

Pharmacobotany, Phytochemical Analysis and Anti-inflammatory effect of the Ethanolic Extract of *Luffa operculata*

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ABSTRACT

Background: *Luffa operculata* is a vegetable species well known in the traditional peruvian medicine for its many medicinal properties and cosmetic applications. **Objective:** The aim objective was to determine the pharmacognostic characteristics of *L. operculata* as well as observing the pharmacological effect of the ethanol extract of *L. operculata* fruit on chronic inflammation in rats. **Materials and Method:** Phytochemical analysis was carried out by using specific chemical reagents for each constituent chemical, the pharmacobotanical study was done with a histological tinction (fruit, stem and leaves), which were stained with Safranin 1% and Toluidine blue 1%; the chronic inflammation was assessed by air bag method in Holtzman male rats. Doses of 100, 250 and 500 mg/Kg were tested in order to determine the anti-inflammatory effect, which was demonstrated with histopathological evaluation and lymphocytes reduction. **Results:** The main findings indicate that the ethanolic extract presented saponins, alkaloids, carbohydrates, terpenes and steroids. The efficiency of lymphocyte reduction per field in the histopathological study of the granuloma was 58.4% with the middle dose of 250 mg/kg ($p < 0.0001$), which gives a dose-independent anti-inflammatory effect in rats. **Conclusion:** *Luffa operculata* presented anti-inflammatory effect at 250 mg/Kg by oral administration in a chronic experimental model of inflammation in rats.

Key words: Pharmacobotany, Saponins, *Luffa operculata*, Anti-inflammatory.

INTRODUCTION

Nature medicine plays a crucial role in the prevention and treatment of several pathologies (acute and chronic diseases).^{1,2} As of medicinal plants are obtained numerous bioactive phytochemicals, which could be studied as promising drugs passing by preclinical and clinical steps.³ These compounds generally are tested based on its traditional uses known as ethnopharmacology. Being necessary validate this information, pharmacological studies are carried out in order to give information scientific to population.⁴

Inflammatory process is produced as a response to cell injury and against pathological microorganism (parasites, bacteria, fungi, virus, etc), trauma, or toxins participating as a critical host-defense mechanism.⁵ Additionally, acute inflammatory may lead to a chronic inflammation, which carry out a subsequent event since tissue destruction until fibrosis, and necrosis. Several kinds of cells are involved releasing inflammatory mediators such as prostaglandins, histamine, nitric oxide, interleukins among other. Otherwise, in acute inflammation, neutrophils are the major cell types whilst mononuclear cells (mostly macrophages, lymphocytes and plasma cells) are involved in chronic inflammation.⁶

Luffa operculata belongs to *Cucurbitaceae* family, which is a medicinal plant with different popular names: in Brazil buchinha-do-norte or cabacinha, or sponge (esponja; translated to Spanish) in other Latin American countries.⁷ The community has

given it different uses, which also depend on the area or region: purgative, abortifacient, due to its irritating action on the mucosa; in Brazil there are preparations for sinusitis.⁸ Phytochemical analysis of *L. operculata* evidenced mainly saponins such as Neocucurbitacins A and B⁹ opercurins A and B,¹⁰ Jarvenins I and III. Furthermore, other chemical groups such as resin, free sterols, aliphatic esters, quinones, organic acids and phenols. However, tannins and flavonoids have not been reported. Elasterin A, B and D, cucurbitacins and isocucurbitacin were found in its resin.¹¹

On the other hand, cucurbitacins are highly oxygenated triterpenic derivatives, which occur mainly, but not exclusively in plants of the cucurbit family, are intensely bitter and some can be highly toxic.¹² Recent works have reported that cucurbitacins are cytotoxic substances, its main mechanism is mediated by suppression of phosphorylation of STAT3 (signal transducer and activator of transcription 3).^{13,14} At present, in Peru no more scientific information has been found on *Luffa operculata*. For this reason, this research seeks to determine the pharmacological and phytochemical characteristics of the ethanol extract of the *Luffa operculata* Cogn fruit. (field soap) as well as observing the effect on chronic inflammation in rats.

MATERIALS AND METHODS

Plant material

Luffa operculata plant was collected, in January 2019 from San José Bajo, Santiago de Cao district,

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Ascope Province, La Libertad Department, Peru. The material plant was classified (043-USM-2019) at the National Herbarium of the Universidad Nacional Mayor de San Marcos (UNMSM), Lima, Peru.

