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RESEARCH ARTICLE

Evaluation of Nutraceutical Properties of *Laportea interrupta* (L.) Chew

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Abstract In the present study *Laportea interrupta* was analysed for nutritional, antioxidant, and antipyretic properties. Leaves contained significant amount of carbohydrates (19.80 g/100 g), proteins (31.30 g/100 g), starch (15.40 g/100 g), essential amino acids, and minerals. Ethanol extracts of flowers and roots revealed high total phenolic (46.35 mg gallic acid equivalents/g of extract) and flavonoid contents (96.67 mg rutin equivalents/g of extract) respectively. Antioxidant assays showed that ethanol root extract possessed a strong 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (IC_{50}: 32.34 μg/mL), a 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation scavenging activity (1,072.57 μM trolox equivalents/g of extract), and a ferric reducing ability (9,456.01 mM Fe(II)/g of extract). Antipyretic studies revealed that ethanol root and leaf extracts at a dosage of 400 mg/kg in rats reduced the pyrexia induced by Brewer's yeast by 68.0 and 57.4%, respectively. Thus, nutraceutical potential of *L. interrupta* and ethnobotanical information about its use as an antipyretic was confirmed.

Keywords: antioxidant, antipyretic, flavonoid, *Laportea interrupta*, phenolic

Introduction

Nutraceuticals and functional foods have received considerable attention because of their acknowledged safety and potential nutritional and therapeutic effects (1). Use of nutraceuticals to achieve desirable therapeutic outcomes with minimal side effects, as compared with other therapeutic agents, has been a huge monetary success. However, scarcities of food commodities and a rising demand for medicinal plants have spurred a search for cheaper and easier resources.

Carbohydrates, proteins, and fats are commonly referred to as proximate principles and form the major portion of the human diet while minerals play an essential role in regulation of metabolic activity in the body. Secondary metabolites, such as alkaloids, phenolics, flavonoids, tannins, iridoids, steroids, and terpenoids, which are generally produced by plants for defense, have mostly been associated with the therapeutic properties of medicinal plants (2). Over-production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in humans can result in tissue injury and has been implicated in disease development and oxidative damage to nucleic acids, proteins, and lipids. There is a natural, dynamic balance between the amount of free radicals produced by the body and the amount of antioxidants to scavenge the free radicals in order to protect the body against the harmful effects of these radicals (3). When a lack of antioxidants to quench excess reactive free radicals exists, oxidative stress occurs that can lead to cancer, cardiovascular, neurodegenerative, inflammatory and Alzheimer's diseases (4). Reports reveal that synthetic antioxidants can be toxic and expensive which have resulted in an increasing consumer awareness regarding food additive safety. This has generated a need to identify alternative, natural, and, probably, safer sources of food antioxidants (5). Compounds usually responsible for antioxidant actions possess various pharmacological properties. Non-steroidal anti-inflammatory drugs (NSAIDs) exert antipyretic effects by inhibition of prostaglandin E2 (PGE2) synthesis, which is responsible for triggering the hypothalamus to increase body temperature during

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inflammation. The search for herbal remedies with potent antipyretic activities has recently received a boost as the common antipyretics paracetamol and nimusulide have been found to have toxic effects on liver, kidney and gastrointestinal tract of the body (6).

Laportea interrupta of the family Urticaceae has been widely reported for potential medicinal uses. It is a common weed which grows where it is not wanted and in competition with cultivated plants. The leaves of this nutritional weed are cooked and eaten. Reports have revealed that leaves of *L. interuupta* are used as a leafy vegetable by the Paniya, Kattunaikka, and Kuruma tribes of Wayanad, Kerala, India (7). Moreover, the plant is used for joining broken bones, for treating joint diseases like gout and rheumatism (8), against skin inflammation, and as a local application for carbuncle (9). The leaves are used to treat infective hepatitis (10), malaria, muscular pains, liver pain, joint pain, fatigue, and are used against microbes. Ethnobotanical reports have also revealed that roots of the plant are used as a diuretic (9) and for reducing cold fevers and intermittent fevers, young shoots are used against influenza (10), and fruits are used for curing headaches. These reports suggest that *L. interrupta* can be used as a nutraceutical with a wide range of applications in herbal drug research.

Materials and Methods

Collection and identification of plant material *Laportea interrupta* plants were collected during the month of July 2011 in the Kannur district of Kerala, India. The plant was identified by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu (No. 1467), India. Fresh plant materials were washed under running tap water to remove surface contaminants and were shade dried at room temperature. Leaves, stems, roots, and flowers were separately homogenized into a fine powder using a mixer (MG 172; Preethi Kitchen Appliances Pvt., Ltd., Chennai, India) for further study.

Chemicals DPPH, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and rutin were procured from Sigma Aldrich (Bengaluru branch, Karnataka, India). ABTS diammonium salt, butylated hydroxy toluene (BHT), potassium persulfate, gallic acid, ferrous chloride, ferric chloride, TPTZ, and carboxy methyl cellulose were obtained from Himedia (Mumbai, Maharashtra, India). All chemicals and solvents used were of the highest purity and analytical grade. Millipore water (Direct-Q3; Millipore Corporation, France) having resistivity 18.2 MΩ·cm was used in all the analysis.

