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PHYTOCHEMICAL PROFILING AND ANTIULCER POTENTIALS OF ETHYLACETATE EXTRACT OF STEM BARK OF *KHAYA GRANDIFOLIOLA* C.DC (MELIACEAE)

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ABSTRACT

Khaya grandifoliola of the family Meliaceae is a medicinal plant that have been implicated in traditional medicine in the West African region for the treatment of illnesses like fever, malaria, cough, bacterial Infections, Stomach aches, gastric pains and remedy against worm infestations. The plant is also known for its characteristic antiulcer properties which are believed to be attributed to phytochemicals such as Alkaloids, saponins and Flavonoids. Synthetic drugs used in the treatment of gastrointestinal tract infections are known to cause adverse drug reactions as a result of synthetic chemicals associated with them hence, the need to sought traditional and safe drugs for treating infections of the gastrointestinal tract. *Khaya grandifoliola* was used for this study, and the plant was evaluated for its antiulcer properties. A 500g weight of the plant was obtained after chopping, drying and grinding the bark for the extraction process. The plant extract was obtained using ethyl acetate by cold maceration and a toxicity test was carried out to determined the doses that will be administered to the mice. Indomethacin was used to induced pectic Ulcer in 20 albino rats and ethanol for another 20 albino rats. The rats were then divided into 5 groups (A, B, C, D and E) for both models. Groups A and B received 100

and 200 mg/kg of plant extract respectively, Group C 400mg/kg, group D received Omeprazole (20 mg/kg) for indomethacin induced and ethanol induced respectively and Group E which was the negative control received Tween 80. After 8 hours of the treatment the animals were sacrificed and their stomach examined with a hand lens to determine the extent of the ulcer inhibition. Phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, proteins, reducing sugar and oils were found in the crude extract. Acute toxicity revealed a high therapeutic index and the plant is relatively safe for consumption. The percentage ulcer inhibition of the crude extract was highest (92.11 %) at 400mg/kg for indomethacin induced and highest (97.56 %) at 400mg/kg for ethanol induced. These results are statistically comparable with the standard Omeprazole (88.16 %) and (65.85 %) at 20mg/kg respectively. The plant has a profound antiulcer activity. The crude extract was a claim by traditional leaders in Akure. It is also relatively safe for consumption as indicated by the toxicity test.

KEYWORDS: *Khaya grandifoliola*, antiulcer, phytochemical analysis, acute toxicity, extract.

INTRODUCTION

Traditional medicine can be described as the combination of knowledge and practice used in the diagnosis and prevention or elimination of physical, mental or social diseases which may rely on the past experience or other observation handed down from generation to generation, verbally or in writing.[1] Herbal medicine is the oldest form healthcare known to mankind and over 50 % of all clinical drugs are of natural products origin, and natural products play important roles in drug development in the pharmaceutical industry. Plants are veritable source of bioactive principles with profound therapeutic and medicinal value About 21,000 plant species have the potential for being used as medicinal plants. About 25 % of drugs prescribed worldwide are derived from plants. These are almost free from side effects normally caused by synthetic chemicals.[2] A wide variety of such plants have been used to treat gastrointestinal disorders, diuretic and hypertensive complications, because it lowers arterial pressure. These plant medicines, otherwise referred to as herbal medicines contains active ingredient that are usually in crude form or as standardized plant preparations.[3] Medicinal plants have a renewable source; also availability of medicinal plants in developing countries (like Nigeria) is not a problem. Herbal medicines are used in the treatment of both chronic and acute diseases such as cardiovascular diseases, prostrate problems, depression,

inflammations and ulcers. Moreover, there are still some disadvantages of herbal medicines; some herbal medicines have been found to be toxic at certain doses and so the dosage or mode of administration should be guided by research (clinical trials) result.

Peptic ulcer is simply descriptive of erosion in the gastric or duodenal mucosa that penetrates to or through the muscularis, a lesion observable by a radiologist or pathologist. A peptic ulcer may result from either excessive vagal drive or excessive activity of gastrin-producing cells, as in the tumor or hyperplasia of Zollinger-Ellison syndrome, or possibly from defective mucus protection of the lining epithelium. Duodenal ulcers occur in the first inch or two beyond the pylorus where the gastric juice is still unneutralized and pepsin can be activated. Despite extensive research, the etiology of peptic ulcer remains dubious. It is likely that the cause of ulceration differs between individuals. Indeed, research conducted since the mid-1980s has shown that the bacterium Helicobacter pylorus present in more than 90 % of duodenal ulcers and about 80 % of gastric ulcers. Eradication of the microorganisms seems to be curative for this disease. Against the backdrop of increasing antimicrobial resistance present in this pathogen, the search for new drugs, especially herbal remedies would be a veritable option.[4]