Animals

An integral of 36 male Holtzman rats (110 ± 20 g) were purchased from Bioterio of the Center of Biological products of the National Institute of Health (Lima, Peru). Animals were acclimatized at room temperature during 15 days before the experiments, they were kept in plastic cages with free access to pelletized food and water with 12 h light/dark cycle. Rats were randomized into six groups of six animals per plastic cages.

Chemicals

Carrageenan was purchased from Sigma-Aldrich, USA, ethylenediaminetetraacetic acid, toluidine, safranin, ethanol absolute, glacial acetic acid and trichloro acetic acid (TCA) were obtained of local chemical companies.

Pharmacobotanical study

Stems, leaves, flowers and fruits were fixed in FAA (formaldehyde, ethyl alcohol, glacial acetic acid, 10: 50: 5: 35). The samples were processed, at the level of the middle parts of the stem, leaf, female flower and fruit, with the following techniques:¹⁵ transverse and longitudinal freehand cuts of the stem, leaves, flower and fruits, as well as superficial cuts of leaves; all histological sections were rinsed in 50% sodium hypochlorite, washed and then stained in 1% toluidine blue and 1% cresyl violet stains, with mounting in carbolic glycerin. Photomicrographs at 40, 100 and 400 magnification on a Leica DM50 microscope with built-in camera and the use of Lasez[®] software. The final images were processed with Adobe Photoshop CS3 10.0.

Extraction of plant material

Fruits of *Luffa operculata* (500 g) were cleaned, selected and dried at 40°C, then were pulverized and soaked with 96 % ethanol for 7 days. The liquid extract was filtered with a filter paper Whatman N° 1 and evaporated using a rotavap. The crude ethanolic extract obtained (10 g) was stored until further real study in an amber flask at 4°C.¹⁶

Phytochemical screening

Chemical constituents were confirmed by chemical reaction with specific reagents for each metabolite such as: sterols, terpenoids, alkaloids, carbohydrates, flavonoids, tannins, phenols, glycosides, saponins according to the validated methodology.¹⁷

Antiinflammatory effect in an experimental model of chronic inflammation

Experimental design

For this, chronic inflammation was induced by the experimental model of Sedwick *et al.*¹⁸ or also known as the air bag method in rats. The animals were acclimatized for a week, between 21 and 25 °C and 50 and 60% humidity, with 12 hours of light and darkness. On the first day the skin was shaved, on the second day 20 mL of air was injected subcutaneously into the intracapsular area of the loin, forming an oval air pocket, on the fourth day 10 mL of air was added, on the fifth day the animals were randomly distributed in 6 groups of 6 rats and treated orally: 1) Physiological saline (PS) 2 mL / kg; 2) Carrageenan (C) + PS 2 mL / kg; 3) C + Dexamethasone 4 mg / kg; 4, 5 and 6) C + Extract 100, 250 and 500 mg / kg respectively. Three hours later, 2 mL of 1% carrageenan dissolved in saline solution was injected, directly in the bag; the animals were sacrificed after 24 hours using 100 mg / kg pentobarbital. An injection of 5 mL of saline solution containing 0.1% ethylenediaminetetraacetic acid (EDTA) was applied to the air bag and

then a small incision was made in the wall of the bag, and the contents of the air bag were removed. carefully using a sterile Pasteur pipette. 3 mL of exudate was obtained, to which the total proteins were measured, and a histopathological study of the carrageenan-induced granuloma was carried out.

Histological analysis

The histopathological evaluation, according to Devi, was carried out with pieces of skin containing the granulomas, which were gently washed with saline solution, to remove the blood and the debris adhered to the tissue, being fixed in a buffered 10% formaldehyde solution; later, 3 μ thick sections were made with the help of a microtome, and stained with hematoxylin and eosin; After dehydration and cleaning of the slides, microscopic observation was made: qualifying inflammatory infiltrate (absent, mild, moderate, severe); number and percentage of lymphocytes. The slides were examined under a light microscope (Olympus BX51).¹⁹

Statistical analysis

Statistical analysis was carried out using SPSS v. 21. Normality test by the Shapiro-Wilk statistic and one-way analysis of variance (ANOVA) followed by Tukey multiple comparison. A P-value of 0.05 was considered statistically significant. Experimental data are presented as mean ± standard deviation (SD).

Ethical considerations

All experimental procedures were considered following international guidelines (CCE Council 86/609) and approved by the ethical committee of the Universidad Nacional Mayor de San Marcos (01414-R-12-UNMSM), animals were euthanized by intravenous injection of pentobarbital (100 mg/kg).

