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Anti-inflammatory, Antipyretic and Antioxidant Properties of a hydroalcoholic Leaf Extract of Palisota hirsuta K. Schum. (Commelinaceae)

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ABSTRACT

Background: Leaves of *Palisota hirsuta* K. Schum. (Commelinaceae) have been used to relieve inflammation and rheumatism in Ghanaian and West African traditional medicine. Nevertheless, scientific information regarding this species is scarce.

Objectives: This study evaluated the scientific basis for the traditional use of *Palisota hirsuta* leaves as an anti-inflammatory, antipyretic and antioxidant agent.

Methods: Effect of the extract on acute inflammation was assessed in the carrageenan-induced foot edema in 7-day-old chicks. *In vitro* antioxidant properties of the extract were evaluated using the reducing power test; 2, 2-diphenyl-1-picrylhydrazyl hydrate radical scavenging assay and the lipid peroxidation assay. In all tests, *n*-propyl gallate was used as the reference antioxidant.

Results: Pre treatment with the extract significantly inhibited foot edema in the chicks with maximal inhibition of 54.71 ± 11.04 %. *P. hirsuta* also dose-dependently decreased baker's yeast induced fever in rats. The extract showed a reducing power potential (EC_{50}; 133.7±7.59 mg ml^{-1}), antioxidative activity in the DPPH de-colorization assay (EC_{50}; 1.77 ± 0.40 10^{-1}) and exhibited a concentration dependent inhibition of lipid peroxidation (EC_{50}; 4.29 ± 0.95 10^{-1}).

Conclusion: These findings present the extract with potent antioxidant properties which may account in part for its anti-inflammatory and antipyretic effects which support its traditional uses.

Keywords: carrageenan, baker's yeast, *n*-propyl gallate, edema, lipid peroxidation

INTRODUCTION

Palisota hirsuta K. Schum. (Family: Commelinaceae), is a robust herb found in forest regrowths and is about 2-4 m high, reproducing from seeds. The whole plant and various parts are used extensively in Ghanaian and West African medicine for various painful inflammatory conditions. In Ghana, the whole plant is used for stomach pains and the sap from the roasted leaves is instilled in the ear for earache while heated leaves are applied over the lumbar region for kidney pains.[1] Also, a leaf infusion or poultice is taken orally or applied locally for piles.[1-2] In Nigeria, the Igbos of Obomkpa prepare an ointment made from this plant for gunshot wounds and swellings.[1]

The methanolic leaf extract of this plant has been reported to have antiviral activity.[3-4] Previous results from our laboratory have also demonstrated the analgesic and anti-arthritic potential of an ethanolic leaf extract of this plant in rodent models[5-6] as well as the anti-inflammatory and antipyretic effect of an ethanolic root extracts.[7]

Inflammation underlies almost every disease process thus making the inflammatory response to receive a great deal of interest in the field of medical research.[8] Since fever has been established to be one of the most prominent systemic manifestations of acute inflammation, the yeast-induced hyperthermia in

young rats previously described was also employed to investigate the anti-pyretic activity of the plant extract.[9] Moreover, most of the drugs currently used as anti-inflammatory agents also have anti-pyretic effects . The role of free radicals and active oxygen in the pathogenesis of human diseases has been recognized.[10] Electron acceptors, such as molecular oxygen, react rapidly with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS) which include superoxide anions (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (-OH) [11]. Oxidants including large amounts of superoxide and hydrogen peroxide are produced during inflammation when polymorphonuclear leukocytes and macrophages are stimulated.[12]

Based on the traditional claims surrounding *P. hirsuta* leaves, the present study was aimed at investigating the scientific basis for the traditional use of *P. hirsuta* leaves as an anti-inflammatory, antipyretic and an antioxidant using animal models.

MATERIALS AND METHODS

Plant material

Leaves of the plant *Palisota hirsuta* were collected from the Botanic Gardens of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, between January and February, 2007. The leaves were authenticated by Mr. Amissah, the curator of the garden, and a voucher specimen (No. FP 10081) has been kept in the Faculty of Pharmacy Herbarium, KNUST, Kumasi.