A variety of botanical products have been reported to possess antiulcer activity; Ficus arnottiana Family: Moraceae Part used: Fruits, leafs Common name: paras papal. Active constituent: Fruits of the plant contain β-sitosterol, Gluanol acetate and Glucose, Friedelin, Sterols, alkaloids, Carbohydrates, Tannins, Phenols. It has several vernacular names including Para's pipal and kodiarasu. Alstonia Scholaris R. Local name: Saptaparna Family: Apocynaceae. Active constituent: Alkaloids, coumarins, flavonoids, phlobatannin, reducing sugars, simple phenolic, steroids, saponins and tannins. The saponin and flavonoids are used in ulcer treatments. Asparagus racemosus Willd Local name: Satawari, satavari. The principal chemical constituents exhibiting medicinal properties are Shatavarin (steroidal saponin). Four types of Shatavarin, Shatavarin I–IV are present in roots. Apart from this, quercitin-3-glucorinide, rutin in shoots; sitosterol, stigma sterol and some other unidentified saponins are found in fruits and seeds. Root powder is used to increase vigor and strength; roots are used as an antiinflammatory, antiulcerogenic, antitumor activity. Bauhinia variegata Linn. Local name: Kachnar, Kaniar. Principal constituents: The seeds yield fatty oil, the bark yields fiber. Five flavonoids isolated from the different organs of B. variegata were identified as Quercetin, rutin, Quercetin, apigenin and apigenin 7-O-glucoside. Saponins, steroids,

flavonoids, alkaloids, tannins, sugars are also present. The bark is astringent, tonic and anthelmintic. It is useful in scrofula and skin diseases. It is also used for ulcers and leprosy. Carica papaya L. Local name: Papitta. Medicinal properties: Papaya is used in tropical folk medicine. It contains many biologically active compounds. Two important compounds are chymopapain and papain, which are widely known as being useful for digestive disorders and disturbances of the gastrointestinal tract. Chemical constituents and components: Main chemical components are papain, chymopapain, pectin, carposide, carpaine, pseudocarpaine, dehydrocarpines, carotenoids, crypto glavine, cis-violaxanthin and antheraxanthin. The milky white sap produced by the trunk of the papaya tree is also a useful remedy and is applied externally to accelerate the curing of abrasions, ulcers, boils, warts and cancerous growth. Annona squamoza Common name: Seetha, Anonang-baker, Anono, Guma. The bark contains a large amount of tannic acid. Fruit pulp contains sugar, gum, extractive matters and ash.[5]

Khaya grandifoliola (Meliaceae) commonly called African Mahogany is a medicinal plant endemic in Nigeria.[6] It is a tall, woody tree widely distributed across West Africa from the Guinea coast to Cameron and extending eastward through Congo Basin to Uganda and some parts of Sudan. It grows up to 40 m high and 5 m girth. The bark is grey in colour and yield a bitter gum when wounded. Khaya species are valuable indigenous traditional medicine in West Africa. Its bitter bark is mostly the part that is used to make concoctions to treat some illness like fever, lumbago, cough, rheumatism, stomach ache, gastric pains and as remedy against worm infestation. The anti malarial activity of the stem bark was also reported.[7] The stem bark was also found to possess antianaemic[8], hypoglycaemic, hypoproteinaemic and hypocholesterolaemic.[9] It contains some chemical constituents such as limonoids.[10] In many malaria endemic countries, like the tropics, the extract of *Khaya grandifoliola* is used as an antimalarial herbal remedy. The bark and seeds of *Khaya grandifoliola* are the most common parts used for treatment and are extracted by infusion or decoction. The extracts have proven to fight against the *P. falciparum* parasite, one of the vectors of malaria in humans. The aqueous extract of *khaya grandifoliola* is used in traditional settings in Nigeria as remedy in cough, mycobacterium tuberculosis and bacterial infections. *Khaya grandifoliola* is used in Cameroonian folk medicine for the treatment of pneumonia, intestinal helminthiasis hepatitis and other liver related-diseases.

