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ANTIULCEROGENIC ACTIVITY OF STEM EXTRACT AND FRACTIONS OF *HOMALIUM LETESTUI*

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ABSTRACT: *Homalium letestui* Pellegr (Flacourtiaceae) used traditionally by the Ibibios of Southern Nigeria to treat stomach ulcer, malaria and other inflammatory diseases were evaluated for antiulcer activities of the stem extract and its fractions. The effects of ethanol stem extract of *H. letestui* (200-600 mg/kg) and fractions (Aqueous and dichloromethane, 400 mg/kg) on experimentally induced ulcer were studied in rats using ethanol, indomethacin, reserpine, and histamine-induced ulcer models. The extract (200-600 mg/kg) inhibited ethanol, indomethacin, reserpine, and histamine-induced ulcer models in a dose-dependent fashion. The various degrees of inhibitions were statistically significant ($P<0.05$, 0.01, 0.001). The effects of the extract/fraction were comparable to that of the standard drugs used in indomethacin and ethanol-induced ulcer models with the dichloromethane fraction having the highest activity. Thus, *H. letestui* extract demonstrated a good antiulcer activity which supports the use of this plant in traditional medicine.

INTRODUCTION: *Homalium letestui* Pellegr (Flacourtiaceae) is a forest tree growing up to 80-100 ft and found in the rainforest of West Africa [1, 2]. The plant parts; particularly stem bark and root are used in various decoctions traditionally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, malaria, and other inflammatory diseases as well as an aphrodisiac [3].

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Reports of antiplasmodial [3], antidiabetic [4], anti-inflammatory and analgesic [5], cellular antioxidant, anticancer, and antileishmanial [6], depressant and anticonvulsant [7] activities of the plant have been published. We report in this study, the antiulcerogenic activity of this plant in order to provide a scientific basis for its use in traditional medicine in the treatment of stomach ulcer.

MATERIALS AND METHODS:

Plants Collection: The plant material *Homalium letestui* (stem) was collected in a forest in Uruan area, Akwa Ibom State, Nigeria in July 2014. The plant was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo,

Nigeria. Herbarium specimen (FPUU 382) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

Extraction: The stem was washed and shade-dried for two weeks. The dried plant material was further chopped into small pieces and reduced to powder. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness *in vacuo* 40 °C using rotary evaporator. The crude ethanol extract (10 g) was partitioned with a 50:50 mixture of distilled water and dichloromethane. The aqueous fraction was evaporated to dryness in a water bath at 60 °C and the dichloromethane fraction air-dried. The ethanol extract, the aqueous and dichloromethane fractions were stored at -4 °C until used.

Animals: Albino wistar rats (168-175 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Indomethacin-Induced Ulcer: Male adult Albino rats were used for the experiment. They were randomized into seven groups of six rats each. Food was withdrawn 24 h and water 2h before the commencement of experiment [8, 9, 10, 11]. Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2CO_3); Groups 2-4 were pretreated with *H. letestui* stem extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg); Group 6 received dichloromethane fraction (400 mg/kg), and Group 7, cimetidine (100 mg/kg p.o. dissolved in 5% Tween 80).

One hour later, groups 2 - 7 were administered with indomethacin. Four hour after indomethacin administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens, and the presence of ulcer lesion was scored [12]. Ulcer index (UI), the preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract were calculated using standard methods [9, 10].

Ethanol - Induced Gastric Ulceration: The procedure was similar to that used in indomethacin-induced ulceration. The rats randomly assigned to were randomized into eight groups of six rats each. Food was withdrawn 24 h and water 2 h before the commencement of experiment [8]. Group 1 (Control) received only ethanol (2.5 ml/kg p.o), Groups 2- 4 were pre-treated with *H. letestui* stem extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg); Group 6 received dichloromethane fraction (400 mg/kg) and Group 7, received propranolol (40 mg/kg p.o. dissolved in distilled water). One hour later, groups 2-6 were administered with ethanol. Four hours after ethanol administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens, and the presence of ulcer lesion was scored [10].

Histamine-Induced Gastric Ulceration in Rats: The procedures were similar to that used in indomethacin-induced ulceration except that the negative control group (Group 1) received only histamine acid phosphate (Sigma, 100 mg/kg i.p. dissolved in distilled water) [11], Groups 2-4 were pretreated with *H. letestui* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg), Group 6 received dichloromethane fraction (400 mg/kg) and Group 7 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), One hour later, groups 2-7 were administered with histamine acid phosphate,100 mg/kg i.p). Eighteen (18) hours after histamine administration, animals were killed by cervical dislocation.

