at reduced pressure. 27.15 g of the concentrated extract was obtained. The extract was stored in a refrigerator at -4°C to prevent deterioration. Dilutions were prepared fresh each day for the experiments.

Phytochemical screening

The freshly prepared aqueous extract was subjected to chemical analysis testing for the presence of alkaloids, saponins, flavonoids,

*Corresponding authors E-mail: faloabi25@yahoo.com. Tel: +234-8032396550.

reducing agents, tannins and terpenes. The method of Trease and Evans (1989) and Harbone (1973) were used.

Pharmacological evaluation

Wistar rats (150 - 180 g) of either sex kept at the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water (plant extracts were administered orally by gavage in 5% Tween 80 suspension at different dose levels. Ethical approval was obtained from the Animals Use and Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Determination of the LD₅₀ of the aqueous extract

The method of Lorke (1983) was employed. Twenty (20) mice (20 – 22 g) of either sex were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin City. The animals were randomly divided into five groups of four mice each. Prior to testing, the animals were feed with mice pellets and had free access to drinking water but starved for 12 h before testing. The first four groups were orally administered with 1, 2, 3 and 5 g/kg of the extract. General symptoms of toxicity and mortality were first observed for 24 h after which the animals were left for further 14 days for any delayed toxicity.

Anti-edematogenic activity

Anti-edematogenic activity was measured using carrageenan-induced rat paw oedema assay (Winter et al., 1962; Adeyemi et al., 2002). Groups of 6 rats of both sexes (pregnant females excluded) were given a dose of the extract. After one-hour 0.1 ml of 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for four hours (Bamgbose and Noamesi, 1981). Two groups of drug treated rats and one control group were used each test day. The mean paw oedema value for the test group being compared with its mean value for the control group for that day.

Anti-edematogenic activity (Amir and Kumar, 2005) was measured as the percentage reduction in oedema level when drug was present, relative to control. Indomethacin (10 mg/kg) was administered orally as reference drug while 5% Tween 80 was used as negative control.

Statistical analysis

All data were expressed as mean \pm SEM; the student's t-test was applied to determine the significance of the difference between the control group and the test extracts.

RESULTS AND DISCUSSIONS

The phytochemical studies revealed the presence of saponins and reducing sugars. The presence of steroidal saponins was confirmed (Table 1). The aqueous extract was found to be partially non toxic at the doses range of 1 – 5 g/kg.

Inflammation is the response of living tissues to injury;

Table 1. Photochemical compositions of the aqueous extract of *Leea guineensis*.

Chemical constituents	Aqueous extract
Alkaloids	+
sapomns	+
Flavonoids	-
Tannins	-
Reducing sugars	+
Anthraquinone gJycosides	-

+ Present; - absent.

it involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Vane and Bolting, 1995). It is known that anti-inflammatory effects may be elicited by a variety of chemical agents and that there is no remarkable correlation between their pharmacological activity and chemical structure (Sertie et al., 1990).

Carrageen -induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug (Manueli et al., 1994) and appeared to be the basis of the discovery of indomethacin, an anti-inflammatory drug (Winter et al., 1963). The inflammation can be induced in two phases; the first phase is mediated by the release of histamine and serot-onin, followed by kinase release and then prostagladins while the second phase involves the release of arachidonic acid from the tissues (Ogonowsk et al., 1997). Thus, the *in vivo* anti-edematogenic activity of the aqueous extract of the leaf of *L. guineensis* was evaluated by carrageenan-induced rat paw oedema method (Winter et al., 1962; Adeyemi et al., 2002). The results are shown in Table 2. The extracts were screened at three different dose ranges to ascertain the dose-depen-dent level. From the results obtained, the dose at 400 mg/kg caused 73% inhibition of the oedema level, which was the highest activity and even greater than the reference drug. The dose at 200 mg/kg also showed significant anti-inflammatory activity ($P < 0.05$).

The presence of copious amount of saponins probably was responsible for the activity. This is because the anti-edematogenic activities of most medicinal herbs have been closely related to the high content of saponins.

CONCLUSION

The positive response on the anti inflammatory effect of *L. guineensis* justifies the traditional use of the extract in the treatment of splenomegaly. Further work will be carried out to identify and characterized the chemical components responsible for the pharmacological action using a bio-activity guided isolation techniques.

Table 2. Carrageenan rat paw oedema: Anti-edematogenic activity of the aqueous extract of the leaves of *Leea guineensis*.

Drugs	Doses (mg/kg, p.o.)	Change in paw oedema (mm)	Oedema inhibition (%) relative to control at the 4 th h
Control 5%Tween 80	0.3 ml	2.75±1.35	-
Indomethacin	10	1.52±0.29	46
Aqueous extract	100	2.60±0.57	6
	200	1.25±1.45*	56
	400	0.75±1.47**	73

Values are mean ± SEM.

*P<0.05, **P<0.001, significantly different from control (paired t-test, n = 6).

p.o. = per oral.

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