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ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF *HIPPOCRATEA INDICA* ROOT BARK AND *POGA OLEOSA* FRUITS

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Abstract

The methanolic extracts of *Hippocratea indica* root bark and *Poga oleosa* fruits were investigated for anti-inflammatory and antimicrobial activities. Both extracts inhibited carrageenan-induced paw oedema significantly in rats in a dose-dependent manner in 4 h. *H. indica* inhibited oedema significantly at the minimal dose (125 mg/ml, $p < 0.05$) from 2 h onward, and gave 100% inhibition in 4 h. at 250 mg/kg. It was shown to be a more potent anti-inflammatory agent than *P. oleosa.* Also, *H. indica* extract exhibited greater antimicrobial activity against tested bacteria, with *Staphylococcus aureus* being the most susceptible bacterium (MIC, 16 mg/ml). Both plants were inactive against *Candida albicans.* These results confirm the anti-inflammatory and antibacterial activities of the two plants.

Keywords*: Hippocratea indica*, *Poga oleosa*, anti-inflammatory, antimicrobial

Introduction

The inflammatory process occurs as a natural immune response to infection, mechanical injury and heat. Inflammation is caused by the rapid increase in white blood cells (T-cells, B-cells etc), which are drawn to the injured or infected area in order to destroy the foreign cells. As it were, the primary indication of infection in most cases is inflammation (Hugo and Russell, 1994).

The symptoms of inflammation like warmth, redness, swelling and pain are the direct results of this increased concentration of blood cells. *Hippocratea indica* and *Poga oleosa* were two plants which were indicated along with other plants in the treatment of infectious diseases in an earlier survey (unpublished). The aim of this work is there fore to ascertain if the mode of action of the plants in treatment of infectious diseases is by reversing or inhibiting the inflammatory processes, and also the antimicrobial activities of the two plants were carried out to ascertain their effectiveness on some microorganisms that are usually implicated at the sites of infection.

Hippocratea indica Willd. (Celastraceae) is a shrub or slender climber up to 4 m long occurring from Guinea and Mali to Southern Nigeria. The plant has reputation in ethnomedicine for treating various illnesses, like guinea worm infestation and respiratory troubles (Burkill, 1985). In Liberia, the plant decoction is used in cleaning wounds and sores. The plant is prepared as a pulp for application to guinea-worm sores, and as a draught for respiratory troubles by the Baule of Ivory Coast. The leaves are steeped in water in Casamance (Senegal) for washing – a common practice for women and children, and in decoction along with the roots for washing infants

with various illnesses and fevers, and as a strengthening tonic for adults. The macerated root is applied as a poultice to sores in Liberia. African Brazil nut, *Poga oleosa* Pierre (Rhizophoraceae) is a medium-sized tree up to 30 m high (Burkill, 1997). It is widely distributed from Nigeria to the Congo region in the dense equatorial forests, often along riverbanks and coastland. The tree produces edible nuts used as a condiment and cooking oil (Burkill, 1997). In human medicine, the oil is employed as a laxative in the treatment of gonorrhoea and as a massage oil. An ointment of the bark with palm oil is used in certain cutaneous conditions like inflammation. Literature information on these two plants appears very scanty. In order to validate some of the ethnomedicinal claims on these two plants, they were investigated for anti-inflammatory and antimicrobial activities, and our findings reported in this paper.

Material and methods

Plant materials and Extraction

Root bark of *H. indica* and fruits of *P. oleosa* were harvested from trees growing in Abeokuta, Ogun State in October, 2005, and were authenticated by Mr. T. K. Odewo of Forestry Research Institute of Nigeria (FRIN) Ibadan. Voucher specimens (*H. indica* FHI 107403; *P. oleosa* FHI 107404) were deposited at the herbarium. The plant samples were cut into pieces and air-dried in an air-circulating oven at 40^{o}C. Dried plant samples were reduced to powdery form using a milling machine. 700 g and 900 g of *H. indica* and *P. oleosa* respectively, were exhaustively extracted by maceration using cold methanol. The solvent was removed at 35^{o}C under reduced pressure, to give 11.4% (*H. indica*) and 1.22 % (*P. oleosa*) yields respectively, on dry weight basis. Residual extracts were stored at 4^{o}C prior to experimentation.

Animals

Male and female albino rats (weighing 100-200 g) used for this study were obtained from the breed of the animal house of Department of Veterinary, Physiology and Pharmacology, University of Ibadan, Nigeria. The animals were kept in well ventilated cages under standard conditions throughout the experiment, and were fed with pellets purchased from Livestock Feeds Nigeria Plc., and also with water. The animals were deprived of food, but only provided with tap water 24 h prior to experimentation.