The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens, and the presence of ulcer lesion was scored [12], stomach processing and examination, as well as ulcer scoring, were similar to that used in indomethacin-induced ulceration.

Reserpine-Induced Gastric Ulceration in Rats: Male adult Albino rats weighing 120-170 g were used for the experiment. They were randomized

into six groups of six rats each. Food was withdrawn 24 h and water 2h before the commencement of experiment [8]. Group 1(control) received only reserpine (Sigma, 8 mg/kg p.o. dissolved in Tween 80); Groups 2 - 4 were pretreated with *H. letestui* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg), Group 6 received dichloromethane fraction (400 mg/kg) and Group 7 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), One hour later, groups 2 - 5 were administered with reserpine, 8 mg/kg i.p dissolved in 10% Tween 80 [11]. Eighteen hours (18 h) after reserpine administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens, and the presence of ulcer lesion was scored [12]. Ulcer index (UI), the preventive ratio (PR) and the degree of ulceration (DU) of each of the groups pretreated with extract were calculated using standard methods [9, 10].

Statistical Analysis: Data are reported as the mean ± standard error of the mean (SEM) and were analyzed statistically using One way ANOVA followed by Tukey-kramer multiple comparison test and values of P<0.01 were considered significant.

RESULTS:

Indomethacin-Induced Gastric Ulceration: The extract and fractions (p.o.) pretreatment on indomethacin-induced gastric ulceration showed a dose-dependent reduction in ulcer indices in pretreated groups relative to control. The reductions were statistically significant (P<0.05, 0.001) compared to control. The dichloromethane fraction exerted the highest antiulcerogenic effects which were comparable to that of the standard drug used, cimetidine **Table 1**.

TABLE 1: EFFECT OF *HOMALIUM LETESTUI* EXTRACT ON INDOMETHACIN - INDUCED ULCERATION IN RATS

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control (Indomethacin)	60	18.34 ± 2.72	-
Homalium letestui	200	10.05 ± 1.22[a]	45.20
extract p.o.	400	2.10 ± 0.60[c]	88.54
	600	2.00 ± 0.76[c]	89.09
Dichloromethane fraction	400	1.60 ± 0.22[c]	91.27
Aqueous fraction	400	3.83 ± 0.25[c]	79.11
Cimetidine	100	1.52 ± 0.88[c]	91.71

Data were expressed as mean ± SEM. significant at a P<0.05, b P<0.01,c P<0.001 when compared to control n = 6.

Ethanol-Induced Gastric Ulceration: The extract and fractions significantly protected rats from ethanol-induced ulcer **Table 2**. There was a significant (p<0.001) dose-dependent reduction in the ulcer indices relative to control. The effect of the extract and fractions were less than that of the standard drug, propranolol.

Histamine-Induced Ulceration: Administration of the extract and fractions significantly (p< 0.001) reduced histamine-induced gastric ulceration in a dose-dependent fashion compared to control **Table 3**. The dichloromethane exhibited a higher antiulcer potential than the aqueous fraction but less than that of the standard drug, cimetidine.

TABLE 2: EFFECT OF *HOMALIUM LETESTUI* EXTRACT ON ETHANOL - INDUCED ULCERATION IN RATS

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio (%)
Control (Ethanol)	-	4.63 ± 0.33	-
Homalium letestui	200	2.66 ± 0.33[a]	42.54
extract p.o.	400	2.00 ± 0.61[b]	56.80
	600	1.34 ± 0.57[c]	71.05
Dichloromethane fraction	400	1.20 ± 0.25[c]	74.08
Aqueous fraction	400	2.66 ± 0.22[a]	42.54
Propranolol	40	0.82 ± 0.15[c]	88.76

Data were expressed as mean ± SEM. significant at a P<0.05, b P<0.01, c P<0.001 when compared to control n = 6.