MATERIALS AND METHODS

Plant Collection, Identification and Authentication

Khaya grandifolia stem bark was collected in Akure, Ondo state, Nigeria. It was identified and authenticated by Bernard Omomoh at Obafemi Awolowo University (OAU) on 16th April, 2015 with herbarium number 17459 after collection by Osemenam Henry on 15th April, 2015. A Voucher specimen was deposited at both Departmemt of Pharmacognosy OAU and Madonna University, Nigeria. The bark of the plant sample was chopped and dried. The bark was now grinded to a coarse powder and later stored in an airtight container prior to extraction.

Chemicals and Drugs

The following chemicals used were of analytical grade: Omeprazole , Tween 80, chloroform, Fehling solution 1 and 11, Dragendorff's reagent, Mayer's reagent, sulphuric acid, Ammonium hydroxide, sodium hydroxide, Ferric chloride, 50 and 96 % Ethanol, potassium hydroxide, Ethyl acetate and Millon reagent.

Experimental animals

Adult albino mice (20-27 g), adult swiss albino mice(120-200 g) of both sexes, obtained from the animal house of Faculty of Veterinary Medicine, University of Nigeria, Nsukka.The animals was acclimatized for 5 days and housed under standard conditions of temperature (25+-2) and 12-h light/dark cycle. All animals will be allowed unrestricted access to drinking water.

Preparation of plant Crude Extract

A 500 g of the stem bark coarse powder was soaked in 95% of ethylacetate 2.5 litres. This was distributed into 2 stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation. The process is intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration.[11] The residue was subjected to several parts of rinsing and filtration with fresh solvents to attain some level of exhaustive maceration (extraction) as judged by loss of colour of the filtrate. The collective filtrate was evaporated to dryness using a rotary vaccum evaporator at a controlled temperature of 40-45°C. The extracts were transferred into sterile sample containers and preserved in a refrigerator at 4c until required for use.

Preliminary phytochemical screening of *Khaya grandifolia* crude extract

Preliminary phytochemical qualitative analysis of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as Reducing sugars, Alkaloids, Steroidal compounds, phenolic compounds, cardiac glycosides, flavonoids, saponins, and tanninins using standard procedures.[12]

Acute Toxicity Studies (LD_{50})

The Lorke method[13] of LD_{50} determination was used, this method employs 13 albino rats with the following assumption made. The first phase is determination of the toxic range. The mice will be placed in three groups (n = 3) and will be given EE (10, 100 and1000 mg/kg; p.o.) solubilized in a solution of 3 %, v/v Tween 85. The treated mice will be observed for 24 h for number of deaths. The death pattern in the first phase determines the doses that will be used for the second phase. If there is no death recorded in the first phase, a fresh batch of four mice would receive1000, 1600, 2900, and 5000 mg/kg of the extract. The treated animals will be observed for lethality or signs of acute intoxication for 24 h. The LD_{50} is the geometric mean of the highest non-lethal dose and the least toxic dose.

The LD_{50} was calculated by the formula

$LD_{50} = \sqrt{(Do \times D100)}$

Do = Highest dose that gave no mortality

D100 = Lowest dose that produced mortality

Evaluation of ulcer activity using Indomethacin Model in rats

The albino rats weighing 120-200 g were fasted for 18hours but allowed to take water. Groups A and B received (100 and 200 mg/kg) of plant extract respectively, Group C (400 mg/kg) group D received Omeprazole (20 mg/kg) and Group E received Tween 80 then indomethacin was administered to each of the rat to induce ulcer after 30 min. The animals were sacrificed with Chloroform after 8 h. The stomachs were dissected out and opened along the greater curvature, carefully rinsed with distilled water, fixed in 10 % formalin and pinned on a cork board to examine the lesion with the help of hand lens. The number and severity of the lesion were observed and scored.[14]

Evaluation of ulcer activity using Ethanol Induced Model

The albino rats weighing 120-200 g were fasted for 18hours but allowed to take water. Groups A and B received (100 and 200 mg/kg) of the plant extract respectively, Group C

(400 mg/kg) group D received Omeprazole (20 mg/kg) and Group E received Tween 80 (5 ml/kg).The above doses were administered with ethanol (1 ml of 96 %, v/v) 1 h later. The animals were sacrificed 1 h after the administration of the ethanol and the stomach was incised along the greater curvature and ulceration was scored as for the pyloric ligation-induced ulcer model.[14]

Statistical Analysis

All values are expressed as Mean ± SEM. Data obtained was analysed using one-way analysis (ANOVA) in SPSS values of test group were compared with those of control using LSD post hoc test. The differences between means were regarded significant at $p < 0.05$.