RESULTS

Description of the internal tissues of the flower, fruit and seed

The female flower is largely pedunculated, with an inferior ovary. In cross section it is shown tetralocular with divided locules and parietal ovules. Abundant pubescence of elongated multicellular trichomes and presence of shortly pedunculated glandular trichomes.

Fruit with epicarp fused with epidermis of the floral thalamus; thick subepidermal layer with sclereids (corresponding to the epicarp), parenchymal mesocarp. Seed with external seminal covering consisting of an epidermal layer of elongated cells arranged in a palisade and a strong cuticular covering; a sclerenchyma layer and an inner covering with elongated sclereids. Endosperm with reserve parenchyma.

Determination of phytochemical constituents

In table 1 is showed the main chemical groups identified by precipitation and color change in the ethanol extract of *Luffa operculata*.

Table 1: Qualitative phytochemical screening for *Luffa operculata*.

S. NO	Constituents	Tests Performed	Results
1	Terpenoids	Salkowski Test	+
2	Alkaloids	Mayer's Test	+
		Wagner's Test	+
3	Carbohydrates	Molisch's Test	+
		Fehling's Test	+
4	Flavonoids	Shinoda's Test	-
5	Tannins	Gelatin Test	-
6	Phenols	Ferric chloride	+
7	Saponin	Foam Test	+

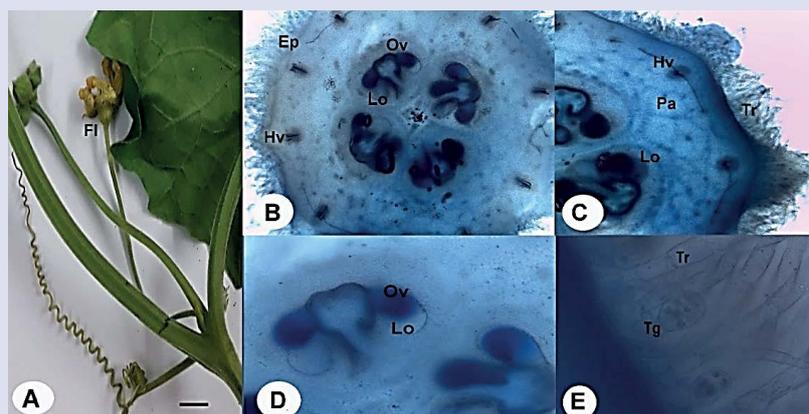


Figure 1: Internal structure of the *Luffa operculata* flower. A: detail of the female flower, B and C: general view of the ovary in cross section, D: detail of locules and ovules in the ovary, E: Pubescent epidermis of the ovary with elongated multicellular (tr) and glandular (Tg) trichomes (Tr). Cuticle (Cu). A. bar 1 cm, B and C. 100x, D and E. 400x.

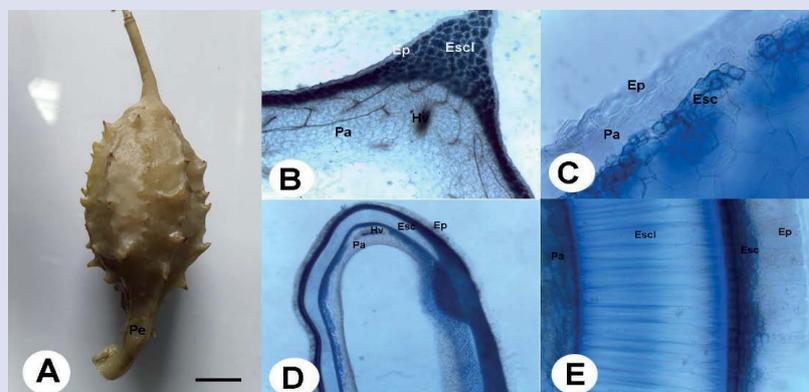


Figure 2: Internal structure of the fruit and seed of *Luffa operculata*. A: General view of the fruit. B: panoramic view of the external part of the fruit. C: detail of the thalamus and epicarp of the fruit. D: general view of the seed (without the embryo). E: detail of the inner seed coat. Pe, peduncle; Ep, epidermis; Escl, sclereids; Esc, sclerenchyma; Pa, parenchyma; Hv, vascular bundle. A. bar 1 cm, B and D. 100 X. C and E. 400 X.