TABLE 3: EFFECT OF *HOMALIUM LETESTUI* EXTRACT ON HISTAMINE - INDUCED ULCERATION IN RATS

Treatment	Dose(mg/kg)	Ulcer Indices	Preventive Ratio
Control (Histamine)	100	16.01 ± 0.81	-
Homalium letestui	200	12.62 ± 1.40[a]	21.17
extract p.o.	400	9.25 ±0.82b	42.22
	600	3.12 ± 0.12c	80.51
Dichloromethane fraction	400	2.25 ± 0.14c	85.94
Aqueous fraction	400	10.25 ± 0.66	35.97
Cimetidine	100	1.00 ± 0.11*	94.12

Data were expressed as mean ± SEM. significant at *P <0.001 when compared to control n = 6.

Reserpine-Induced Ulceration: Administration of the extract and fractions significantly (P<0.001) reduced reserpine-induced gastric ulceration in a dose-dependent fashion compared to control **Table 4**. These effects were incomparable to that of the standard drug, cimetidine.

TABLE 4: EFFECT OF *HOMALIUM LETESTUI* EXTRACT ON RESERPINE - INDUCED ULCERATION IN RATS

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control (Reserpine)	8	16.22 ± 0.65	-
H. letestui extract p.o.	200	12.56 ± 0.94[a]	22.56
	400	8.21 ± 0. 53[b]	49.38
	600	3.44 ± 0.67[b]	78.79
Dichloromethane fraction	400	2.75 ± 0.34[b]	83.04
Aqueous fraction	400	7.56 ± 0.52[b]	65.72
Cimetidine	100	1.10 ± 0.84[b]	93.21

Data were expressed as mean ± SEM. significant at [a] P<0.01; [b] P <0.001 when compared to control n = 6.

DISCUSSION: *Homalium letestui* stem has been reported to be used traditionally in the treatment of ulcer [3]. For this reason, the antiulcer activity of the stembark extract and fractions were evaluated using indomethacin, ethanol, reserpine, and histamine-induced ulcer models. Indomethacin, a known ulcerogenic especially in an empty stomach [13] causes ulcer mostly on the glandular (mucosal) part of the stomach [12, 13] by inhibiting prostaglandin synthetase through the cycloxygenase pathway[14]. Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turnover and repair [15, 16].

Suppression of prostaglandin synthesis by indomethacin results in increased susceptibility of the stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to significantly reduce mucosal damage in the indomethacin-induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti-ulcer effect of the extract. Administration of ethanol has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production [17]. This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen-derived free radicals have been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa [18]. It was observed in this study that the extract reduced significantly ethanol-induced ulcer. This may be due to the cytoprotective effect of the extract via antioxidant effects which the stem extract has been reported to exhibit [6].

Okokon *et al.,* [5] reported the presence of α-terpineol, Vanillin, 4-phenyl isocoumarin, 3, 4, 5-trimethoxy phenol, 2-Coumaranone, and xanthones in the stembark extract of *H. letestui*. Vanillin, a phenolic aldehyde has been reported to possess antioxidant and free radical scavenging ability [19, 20, 21] which could account for the anti-ulcer property of this plant. α-terpineol present in this extract is an isomer of the monoterpene, terpinen-4-ol. α-terpineol and terpinen-4-ol have been reported to possess anti-ulcer activity [22, 23]. The antiulcer activity of this extract could also be due to the presence of α-terpineol. Similarly, xanthones have been reported to demonstrate antiulcer activity [24]. These compounds present in the stem extract may be responsible for the observed antiulcer activity. Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene

C4 (LTC4) [25]. The gastroprotective effect of the extract may in part be due to the suppression, by the extract of lipoxygenase activity [12]. Histamine-induced ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine [26]. The inhibition of ulcer due to histamine by the extract may be due to its suppression of histamine-induced vasospastic effect and gastric secretion.

Okokon *et al*., [4] had reported the presence of flavonoids, saponins, terpenes, and tannins in the stem extract of *H. letestui*. Flavonoids such as quercetin have been reported to prevent gastric mucosal lesions in various experimental models [27, 28] by increasing the amount of neutral glycol-proteins [27]. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion [29]. Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by the formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting PGF2α [30, 31].

In conclusion, the results of the present study show that stem extract and fractions of *H. letestui* displays gastroprotective activity as demonstrated by significant inhibition of the formation of ulcers induced through four different ulcer models studied. The antiulcer activity of the extract may be due to the action of its phytochemical compounds present in the extract. The observation justifies the ethnomedical uses of the plants as antiulcer and antacid in addition to its nutritional values.

CONCLUSION: The present study demonstrates that stem extract of *Homalium letestui* might to be useful for the treatment of ulcer.

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