RESULTS

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done for the ethyl acetate extract of *Khaya grandifolia* resulted in the presence of Alkaloids, glycosides, terpenoids, flavonoids, steroids, tannins, saponins, resins and reducing sugars (Table 1). The extract was safe as no death was recorded at the dose above 5000mg/kg which is regarded as safe dose for crude extract. The result of the antiulcer activity using the indomethacin-induced model showed a dependent significant anti-ulcer effect better than that of the standard, omeprazole. The percentage ulcer inhibition presents 72.37, 86.84, and 92.11% for 100, 200, and 400 mg/kg respectively with omeprazole 65.85 % at 20mg/kg (Table 2). For the ethanol-induced model, the result showed a dependent significant anti-ulcer effect better than that of the standard, omeprazole. The percentage ulcer inhibition presents 56.10, 87.80 and 97.56 % for 100, 200, and 400 mg/kg respectively with omeprazole 88.16 % (20 mg/kg) (Table 3).

Table 1: Results of Phytochemical analysis of *Khaya grandifolia* ethylacetate extract

Constituents	**Relative abundance**
Glycosides	+++
Alkaloids	+
Steroids	+++
Terpenoids	+++
Flavonoids	++
Proteins	+++
Carbohydrates	+++
Reducing Sugars	+++
Tannins	++
Saponins	+
Resisns	+++

Keys: = - Absence, +Slightly Present, ++ Present, +++ Abundantly present

Table 2: Result of peptic ulcer inhibition of ethylacetate extract of the stem bark of *Khaya grandifoliola* on albino rats induced with Indomethacin

Extract	Doses (mg/kg)	Ulcer count	UI (%)
Extract	100	5.2500±1.43614*	72.37
Extract	200	2.5000±.64550*	86.84
Extract	400	1.5000±.95743*	92.11
Omeprazole	5	2.2500±1.43614*	65.85
Neg. Control (5 ml/kg)	-	19.0000±2.04124	0.00

Values are mean ± SEM, * significant at p< 0.05, n=5.

Table 3: Result of peptic ulcer inhibition of ethylacetate extract of the stem bark of *Khaya grandifoliola* on albino rats induced with Ethanol.

Extract	Doses (mg/kg)	Ulcer count	UI (%)
Extract	100	4.5000±1.50000*	56.10
Extract	200	1.2500±.62915*	87.80
Extract	400	0.2500±.25000*	97.56
Omeprazole	10	3.5000±1.84842*	88.16
Neg. Control (5 ml/kg)	-	10.2500±1.65202	0.00

Values are mean ± SEM, * significant at p< 0.05, n=5.

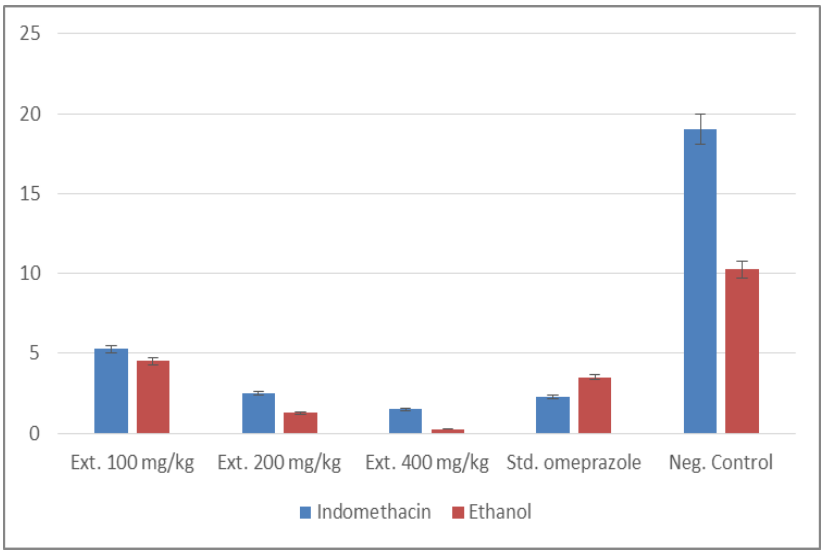

Figure 1: The Anti-ulcer effect of ethylacetate extact of *Khaya grandifoliola* on albino rats induced with Indomethacin and Ethanol.