Carrageenan-induced paw oedema in rats

The rats were divided into five groups (5 animals/ group) for each plant under investigation. Anti-inflammatory experiment was conducted using the method of Devi et al (2003). Oedema was induced in the rats by injection of carrageenan (0.1ml, 1 %w/v in distilled water) into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured using the cotton thread method (Bamigbose and Naomesi, 1981) before (at 0 h) the injection of carrageenan, and at hourly intervals for 4 h afterwards. Three test groups of animals received oral administration of three different doses- 125 mg/kg, 250 mg/kg and 500 mg/kg of methanol extract of *H. indica* root bark in 40 % Tween 80, respectively. The control group received 40 % Tween 80 (1 ml/kg) and the last group was administered with the reference drug, indomethacin (MSD Canada, 10 mg/kg) dissolved in distilled water respectively. Drug administration was done 30 min prior to carrageenan injection (Olajide et al., 2000; Devi et al., 2003; Esteves et al., 2005). *P. oleosa* extract was similarly administered to the experimental animals. The inhibitory activity was determined at hourly intervals according to the formula used by Olajide et al (2000).

Antimicrobial assay

The antimicrobial assays were performed following standard procedures as described by Adesina et al (2000), with nutrient agar and Sabouraud dextrose agar as medium for the bacteria and fungus, respectively. Clinical isolates of five test organisms (Gram positive bacteria, *Staphylococcus aureus, Bacillus subtilis,* and the Gram negative bacteria, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*, and the yeast, *Candida albicans*) used in the experiment were obtained from the Department of Microbiology at the teaching hospital of the Olabisi Onabanjo University (OOU). Similar concentrations (31.25 -1000 mg/ml) of each extract dissolved in 50 % MeOH were used for the assay in the well diffusion method. Ciprofloxacin and tioconazole (at 5 mg/ml each) respectively, were the reference antibacterial and antifungal agents, while 50 % Methanol served as negative control. All the antimicrobial determinations were carried out in duplicates. Antimicrobial activity was assessed by measuring the diameter of zones (less well size, 6mm) of inhibition of growth of organism after incubation at 37^{o}C for 24 h for bacteria, and

25°C for 24 h for the fungus. The minimum inhibitory concentration (MIC) for the bacteria was determined according to Adesina et al (2000).

Statistical analysis

Values were expressed as mean ± S. E. M. Statistical significance was determined by the student's *t*-test compared with control. Values with $P<0.05$ were considered significant (Woodson, 1987).

Results and Discussion

Anti-inflammatory activity

Injection of carrageenan in the control group (Tween 80) caused the development of high intensity oedema which lasted throughout the period of study. Maximum peak was observed between 3-4 h after injection of the phlogistic agent (Table 1). Corresponding decreases in % paw size in a dose-dependent manner, with reference to 0 h., were evident for all the tested doses of *H. indica* root bark during the 4 h experimental period. A similar observation was noticed with higher doses of *P. oleosa* fruit (250 and 500 mg/kg). However, only *P. oleosa* extract at the least dose (125 mg/ml) gave an initial rise in oedema formation which peaked in 2h, followed by a gradual fall until the end of the experiment. Similar changes in oedema size over specific experimental periods has been described by Carvalho et al (1999), Olajide et al (2000) and Devi et al (2003) for other anti-inflammatory plants.

Both plants investigated in this study produced significant inhibitions of carrageenan-induced oedema in experimental rats when compared with the control. This inhibition also followed a dose-dependent fashion, and also increased with time after administration of carrageenan. Complete inhibitions were recorded only with higher doses of *H. indica* between 3-4 h of experimentation (Table 2). The reference drug, indomethacin at the tested dose (10 mg/kg), was comparable with 125 mg/kg and 500 mg/kg of *H. indica* and *P. oleosa* extracts respectively, in anti-inflammatory potency. Based on these doses of the two plants, *H. indica* root bark is four times as active as *P. oleosa* fruit as an anti-inflammatory agent. The control group administered with Tween 80 did not exhibit inhibition of oedema.

Mechanisms of oedema formation due to carrageenan and anti-inflammatory activity of potential agents have been reported by earlier workers (Olajide et al., 2000; Devi et al., 2003; Esteves et al., 2005). Furthermore, Choi and Hwang (2001) have linked reduction in serum levels of aspartate amino tranferase and alanine amino tranferase in experimental animals as indicative of anti-inflammatory activity. It is also apparent from this study that combination of both plants in an anti-inflammatory herbal remedy could produce better results. A similar herbal preparation containing *Alstonia boonei* root bark, already reported as a potent anti-inflammatory agent (Olajide et al, 2000), in addition to two other plants has been reported by Kweifo-Okai et al (1995) to be a potent anti-inflammatory remedy. These workers have established a relationship between anti-inflammatory activity, and analgesic and antipyretic activities. This implies that analgesic and antipyretic activities could be predicted for *Hippocratea indica* root bark and *Poga oleosa* fruits, in addition to the anti-inflammatory activity investigated in this paper.

