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ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF LEAF EXTRACT OF *HISBISCUS ASPER* HOOK.F (MALVACEAE)

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Abstract
The antibacterial and anti-inflammatory activities of crude methanolic leaf extract of *Hibiscus asper*, Hook .F. (Malvaceae) were investigated. Egg-albumen-induced rat hind paw oedema model was used to evaluate the anti-inflammatory activity of the extract. The in- vitro antibacterial activity was assessed by agar well diffusion method against clinical bacterial strains, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Preliminary phytochemical analysis revealed the presence of glycosides, carotenoids, alkaloids, tannins, saponins, flavonoids, terpenoids, carbohydrates, resins and reducing sugars. The extract was effective against *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* while the values for their minimum inhibitory concentration (MIC) were 60.07, 34.67 and 52.48 mg/ml respectively. *Escherichia coli* and *Salmonella typhi* were not susceptible to the extract. The extract at doses 100 and 400mg/kg significantly ($p< 0.05$) inhibited the development of egg-albumen-induced paw oedema after three hours and at doses 100, 200 and 400 mg/kg also significantly ($P< 0.05$) inhibited paw oedema after four hours. The extract showed a dose- dependent inhibition of oedema with LD_{50} greater than 5000 mg/kg. The present studies indicate that *Hibiscus asper* leaf extract has both antibacterial and anti-inflammatory activities and lend scientific credence to the use of the plant to control veneral diseases, urethritis, painful menstruation and the like in folkloric medicine.

Keywords: *Hibiscus asper*; leaf; phytochemical constituents, antibacterial; anti-inflammatory activity.

INTRODUCTION

Hibiscus asper Hook .F. (Malvaceae) (Burkill, 1985) with vernacular names "Wild sorrel", "False roselle," "Roselle savage" is widely distributed throughout tropical Africa and in Madagascar. It is found in fallow fields, grassland and edges of gallery forest. As a weed it is not considered very harmful in its area of origin. *Hibiscus* comprises 200-300 species and *H. asper* belongs to section Furcaria, a group of about 100 species of which about 30 are in tropical Africa (Grubben and Penton 2004). Some members of the *Furcaria* section include *H. sabdariffa*, *H. cannabinus* and *H. surattensis*.

H. asper is a perennial herb growing up to 2m tall; stem having fine prickles and simple or stellate hairs. The leaves are alternate and simple, and have threadlike stipules up to 6mm long. The leaf petioles are 0.5 – 18cm long, leaf blade is lanceolate to ovate and unlobed or shallowly to deeply palmated 3-5 (-7) – lobed. The flowers are yellow with purple centre (Saunders, 1966). The fruits are ovoid or globose capsules about 3cm in diameter, hairy often speckled red and covered by calyx with bristles. The seeds are brown and about 3mm long (Akobundu and Agyakwa, 1987).

The leaves of *H. asper* dried over a fire are applied to eczematous sores in Senegal. Various other skin problems of humans and domestic animals are treated with the leaves in Senegal, Guinea and Mali. The plant is used to treat urethritis in Northern Nigeria, anaemia and jaundice in Benin and leucorrhoea in Cameroon. In addition, it is used as a poison antidote (venomous stings, bites, etc) in Nigeria. In Mali and Benin it is used to treat malaria, angina in the Central African Republic, and painful and irregular menstruation in Benin. It is used as a depurative and diuretic in Mali, for veneral diseases, subcutaneous parasitic infections, and to control internal parasites in veterinary medicine in Guinea (Grubben and Penton 2004; Burkill, 1985).In some tropical regions of Africa, it is also used as vegetable, potent sedative, tonic and restorative, and antidepressive drug (Foyet *et al*, 2011)

Most human diseases result from bacterial infection, which sometimes are the underlying causes of inflammation and pain (Otimenyin *et al*, 2006). Fresh leaves of *H. asper*, after mashing are placed on boils to reduce swelling pain and hasten pus formation.Although, Foyet *et al* (2011) recently documented the antioxidant activity and the neuroprotective effect of *H. asper* leaves, there is still little scientific data on the plant. Thus, this work reports the phytochemical, antibacterial and anti-inflammatory properties of the leaves of *H. asper*.