DISCUSSION

In the present study ethyl acetate extract of the stem bark of *khaya grandifolia* were evaluated in vivo to determine the anti ulcer effect of the extract on the adult rats as compared to the standard Omeprazole in order to validate their effect. Gastric Ulcer bears a lot of

complications if it is not properly managed. Unavailability of suitable anti- ulcer agents and side effects of associated with synthetic drugs prompt to search out newer anti-ulcer agents from natural resources. This study sketches out the anti-ulcer potential of medicinal plants from Akure, ondo State Nigeria. Preliminary phytochemical analysis showed the presence of secondary metabolites as flavonoids, saponins, terpenoids, alkaloids, glycosides, steroids, resins and tannins in the bark of the plant.

These secondary metabolites are effective as antioxidant, antineoplastic, anti-ulcer, anti-inflammatory, and immune stimulating agents.[15-20] Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit *Helicobacter pylori* growth, act as free radical scavengers, and inhibit H+/K+-ATPase.[17,19,21] Saponins may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation.[19] In addition, terpenoids and alkaloids compounds are also reported to have potent activity against gastric ulcers.[22,23]

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis.[15,21,24–26]

The antiulcer effect of the ethyl acetate extract of the stem bark of *Khaya grandifolia* showed that the ulcer inhibition of the crude extract was highest (92.11%) at 400 mg/kg for indomethacin- induced and highest (97.56 %) at 400 mg/kg for ethanol-induced. These results are statistically comparable with the standard Omeprazole (88.16 %) and (65.85 %) at 20 mg/kg for indomenthacin-induced and ethanol-induced respectively. The antiulcer effect of the ethyl acetate extract of the stem bark of *Khaya grandifoliola* may be due to the flavonoids, saponins, alkaloids and terpenoids which have been shown to produce some antiulcer and anti-gastric activity. The series of experiment were designed to determine the effects of *Khaya grandifolia* stem bark extract on ulcer formation and its degree of toxicity. Indomethacin is a selective inhibitor of cyclo-oxygenase and it strongly inhibits the cyclo-oxygenase pathway causing increased level of leukotriene in the gastric mucosa which has been found to cause inflammation and pain. It may also potentiate the secretory response

elicited by Histamine due to the inhibition of prostaglandin E2. However, an anti-secretory effect might be indicated as the extract protected the stomach from NSAID (Indomethacin) induced damage. This damage is elicited by the inhibition of prostaglandin synthesis, which is essential for mucosa integrity. This results to a sustained reduction mucosal blood flow and subsequent generation of ulcer. Among the systems of the body that are affected by prolonged ethanol exposure, the gastrointestinal tract deserves special attention because gastric lesions are a frequent problem in ethanol abusers. Direct contact between ethanol and mucosa induces many functional and metabolic modifications. Damage to the stomach in alcoholics differs from damage to other organs, such as the liver, because ethanol consumption affects the upper gastrointestinal tract through multiple and complex mechanisms. These mechanisms depend on contact with ethanol that can cause direct mucosal damage or nonalcoholic components (e.g., fermentation products). Thus, alcoholism is considered an independent risk factor for the initiation and complications associated with ulcerative disease, similar to smoking, stress. Ethanol digests the mucosal layer and exposes the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin. Omeprazole is an effective agent in the treatment of peptic ulcer disease. Omeprazole is a proton pump inhibitor. Proton pump inhibitors are the most effective anti-secretory agents available for the treatment of gastric acid-related disorders. These drugs dose-dependently inhibit basal and stimulated gastric acid secretion by inhibiting the H+/K+-adenosine triphosphatase (ATPase), also known as the proton pump, that is located in the highly acidic luminal domain of the parietal cell. This study has provided data which suggest that the ethyl acetate of *Khaya grandifoliola* contains biologically active components that produce anti-ulcerogenic activities.

CONCLUSION

The present study confirm the absence of oral acute toxicity at the doses employed, and presence of anti-ulcer pharmacologic activity of the bark of of *Khaya grandifoliola* which could be as a results of the presence of antiulcer compounds.. Its efficacy is comparable to the standard drug Omeprazole. *Khaya grandifoliola* is therefore a good safe option for health care needs of the growing population of especially developing countries like Nigeria. These findings also validate the claims on its use in traditional medicine for gastric ulcer. Further studies shall focus on isolation and characterization of the active constituent(s) as well as elucidating mechanisms of action.to foster specification and specificity of action.

Conflict of interest

The authors report no conflicts of interest in this work.

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