Preparation of extract

The leaves were air-dried indoors for a week and pulverized with a hammer-mill. The powder was extracted by cold maceration using 70 % (v/v) ethanol over a period of 72 hours. The resulting extract was concentrated under low temperature and pressure at 60°C to a syrupy mass in a rotary evaporator. The syrupy mass was then dried to a dark brown semi-solid mass on a water bath and kept in a dessicator till it was ready to be used. The final yield was 10.5%. This is subsequently referred to as PHE or extract

Drugs

Diclofenac sodium was obtained from Troge, Hamburg, Germany, dexamethasone sodium phosphate from Pharm-Inter, Brussels, Belgium, paracetamol powder from Phyto-Riker, Accra, Ghana, carrageenan sodium salt, *n*-propyl gallate, 2,2, diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide solution, trichloroacetic acid (TCA), thiobarbituric acid (TBA) obtained from Sigma-Aldrich Inc., St. Louis, MO, USA and commercially available dried baker yeast (*Saccharomyces cerevisiae*) from Saf do Brasil Produtos Alimenticios Ltd, Brazil.

Animals

Cockerels (*Gallus gallus;* strain Shaver 579, Akropong Farms, Kumasi, Ghana) were obtained 1-day post-hatch and were housed in stainless steel cages (34×57×40 cm^3) at a population density of 12-13 chicks per cage. Food (Chick Mash, GAFCO, Tema, Ghana) and water were available *ad libitum* through 1-quart gravity-fed feeders and waterers. Room temperature was maintained at 29 °C, and overhead incandescent illumination was maintained on a 12-h lightdark cycle. Daily maintenance of the cages was conducted during the first quarter of the light cycle. Chicks were tested at 7 days of age. Group sample sizes of five were used throughout the study.

Sprague-Dawley rats of both sexes (150-200 g) and were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and housed in the animal facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST). The animals were housed in groups of six in stainless steel cages (34×47×18 cm^3) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 24-28°C, relative humidity 60-70%, and 12 hour light-dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services Publication No. 85-23, revised 1985). All protocols used were approved by the Departmental Ethics Committee.

Phytochemistry

The presence of saponins, tannins, alkaloids, triterpenes, flavonoids, glycosides, reducing sugars and tannins were tested by simple qualitative and quantitative methods. [13-14]

Carrageenan-induced edema

The carrageenan foot edema model of inflammation in

the chick previously described [15] and as modified[7] was used to evaluate the anti-inflammatory properties of the extract and compared to a steroidal anti-inflammatory drug, dexamethasone and a non-steroidal anti-inflammatory drug, diclofenac. Carrageenan (10 ml of a 2 % suspension in saline) was injected subplantar into the right footpads of the chicks. Foot volume was measured before injection and at hourly intervals for 5 hours after injection by water displacement plethysmography as described.[16] The edema component of inflammation was quantified by measuring the difference in foot volume before carrageenan injection and at the various time points.

Effect of extract and drugs on carrageenan-induced edema

The experiment was performed to study the effect of the drugs when given preemptively (30 min for i.p. route and 1 h for oral route) before the carrageenan challenge Chicks were randomly selected for the following study groups: control; diclofenac (10, 30 and 100 mg kg^{-1}, i.p.); dexamethasone (0.3, 1.0 and 3.0 mg kg^{-1}, i.p.) and extract (10, 30, 100 and 300 mg kg^{-1}, *p.o.*). Extract was prepared in 2 % tragacanth mucilage. All drugs were freshly prepared

Induction of brewer's yeast pyrexia

Hyperthermia was induced in rats as previously described.[9] Animals were fasted overnight and for the entire duration of the experiment but given water *ad libitum*. Initial rectal temperatures (TR) were recorded before induction of pyrexia with a lubricated digital thermometer inserted about 3 cm into the rectum of each rat. To induce pyrexia, rats were injected with a pyrogenic dose of baker's yeast (0.135 mg kg^{-1} i.p). TR changes were recorded every hour up to 4 h. Animals that showed an increase of not less than 0.5 °C in rectal temperature were selected for the experiment.

Effect of extract on brewer's yeast-induced pyrexia

Animals were divided randomly into seven groups of five animals each. Three groups received the ethanolic extract (30, 100, 300 mg kg^{-1}, *p.o.*) while three other groups were given paracetamol (10, 30, 100 mg kg^{-1}, *p.o.*) which served as the reference drug. Control group received 0.5 ml saline solution. Rectal temperatures were determined before, and at hourly intervals up to 4 h after extracts/drugs administration.