MATERIALS AND METHODS

Collection and authentication of the plant material

Matured leaves of *Hibiscus asper* were harvested from plants growing in wastelands in Awka, Ukwulu and various parts of Anambra State between June and September. The plant was authenticated by Mr. P.O. Ugwuozor, Department of Botany, University of Nigeria, Nsukka. A voucher specimen (UNH no 73F) was deposited at the Herbarium. The fresh leaves were dried at room temperature for about 7 days and pulverized using mechanical means.

Extract preparation

About 300 g of the pulverized leaves were macerated in 1.7 litres of methanol for 48h and filtered using Whatman no.1 filter paper. The marc was further washed with about 400 ml of fresh methanol and the filtrate concentrated *in vacuo* at 40^{O}C in a rotary evaporator to obtain 39.4 g (13.13% w/w) of the crude methanolic extract.

Phytochemical analysis of crude extract

The extract was subjected to phytochemical analysis using standard methods for alkaloids, saponins, tannnis flavonoids, sugars, carbohydrates, oils, resins. (Harborne, 1998; Evans, 2002; Agrawal and Paridhavi, 2007).

Test organisms

The organisms used were clinical isolates obtained from the Department of Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka. They include the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, and the gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. Stock cultures were subcultured on solid agar medium at 37^{O}C for 24 hours and a subculture from each growth reincubated to obtain the pure colonies used.

Antibacterial screening

The sensitivity of the crude extract and standard drug against the listed microorganism were screened using the cup plate agar diffusion method. Culture of the test organism was

evenly streaked on the surface of nutrient agar plate and allowed to equilibrate. Two-fold serial dilution of the crude extract and the positive control (Amoxicillin) were obtained using dimethyl sulfoxide (DMSO) and water respectively.Volumes of 0.2ml of these test solutions were introduced into wells bored (8mm) in the nutrient agar plates already streaked with the test microorganisms. After incubation at 37^{O}C for 24 hours, the inhibition zone diameters (1ZD) were measured and the MIC values obtained from the intercept on the natural-logarithm of drug concentration axis of a graph of natural-logarithm of drug concentration against squared 1ZD (mm^2) (Okore, 2005).

Laboratory animals

Adult Wister albino rats (110-190 g) and albino mice (24-35 g) of both sexes were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in standard Laboratory conditions and fed with rodent commercial diet (Guinea Feed Nigeria, Ltd) and water *ad libitum*. Food was withdrawn 5h before the experiment.

Acute toxicity test

The acute toxicity test was based on the method described by Lorke (1983). Adult mice (24-35 g body weight) of either sex were used for the test. The test was carried out in two stages. The crude methanolic extract was administered to three groups of three mice each. Each group received one of the three different doses-10,100 and 1000 mg/kg of extract and monitored for 24 h for deaths. In the second stage 1600, 2900 and 5000 mg/kg of the extract were administered to three fresh batches of three animals. The LD_{50} of the extract was calculated as the geometric mean of the maximum dose that caused 0% death and the minimum dose that caused 100% death.

Anti-inflammatory tests

Egg-albumen-induced rat paw oedema model was used in the study (Williamson *et al*, 1996; Ekpendu *et al*, 1994). The paw oedema was induced by injecting the phlogistic agent (egg albumen) into the plantar surface of the right hind paw of the animals. The animals were fasted for 5 h and deprived of water only during the experiment in order to minimize the variation in oedematous response by the animals (Winter *et al.*, 1963). The extract was solubilized in 5% Tween 80 and made up to the required volume with normal saline at doses 100, 200 and 400 mg/kg and administered intraperitoneally (i.p.) to three groups of rats. The control groups were given 0.2ml normal saline (negative control) and 100mg/kg aspirin (positive control). All the substances (candidate and controls) were administered intraperitoneally 30 min before the injection of the phlogistic agent (0.l ml of fresh undiluted egg albumen) in the plantar region of the rat right hind paw. Paw volumes were measured by water displacement of 0,1,2,3 and 4 h after egg-albumen injection.