Table 1: Effects of methanolic plant extracts on carrageenan-induced paw oedema in rats

Treatment/ Dose	**Percentage changes in paw size at hourly intervals after phlogistic agent administration**							
	1 h		2 h		3 h		4 h	
	H. indica	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*
125 mg/kg	16.76±1.266	16.57±0.84	10.85±1.34	24.36±1.15	4.80±0.74	22.20±1.29	2.00±0.42	16.69±1.45
250 mg/kg	6.40±0.52	15.59±1.01	4.65±0.54	15.59±1.01	1.83±0.34	13.23±0.50	0.00±0.00	8.38±0.62
500 mg/kg	11.30±1.23	12.06±0.90	4.36±0.76	7.58±0.56	0.00±0.00	4.36±0.61	0.00±0.00	3.36±0.40
40% Tween 80 (1 mg/kg)	25.14±2.77		30.67±2.49		32.57±2.59		36.27±2.45	
Indomethacin 10 mg/kg	14.23±2.81		9.55±1.67		8.60±1.69		3.75±1.50	

Each value represents the mean ± S. E. M (n=5).

$P< 0.001$ significantly different compared with control, Student's *t*-test (40% Tween 80 solution)

Table 2: Percentage oedema inhibition in rats treated with methanolic plant extracts

Treatment/ Dose	**Percentage oedema inhibition (%) at hourly intervals after phlogistic agent administration**							
	1 h		2 h		3 h		4 h	
	H. indica	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*
125 mg/kg	33.30	34.10	64.60	20.60	85.30	31.80	94.50	54.00
250 mg/kg	74.50	38.00	84.80	49.20	94.40	59.40	100	76.90
500 mg/kg	56.10	52.00	85.80	75.30	100	86.60	100	90.70
40% Tween 80 (1 mg/kg)	-		-		-		-	
Indomethacin 10 mg/kg	43.40		68.90		73.60		89.70	

Each value represents the mean ±S. E. M (n=5).
$P < 0.001$ significantly different compared with control, Student's *t*-test (40% Tween 80 solution). Tween 80 did not inhibit oedema.

Table 3: Antibacterial activities of methanolic extracts of *H. indica* root bark and *P. oleosa* fruit

Micro-organisms	**Mean diameter of zone of inhibition* (mm)**												**Ciprofloxacin (5 mg/ml, positive control)**
	31.25 mg/ml		62.50 mg/ml		125 mg/ml		250 mg/ml		500 mg/ml		1000 mg/ml		
	H. indica	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*	*H. indica*	*P. oleosa*	
S. aureus	0	0	13.5	0	15.8	6.25	20.8	6.25	25.3	14.0	20.5	8.8	17.0
B. subtilis	0	0	0	5.25	0	5.25	12.5	7.25	12.0	11.5	14.8	5.75	27.0
Ps. aeruginosa	0	0	2.5	0	3.8	0	12.5	0	10.0	0	15.8	0	15.0
K. pneumoniae	0	0	0	0	0	0	0	0	0	0	9.0	0	17.0

*Already excludes size of well (6mm, n=2).
MIC values were 16, 100 and 100 mg/ml respectively, against *S. aureus, B. subtilis* and *Ps. aeruginosa* using *H. indica* extract. With *P. oleosa* extract, values of 126 and158 mg/ml against *S. aureus* and *B. subtilis* respectively were recorded.

Antimicrobial activity

Root bark extract of *H. indica* was observed to exhibit pronounced antibacterial activity against all bacteria at the tested concentrations (Table 3). *B. subtilis* and *Ps. aeruginosa* showed comparable susceptibility to *H. indica* extract at high concentrations (250- 1000 mg/ml). Among the organisms screened, *S. aureus* was the most susceptible to *H. indica* extract (MIC, 16 mg/ml), followed by *B. subtilis* and *Ps. aeruginosa* which were of equal susceptibilities (MIC, 100 mg/ml). In the case of *P. oleosa* fruit extract, only the Gram positive bacteria, *S. aureus* and *B. subtilis* were susceptible with high MIC values (> 120 mg/kg). The antibacterial activity of *H. indica* was more pronounced than that of *P. oleosa.* However, it was noted that none of these two plant extracts was active against *K. pneumoniae* and the yeast, *C. albicans.* The reference antibacterial agent, Ciprofloxacin at 5 mg/ml has comparable activity with *H. indica* only at 1000 mg/ml, but *P. oleosa* extract was less active than the positive control at all concentrations tested.

According to this investigation, it can be concluded that the methanolic extracts of both *H. indica* root bark and *P. oleosa* fruits possess significant anti-inflammatory activity. In addition, *H. indica* exhibited higher anti-inflammatory and antibacterial activities than *P. oleosa.* This finding has justified the use of these two plants in

ethnomedicine for treating infectious diseases and inflammatory conditions. Both activities investigated for the two plants may be attributed to the presence of tannins, saponins, flavonoids and alkaloids in the plants, as revealed by preliminary phytochemical investigation using established methods (Dahiru et al., 2006).

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