Reducing power

The reducing capacity of the extract was determined by its ability to reduce Fe^{3+} to Fe^{2+} as previously described.[17] One millilitre (1 ml) of various concentrations of PHE (0.1, 0.3, 1, 3 mg ml^{-1}, in methanol) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml 1 % potassium ferricyanide solution ($K_3Fe[CN]_6$) in a test tube. The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10 %; 1.5 ml) was then added to the mixture and centrifuged at 650 *g* for 10 minutes. Two and a half milliliters (2.5 ml) of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1 % ferric chloride solution. The absorbance was then measured at 700 nm in a spectrophotometer (Model CE 7200, Cecil Instrument Limited, Milton Technical Centre, England). Blank samples were prepared as follows: 1 ml distilled water was added to 2.5 ml sodium phosphate buffer and 2.5 ml potassium ferricyanide and the mixture processed as above. Each test was done in triplicate. The greater the reducing power, the higher the absorbance.

Data were presented as concentration-absorbance curves and the EC_{50} (concentration that gives 50% of maximal response) computed.

Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The free radical scavenging activity was determined as described.[17-18] One (1) ml of test drug (in methanol) was added to 3 ml methanolic solution of DPPH solution (20 mg/ml) in a test tube. The reaction mixture was kept at 25 °C for 1 h. The absorbance of the residual DPPH was determined at 517 nm in a spectrophotometer. Methanol (99.8 %) was used as blank.

The free radical scavenging activity of PHE (1, 3, 10, 30 mg ml^{-1} in methanol) was compared to the reference drugs *n*-propyl gallate (0.01, 0.03, 0.1, 0.3 mg ml^{-1} in methanol). One milliliter (1 ml) methanol (99.8 %) was added to 3 ml DPPH solution incubated at 25°C for 1 h and used as control. The absorbance decreases with increasing free radical scavenging ability. Results were expressed as percentages of blank (100 %). The concentration required to cause a 50% decrease in the absorbance was calculated (EC_{50}). Each test was done in triplicate.

Lipid peroxidation

The extent of lipid peroxidation in rat brain homogenate

was determined using thiobarbituric acid (TBA), as previously described.[19-20]

Preparation of Rat Brain Homogenate

Rats (200-250 g) were sacrificed by decapitation and whole brain (except cerebellum) was dissected out and homogenized (100 mg ml^{-1}) in ice-cold phosphate buffer (0.1 M, pH 7.4) using an Ultra-Turrax T 25 homogenizer (IKA Labortehnic, Staufen, Germany). This was used as a source of polyunsaturated fatty acids (PUFAs) for lipid peroxidation.

Assay of Lipid Peroxidation

Two and a half millilitres (2.5 ml) of brain homogenate was mixed with 1 ml phosphate buffer and 0.5 ml of test drug. Lipid peroxidation was then initiated by the addition of 0.5 ml of 0.1 mM ascorbic acid and 0.5 ml of 5 mM ferric chloride. The mixture was incubated in a shaking water bath at 37 °C for one hour after which 0.1 ml of the reaction mixture was taken into a test tube containing 1.5 ml of 10 % trichloroacetic acid (TCA) and allowed to stand for ten minutes. The tubes were then centrifuged at 1150 *g* for 10 minutes. The supernatant was mixed with 1.5 ml of 0.67 % thiobarbituric acid (TBA) in 20 % acetic acid in a test tube. This reaction mixture was heated in a hot water bath at 85 °C for one hour, allowed to cool to room temperature (28 ^{0}C) and absorbance taken at 535 nm in a spectrophotometer. Phosphate buffer was used as blank. The effect of the extract (1-30 mg ml^{-1}) was compared with the reference drug *n*-propyl gallate (0.03-0.3 mg ml^{-1}). PHE and the reference drug were dissolved in methanol. Each test was carried out using three replicates. Percentage inhibition of lipid peroxidation by the test drugs was assessed by comparing the absorbance of the drug test with that of the control (homogenate mixture without any drug).

Data were presented as percentage inhibition of lipid peroxidation against concentration.