Oedema is a measure of mean increase in paw volume at time t, with respect to volume at time=0, before injection of the phlogistic agent, and inhibition as a percentage decrease in oedema volume. The anti-inflammatory activity was calculated at each time of observation as percentage inhibition using the formula:

$$\% \ Inhibition = \frac{(Vo - V_t)}{Vo} \times 100$$

where V_t is the volume of oedema at corresponding time and V_o the volume of oedema of control rats at the same time.

Statistical analysis

Data were analyzed using Student *t-test* (Woodson, 1987).

RESULTS AND DISCUSSION

The crude extract of *H. asper* leaf appeared as a dark paste. The result of the acute toxicity test showed that the plant has a wide margin of safety, with acute toxicity value above 5000 mg/kg. The plant can therefore be considered safe (Lorke, 1983) and evidenced by its use as a cultivated vegetable in Senegal and Democratic Republic of Congo, and an important fodder plant eaten by all livestock in the Sahel (Burkhill, 1985).

The result of phytochemical screening of the crude extract is shown in Table 1, while Table 2 shows the antibacterial activities and the resultant minimum inhibitory concentrations (MIC) of the plant extract. Table 3 gives the anti-inflammatory activities of the plant extract on egg-albumen-induced inflammation in rats and Table 4 shows the percentage inhibition of oedema by the crude plant extract.

Phytochemical screening of the crude extract of *H. asper* leaves indicated that it contains alkaloids, flavonoids, tannins, saponins, glycosides, steroids, terpenoids, carotenoids. However, glycosides and flavonoids are of higher preponderance relative to the other phytochemicals. Flavonoids have been reported to possess antibacterial activity against gram-positive and gram- negative bacteria (Liu *et al.*, 1999) since they have the ability to complex with nucleophilic amino acids in proteins and the bacterial cell walls leading to enzyme inactivation and loss of function (Mason and Wasserman, 1987). Tannins are toxic to filamentatous fungi, yeasts and bacteria (Scalbert, 1991) and inhibitory to viral reverse transcriptase. Their antimicrobial action is made possible by their capacity for protein complexation through hydrogen and covalent bonding and inactivation of microbial adhesions enzyme and cell envelope transport proteins (Stern *et al*, 1996).Tannins at low concentrations have been reported to elicit bacteriostatic activity (Oliver, 1959). Alkaloids such as isopteropodine, pteropopine, isomitraphylline assist the white blood cells dispose harmful microorganisms (Ogunwenmo *et al.*, 2007).

The alkaloids, flavonoids, terpenoids, glycosides have been reported to possess anti-inflammatory properties (Shah *et al*, 2006). This anti-inflammatory activity is elicited by inhibiting cyclooxygenase-enzyme involved in prostaglandin synthesis at the site of inflammation (Wannang *et al*, 2006).

The methanolic extract of *H. asper* leaves showed concentration dependent activity against the gram-positive bacteria *Staph. aureus* and *B. subtilis*, and the gram-negative bacterium *P. aeruginosa*, but had no activity against the gram negative bacteria *E. coli* and *S. typhi* (Table 2). The difference observed in susceptibility among the test organisms may be attributed to differences in the nature of their cell wall structures; gram-positive organisms possessing thick cell walls made largely of proteins compared with the cell walls of gram-negative organisms composed of molecules of polysaccharides, lipid and relatively low amount of peptidaglycan strands that confer more resistance to chemical penetration and subsequent cell inactivation (Baird-Parker and Holbrook, 1971). The MIC value was least for *B. subtilis* (34.67 mg/ml) but higher for *P. aeruginosa* (52.48 mg/ml) and *Staph aureus* (66.07 mg/ml). On the other hand the MIC value of the positive control, Amoxcillin (Am) was least for *Staph. Aureus* ($1.26x10^{-4}$ mg/ml) followed by

Table 1: Phytochemical component of the leaf of *Hibiscus asper*.