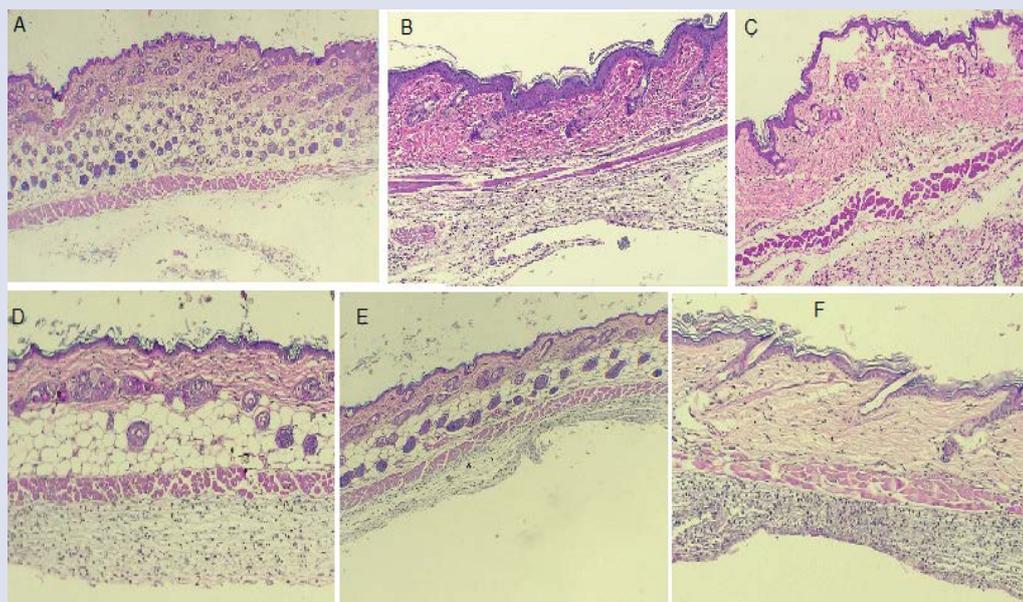


Figure 3: Histopathological analysis in the test of granuloma induced by Carrageenan on the skin of the rat. (10X). Inflammatory infiltrate ((absent, mild, moderate, severe) - number and percentage of lymphocytes): A:PS 2 mL/Kg (Absent – 8%), B: Carrageenan +PS (Moderate to severe – 40%), C: Carrageenan + Dexamethasone 4 mg/kg (mild – 13%), D: Carrageenan + Extract LO 100 mg /Kg (Moderate – 39%), E: Extract LO 250 mg /Kg (Mild – 22%), F: Extract LO 500 mg /Kg (Moderate – 38%).

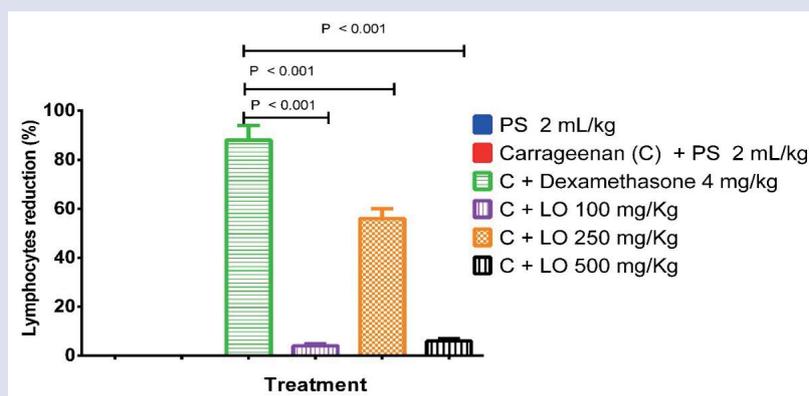


Figure 4: Efficacy of lymphocyte reduction per field to histopathological study of carrageenan granuloma in the skin of the rat back. ($p < 0.0001$).

Histopathological evaluation in the anti-inflammatory effect of *L. operculata*

As are show in figure 3 and 4, there was a reduction in the inflammatory infiltrate of the chronic model in rats induced by air bag. Percentage de lymphocytes was better in the group treated with *L. operculata* at 250 mg/Kg by oral administration. However, animals treated with dexamethasone at 4 mg/Kg had the highest reduction compared with the different treatments carried out with the ethanol extract.