Data analysis

Raw scores for right foot volumes were individually normalized as percentage of change from their values at time 0, then averaged for each treatment group. The time-course curves for foot volume was subjected to two-way (treatment × time) repeated measures analysis of variance with Bonferroni's post hoc t test. Total foot volume for each treatment was calculated in arbitrary unit as the area under the curve (AUC) and to determine the percentage inhibition for each treatment, the following equation was used.

$$\%\, inhibition\ of\ edema \quad \frac{AUC_{control} \quad AUC_{treatment}}{AUC_{control}} \quad 100$$

Data from the antipyretic studies were treated like that in the anti-inflammatory study. Raw scores for basal and changes in rectal temperature were individually normalized as percentage of change from their values at time 0, and then averaged for each treatment group. The time-course curves for changes in rectal temperature was subjected to two-way (treatment × time) repeated measures analysis of variance with Bonferroni's post hoc *t* test. Total change in rectal temperature for each treatment was calculated in arbitrary unit as the area under the curve (AUC).

Differences in AUCs were analyzed by ANOVA, followed by Student's-Newman-Keuls *post hoc* test. Doses and concentrations responsible for 50% of the maximal effect (ED_{50}, EC_{50} and IC_{50}) for each drug were determined using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation.

$$Y \quad \frac{a \quad b \quad a}{1 \quad 10^{\ LogED_{50} \ X}}$$

Where, *X* is the logarithm of dose and *Y* is the response. *Y* starts at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape.

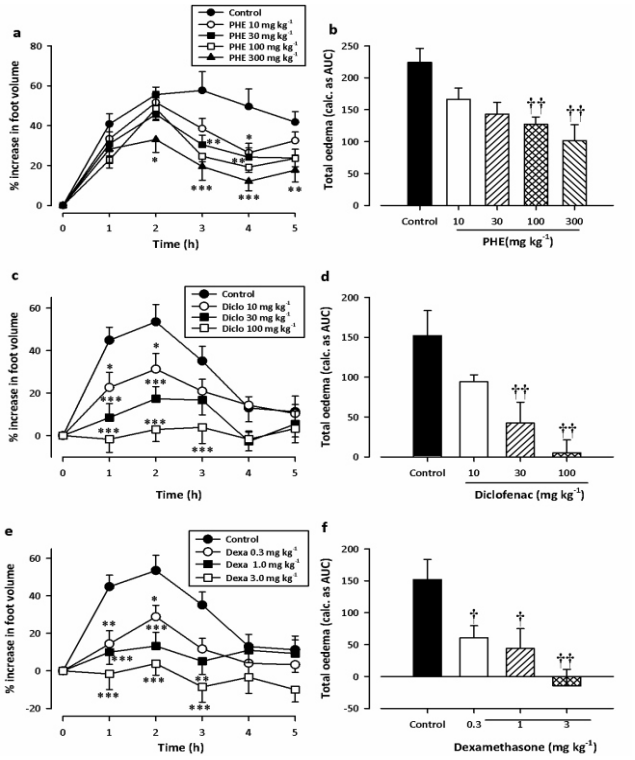
The fitted midpoints (ED_{50}s etc) of the curves were compared statistically using *F* test [21-22]. GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and ED_{50} determinations. $P < 0.05$ was considered statistically significant.

RESULTS

The effect of the extract in acute inflammation was assessed in the chick carrageenan-induced foot edema using dexamethasone and diclofenac as controls. Administration of 10 µl, 2 % carrageenan induced moderate inflammation resulting in foot edema in the 7 day old chicks peaking at 2-3 h.

Figure 1 shows the time courses and the total edema response for the effects of PHE, dexamethasone and diclofenac in carrageenan-induced edema. Two-way ANOVA revealed a significant effect of drug treatment for PHE ($F_{40,100}$= 5.78, P=0.0029), diclofenac ($F_{3,80}$= 5.46, P=0.0089), and dexamethasone ($F_{3,80}$= 1.62, P=0.2241). Total edema produced by each treatment is expressed in arbitrarily as AUC of the time-course curves. PHE (10-300 mg kg^{-1}), dose dependently and significantly reduced the total foot edema with maximal effect of 54.71±11.04 % for PHE administered prophylactically, (Figures 1a and b). Similarly the NSAID diclofenac (10-100 mg kg^{-1}), dose dependently reduced the edema with a maximal effect of 58.78±17.15 % (Figures 1c and d). Dexamethasone (0.3-3 mg kg^{-1}), a steroidal anti-inflammatory agent, on the other hand inhibited the carrageenan-induced edema completely (Figures 1 e and f).