Phytochemical Component	Abundance
Alkaloids	+
Tannins	+
Glycosides	+++
Saponins	+
Flavonoids	+++
Steroids	+
Terpenoids	+
Carotenoids	+
Reducing Sugars	+
Carbohydrates	++
Oil	+
Resins	++

\+ = Low concentration
++ = Moderate concentration
+++ = High concentration

Table 2: Antibacterial activities and the minimum inhibition concentration (MIC) of the crude extract of *Hibiscus asper*.

Microorganism	**Inhibition Zone diameter (mm)**				**MIC (mg/ml)**	
	200mg/ml	**100mg/ml**	**50mg/ml**	**Am 4mg/ml**	***H. asper***	**Am**
Staphylococcus aureus	7.0 ± 0.04.	33 ± 0.6	-	30	66.07	$1.26x10^{-4}$
Escherichia coli	-	-	-	23	-	-
Bacillus sitbtills	7.7 ± 0.6	6.0 ± 0.0	4.0 ± 0.00	19	34.67	$1.0x10^{-2}$
Pseudomonas aeruginosa	6.7 ± 0.06	4.7 ± 0.6	-	26	52.48	$1.32x10^{-3}$
Salmonella typhi	-	-	-	NT	-	-

\- no activity; NT = not tested; Am = Amoxicillin (positive control)

Table 3: Anti-inflammatory activities of the crude extract of *Hibiscus asper* on egg-albumen induced rat paw inflammation in rats.

Treatment	Dose (mg/kg)	Paw oedema volume (ml) (Hours after egg-albumen injection) 1 h	2 h	3 h	4 h
Crude extract	100	0.73 ± 0.20	0.65 ± 0.15	0.42 ± 0.10*	0.42 ± 0.10*
	200	0.82 ± 0.12	0.65 ± 0.08	0.48 ± 0.12*	0.33 ± 0.05*
	400	0.68 ± 0.06	0.56 ± 0.06	0.43 ± 0.08*	0.39 ± 0.08*
Aspirin	100	0.69 ± 0.07	0.53 ± 0.06	0.44 ± 0.04*	0.44 ± 0.04*
Normal saline	0.2ml	0.70 ± 0.09	0.68 ± 0.09	0.64 ± 0.10	0.61 ± 0.10

Each value represents the mean ± SEM; *significant at P< 0.05 compared to control.

Table 4: Percentage inhibition of oedema by the crude extract of *Hibiscus asper*

Treatment	**Dose (mg/kg)**	**% oedema inhibition 1h**	**2h**	**3h**	**4h**
Crude extract	100	-	4.41	34.38*	31.95*
	200	-	4.41	25.00*	45.90*
	400	2.86	17.65	32.81*	36.07*
Apirin	100	1.42	22.06	31.25*	27.86*
Normal saline	0.2ml	-	-	-	-

* significant at P.<.0.05

P. aeruginosa ($1.32x10^{-3}$ mg/ml) and then *B. subtilis* ($1.0x10^{-2}$mg/ml). Amoxicillin obviously appeared to be relatively more effective than the crude extract at this preliminary stage. This can be explained by the fact that the concentration of the active principles in the crude extract may be small compared to that of Amoxicillin, however, a more realistic comparison of the potencies demands that the active principle of the plant extract be isolated and evaluated.

Results of the acute anti-inflammatory effect of the crude extract of *H. asper* at the doses 100 and 400 mg/kg showed significant (P< 0.05) inhibition of the development of paw oedema induced by egg albumen three hours after treatment (Tables 3 and 4) and with activity higher than that of the positive

control, aspirin (acetylsalicylic acid) (100mg/kg). The extract also showed significant ($P<0.05$) acute anti-inflammatory activity in the rats four hours after administration of extract at doses of 100, 200 and 400 mg/kg, with the dose of 200 mg/kg showing the highest activity. These findings may not be unrelated to the presence of flavonoids, alkaloids, steroids and terpenoids which are known to be responsible for the anti-inflammatory properties of most medicinal plants (Shah *et al.*, 2006: Manthey, 2000).

CONCLUSION

These results support the use of the plant as an antibacterial and anti-inflammatory agent in ethnomedical practice. Work is underway to isolate and elucidate fully the compounds whose activities were reported in this paper.

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