DISCUSSION

The *Cucurbitaceae* family makes up an important group of plant species, mostly it develops in tropical climates, with approximately 130 genera and 1,300 species, the vast majority is used for food and medicinal purposes, in Peru around 27 genera and 110 have been reported. species and some of them endemics.²⁰ *L. operculata* is a fruitful and useful vine, with angular branches that can be up to 3 meters high. Its broad, kidney- or heart-shaped leaves have three to five lobes and are 10 to 12 cm long. Its fruits are tuber-like, ovoid to ovoid-oblong, pubescent, less than 10 centimeters long. The interior of the fruit is pulpy, fibrous and with small flat, dark brown seeds that are between 7.5 and 10 millimeters long. It presents unisexual flowers, the corolla is yellow to pale yellow, the lobes of the staminate flowers are close to 12 millimeters long and 6 millimeters wide in our histological findings of the different organs of the species, it corresponds to what has been reported in the literature of this species and fam.²¹

Inflammation, like the host's response to deleterious stimuli, is mediated by pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6, which are secreted by immune cells recruited to injury sites.⁶ Sustained release of inflammatory cytokines and reaction result in chronic inflammation, which is characterized by excessive infiltration of immune cells and is the main cause of many diseases.⁶ The mRNA levels of TNF- α , IL-1 β and IL-6 can also be suppressed in mice and rat model of multiple diseases by saponins like dioscin, such as acute liver injury, liver fibrosis, obesity, brain and intestinal I / R injury and inflammatory lesions of kidney, thus attenuating the inflammatory damage,^{22,23} these histopathological changes have been also showed with the oral treatment of *Luffa operculata* evidenced in Figures 3 and 4, due to its steroid saponin content.

Saponins found in *L. operculata* have allow to understand the mechanism of various biological activities such as dysregulates melatonin and pro-inflammatory cytokines, due to the presence of cucurbitacins.²⁴ In other study the relationship between the chemical structure and pharmacological activity is strongly linked, for example the activity of protopanaxatriol-type saponins from *Panax notoginseng*

is based on the number, length and position of sugar side chains, and the type of glucosyl group in the structure of the molecule affecting its hemolytic activities and adjuvant potentials but have significant effects on the nature of the immune responses.²⁵ Saponins from *Bupleurum rotundifolium* were active in chronic inflammation, reducing efficiently ear weight and neutrophil influx.²⁶

In Brazil, the aqueous extract of *L. operculata* fruit has been used as a remedy for urethritis and edema.²⁷ On the other hand, several compounds were isolated of *L. operculata*, which could be responsible of the anti-inflammatory effect such as Opercurins A and B²⁸ as well as Neocucurbitacins A and B present in the methanol extract.²⁹ Although, we did not isolate the phytoconstituents, the phytochemical screening revealed the presence mainly of steroidal saponins but without any reaction to flavonoids.

CONCLUSION

The ethanolic extract of *L. operculata* showed chronic anti-inflammatory effect against granuloma model induced in rats. Histopathological evaluation showed a reduction of lymphocytes at 250 mg/Kg and saponins present in the ethanol extract could be the bioactive metabolites responsible of the pharmacological effect.

CONFLICTS OF INTEREST

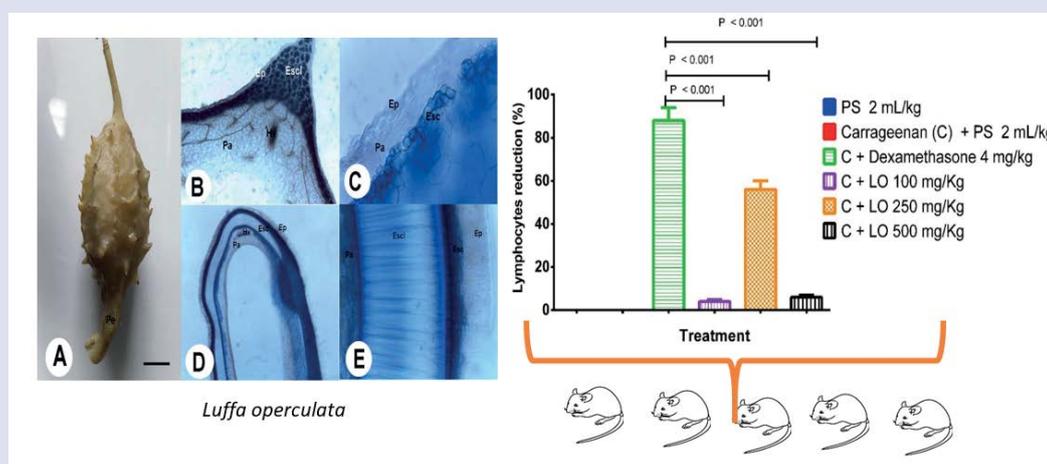
The authors have no conflicts of interest.

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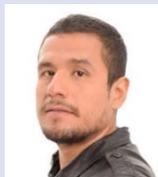
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GRAPHICAL ABSTRACT



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