Figure 2 shows the dose-response curves of the effects of the drugs under test. PHE was found to be approximately 4.15× less potent than diclofenac ($F_{1,33}$= 5.82, P=0.0022) and 348.89× less potent than dexamethasone ($F_{1,33}$= 108.90, P<0.0001). Dexamethasone was also found to be 84.08× more potent than diclofenac ($F_{1,28}$= 65.81, P<0.0001).



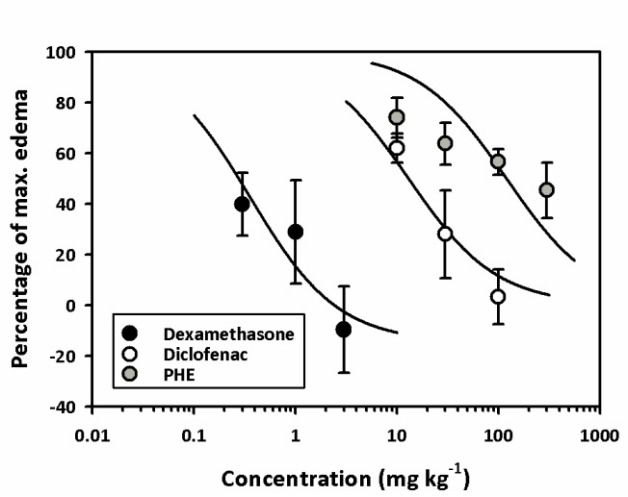


Figure 1 Effect of PHE (10-300 mg kg-1; p.o.), diclofenac (10-100 mg kg-1; i.p.) and dexamethasone (0.3-3 mg kg-1; i.p.) on time course curve (a, c and e respectively) and the total edema response (b, d and f respectively) in carrageenan-induced paw edema in chicks. Values are means ± S.E.M. (n = 5). ***P < 0.001; ** P < 0.01; *P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test). †††P < 0.001; ††P < 0.01; †P < 0.05 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's post hoc test.

Figure 2 Dose response curves for dexamethasone (0.3-3.0 mgkg-1 i.p), diclofenac (10-100 mgkg-1 i.p) and PHE (10-300 mgkg-1 p.o) on carrageenan induced foot edema in the chick.

Rectal temperatures before yeast injection ranged from 35.9 to 37.8 °C with an overall mean ± s.e.m of 36.73±0.05 °C (n=45). Intraperitonial injection of yeast increased significantly (t=16.8; paired t-test), the rectal temperature to between 37.0 to 38.8 °C with a mean of 37.83±0.04. Mean difference in the pre- and post-injection temperatures was 0.89°C, with a 95 % confidence interval of 0.85-1.03. PHE (30, 100 and 300 mg kg^{-1}) administered orally, dose-dependently and significantly, ($F_{4,120}$=37.99; P<0.0001) reduced the increase induced by intraperitonial injection of the yeast as shown in Figure 3. Furthermore, PHE produced a significant ($F_{4,15}$=32.70; P<0.0001) and dose-dependent decrease in total pyrexia represented as AUCs in Figure 3b. Similarly, a two-way ANOVA of time course curves revealed a significant treatment effect for paracetamol ($F_{4,120}$=88.84; P<0.0001) (Figure 3c). Also, paracetamol significantly ($F_{4,15}$=78.87; P<0.0001) and

dose-dependently decreased the area under the curves of the time course curves, compared to that of vehicle-treated group, (Figure 3d). On analysis of the data by non-linear regression, the IC_{50}s obtained were 265.10±63.73 and 18.05±4.08 mg kg^{-1} respectively for PHE and paracetamol. Thus the extract was approximately 15 times less potent than the standard, paracetamol.

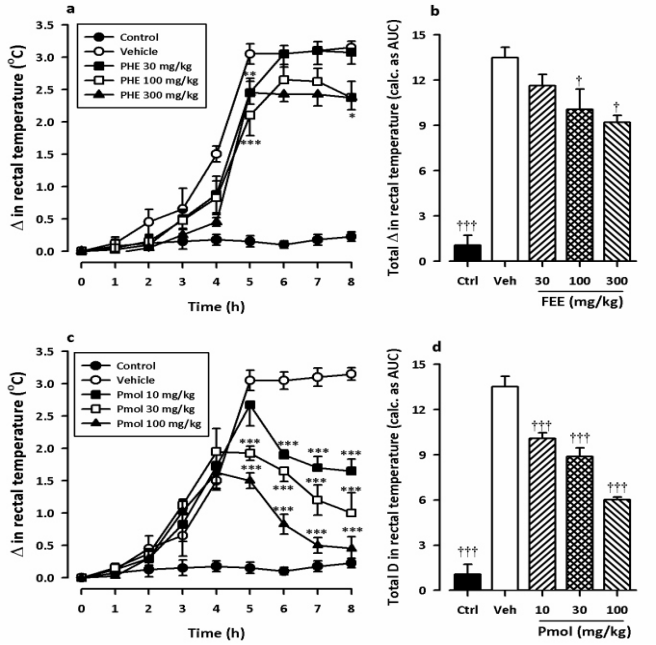


Figure 3 Time course effect of PHE (10-300 mg kg^{-1} p.o) and Paracetamol (10-100 mg kg^{-1} p.o) on Baker's yeast-induced fever in young rats (a and c) and the AUC (total edema) for variation of rectal temperature along time, compared to 0.9% NaCl or vehicle (b and d). Each point in a and c represents the Mean ± S.E.M (n = 5). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 compared to respective controls (two-way repeated measures ANOVA followed by Bonferroni's post hoc); each column in (b and d) represent the mean ± S.E.M. $^{\dagger}P = 0.05$, $^{\dagger\dagger}P \leq 0.01$, $^{\dagger\dagger\dagger}P \leq 0.001$ (one-way ANOVA followed by Newman-Keuls post hoc test)

Both the standard, *n*-propyl gallate (0.00083 - 0.025 mg ml^{-1}) and the extract (0.1- 3 mg ml^{-1}) exhibited concentration dependent free radical scavenging activity, (Figure 4a). The rank order of potency (defined by EC_{50} in mg ml^{-1}; Table 1) was found to be: *n*-propyl gallate (8.02 ± 0.01 x 10^{-4}) greater than extract (1.77 ± 0.40 x 10^{-1}). The extract was however found to be about 220 fold less potent than *n*-propyl gallate.

The extract (0.001- 0.03 mg ml^{-1}) and the standard antioxidant *n*-propyl gallate (0.1-3 mg ml^{-1}) concentration dependently reduced Fe^{3+} to Fe^{2+} resulting in concentration dependent increase in absorbance (Figure 4b). From the EC_{50} (in mg ml^{-1}; Table 1) obtained for the extract (133.70 ± 7.59) and *n*-propyl gallate (3.77 ± 0.07), the extract was found to be about 35 fold less potent than *n*-propyl gallate.

Several concentrations of *n*-propyl gallate (0.01- 0.1 mg ml^{-1}) and the extract (0.1-1.0 mg ml^{-1}) were tested for inhibitory action on ascorbatate/Fe^{3+} induced lipid peroxidation. All drugs showed concentration dependent ability to inhibit lipid peroxidation (Figure 4c). The rank order of potency (defined by EC_{50} in mg ml^{-1}; Table 1) was found to be: *n*-propyl gallate (1.31 ±3.00 x 10^{-2}) greater than extract (4.29 ± 0.99 x 10^{-1}). The extract was found to be about 32-fold less potent than the standard antioxidant, *n*-propyl gallate.

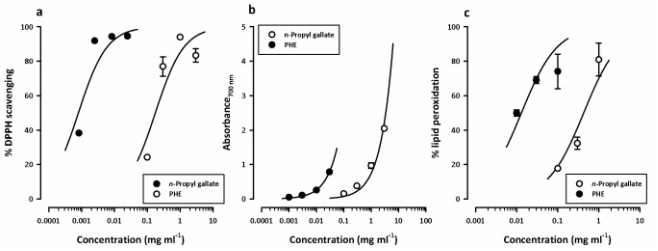


Figure 4 Free radical scavenging ability of PHE (0.1-3 mg ml^{-1}) compared to n-propyl gallate (0.00083-0.025 mg ml^{-1}) in the DPPH radical assay, reducing power of PHE (0.1-3 mg ml^{-1}) compared to n-propyl gallate (0.001-0.03 mg ml^{-1}) and percentage inhibition of lipid peroxidation by PHE (0.1-1 mg ml^{-1}) compared to n-propyl gallate (0.01-0.1 mg ml^{-1}). Each point represents the Mean ± S.E.M

Drug	Ed_{50} (mg ml^{-1})		
	Reducing Power	*DPPH Scavenging*	*`Lipid Peroxidation*
P.hirsuta extract	133.70±7.59***	1.77±0.40×10^{-1}***	4.29±0.99×10^{-1}***
n-Propyl gallate	3.77±0.07	8.02±0.01×10^{-4}	1.31±3.00×10^{-2}

Table 1 EC50s for antioxidant Properties of P.hirsuta extract and n-Propyl gallate.*** P < 0.001, ** P < 0.01, * P < 0.05 compared to reference antioxidant (One-way ANOVA followed by Neuman-Keul's post hoc test).

DISCUSSION

Carrageenan-induced acute footpad edema in laboratory animals[23] has been widely used to screen new anti-inflammatory drugs and remains an acceptable preliminary screening test for anti-inflammatory activity.[24-25] Using chicks, instead of the commonly used rodents is much more economical than rodent models and furthermore, chicks are easier to

handle. Studies have also demonstrated that results from using chicks compare favourably with the more commonly used rodent models (rat and mice) in the screening of drugs with anti-inflammatory activities.[7, 15] The dose-dependent inhibition of carrageenan-induced foot edema by the extract in this model of acute inflammation depicts the anti-inflammatory potential of the extract in acute inflammation

Although the actual mechanism of action of PHE is not known, it is possible that, the anti-inflammatory activity exhibited by the extract could be attributed to the inhibition of the synthesis, release or action of inflammatory mediators that are known to be involved in carrageenan-induced inflammation which include cytoplasmic enzymes and serotonin from mast cells and also bradykinin, prostaglandins and other cyclooxygenase products.[26-28] The extract was compared to the standard drugs diclofenac and dexamethasone which both showed a dose-dependent inhibition of carrageenan-induced edema as already established by other researchers.[29-32]

The extract also exhibited antipyretic activity in yeast-induced pyrexia in rats. Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states.[33] Antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis and an indispensible role of prostaglandins, specifically prostaglandin E_2 (PGE_2) in the febrile response, where it is known to be the final fever mediator in the brain has been demonstrated.[34-36] It may therefore be plausible to conclude that the extract inhibits the synthesis of prostaglandins. However, it must be noted that several biochemical events occur leading ultimately to the synthesis of PGE_2. It may therefore be worthwhile to investigate the exact point in the biochemical events where the extract exerts it antipyretic effect.

In the present study, the antioxidant activity of the extract was evaluated using three different assays: reducing power test, DPPH scavenging activity and the lipid peroxidation assay.

Polyphenols are electron-rich compounds and capable of entering into efficient electron-donation reactions with oxidizing agents.[37] Various plant phenols have been found to interfere with the oxidation of different biomolecules important for life.[38] Prior to this, a routine test using ferric chloride was done to determine the presence of phenols in the extract, which gave a positive results indicating that the extract contains some amount of plant phenols. The potent reductive capabilities exhibited by the extract through the reduction of Fe^{3+} to Fe^{2+} in the reducing power test demonstrates the potential of the leaves extract to donate electrons to free radicals or reactive oxygen species as is exhibited by chain-breaking antioxidants like *n*-propyl gallate and α-tocopherol (Vitamin E).[39] The process gives rise to low energy products that are unable to propagate free radical formation any further.[40] The extract also caused a concentration dependent de-colorization of DPPH solution. This is an expression of the extracts ability to directly interact with free radicals to produce less harmful products. This property is exhibited by chain breaking and scavenging antioxidants like tocopherol (vitamin E) and ascorbic acid (vitamin C) by donating an electron to stabilize an existing free radical.[39-40]

The extract and the standard drug effectively inhibited lipid peroxidation, a proof of their potent antioxidant properties. There are many possible mechanisms involved in the anti-lipid peroxidation property. The effect may be mediated through their scavenging effect on the super oxide anion radical (O_2^-) and the hydroxyl radical (OH^-) formed during the Haber-Weiss reaction and Fenton reaction respectively.

CONCLUSION

The results of this study have provided evidence to support the use of *P. hirsuta* leaves as anti inflammatory and antipyretic agent in traditional medicine. These effects might be partially or wholly be due to the possible inhibition or interference with the production of some inflammatory mediators, especially prostaglandins and/or its potent antioxidant properties as a reducing agent, a free radical scavenger, and a potent inhibitor of lipid peroxidation.

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