Extraction of plant material Powdered leaves, stems, roots, and flowers were packed in small thimbles separately and subjected to extraction successively using the organic solvents petroleum ether, chloroform, and ethanol in an increasing order of polarity with a Soxhlet apparatus (3840; Borosil Glass Works Ltd., Mumbai, India). Petroleum ether and chloroform help to remove lipid constituents, chlorophylls, and other non-polar substances that interfere with the biological activities of polar extracts. Before each extraction step with a new solvent, the thimble was air dried. After extraction with organic solvents, the powered material in the thimble was macerated in hot water using orbital shaker (Rivotek; Riviera Glass Pvt., Ltd., Mumbai, India) at 125 rpm for 24 h. The micella was separated from the plant material by centrifuging (Centrifuge 5430 R; Eppendorf AG, Germany) the content at 3,000×*g* for 10 min at room temperature and the supernatant was collected. All the extracts were concentrated separately using a rotary vacuum evaporator (Equitron; Medica Instrument Mfg., Co., Mumbai, India), air dried, and stored in a deep freezer (BFS-150; Celfrost Innovations Pvt., Ltd., Haryana, India) at −20ºC for further analysis.

Nutritional studies

***Proximate chemical compositions*:** The moisture, ash, and crude lipid contents of the leaves of *L. interrupta* were estimated using Association of Official Analytical Chemists (AOAC) methods (11). Total carbohydrate and starch amounts were estimated using the anthrone method, as described by Sadasivam and Manickam (12). Protein and free amino acid contents were estimated using Lowry's and ninhydrin methods, respectively (12).

***Amino acid analysis*:** The leaf amino acid content was determined following the procedure of Ishida *et al*. (13). Approximately 100 mg of a finely homogenized leaf sample was placed in a test tube into which 10 mL of 6 N HCl was added. The tube was sealed after filling with nitrogen gas and the contents of the tube were digested at 120ºC for 24 h in an oven. The contents of the test tube were filtered using Whatman No. 1 filter paper (Imperial Scientific Works, Coimbatore, India) after cooling. The filtrate was evaporated using a rotary vacuum evaporator (Equitron). Deionized water was then added to the filtrate and evaporation was continued until the contents were acid free. The residue was made up to 10 mL with 0.05N HCl and was filtered through a 0.45 μm PVDF membrane filter (SFPV25X; Axiva Sichem Biotech, Delhi, India). Approximately 20 μL of filtrate was injected into an amino acid analyzer (HPLC- LC 10 AS; Shimadzu Scientific Instruments, Tokyo, Japan) equipped with a Na type cation exchange column (ϕ 5.0 mm×190 mm) (ISC-07/S1504; Shimadzu Scientific Instruments) packed with the strongly acidic cation exchange resin styrene divinyl benzene copolymer with a sulphonic group. The instrument was

equipped with a Shimadzu fluorescence detector (FL 6A; Shimadzu Scientific Instruments) and a Shimadzu Chrompac recorder (CR 6A; Shimadzu Scientific Instruments). The mobile phase of the system consisted of two buffers and a gradient system was used for effective separation of amino acids. The oven temperature was maintained at 60°C with a total run time of 60 min. Amino acid analysis was performed using the non-switching flow method with fluorescence detection after post-column derivatization using *o*-phthaldehyde. The imino groups of proline and hydroxyl proline are converted to amino groups using hypochlorite. An amino acid standard (Sigma Chemical Co., St. Louis, MO, USA) was also run to calculate the concentration of amino acids in the sample. The amount of each amino acid was expressed as g/100 g of protein.

***Mineral quantification*:** The phosphorous content was determined using spectrophotometric measurements (UV-1800; Shimadzu Scientific Instruments). The nitrogen content was estimated using titration after digestion. Calcium and magnesium content measurements used titration only. Estimation of the sodium and potassium content was performed using a flame photometer (VSI-FP1; V.S.I. Electronics Private Ltd., Mohali, India) upon wet digestion of a sample. Amounts of the remaining mineral constituents (manganese, iron, copper, zinc, and boron) were determined using an atomic absorption spectrophotometer (LT-2100; Labtronics, Panchkula, India). The dithiol colorimetric method was used to determine the molybdenum content (12).

Quantification assays

***Quantification of total phenolics*:** The total phenolic content of *L. interrupta* was determined according to the method described by Makkar (14). Approximately 50 μL of plant extract was placed into a series of test tubes and made up to 1 mL using distilled water. A test tube with 1 mL of distilled water served as a blank. Approximately 500 μL of Folin-Ciocalteau Phenol reagent (1 N) was then added to the tubes, including the blank. After 5 min, 2.5 mL of sodium carbonate solution (20%) was added, followed by vortexing (Vortexer; Âscension Innovations, Bangalore, India) to mix the contents, and incubated in the dark for 40 min. Blue color in the incubated test tubes indicated the presence of phenolics and spectrophotometric (UV-1800; Shimadzu Scientific Instruments) reading was performed at 725 nm against the reagent blank. Gallic acid was used as a standard for plotting a calibration curve and results are expressed as gallic acid equivalents (GAE). Analyses were performed in triplicate.

***Quantification of flavonoids*:** The method of Zhishen *et al*. (15) was followed for quantification of flavonoids in the extracts. Approximately 2 mL of distilled water was added to 500 μL of different extracts in test tubes. A blank test tube contained only 2.5 mL of distilled water. An amount of 150 μL of 5% $NaNO_2$ was then added to all tubes, followed by incubation at room temperature for 6 min. After incubation, 150 μL of 10% $AlCl_3$ was added to all tubes, including the blank, and the tubes were again incubated for 6 min at room temperature. Then, 2 mL of 4% NaOH was added to the tubes, which were then made up to 5 mL using distilled water. The tubes were then vortexed (Vortexer; Âscension Innovations) and allowed to stand for 15 min at room temperature. A pink color developed due to the presence of flavonoids. Reading was performed spectrophotometrically (UV-1800; Shimadzu Scientific Instruments) at 510 nm. Flavonoid-rutin was used as a standard for plotting a calibration curve. All experiments were performed in triplicate and results are expressed as rutin equivalents (RE).

***In vitro* antioxidant assays**

***DPPH$^{\bullet}$ scavenging activity*:** The antioxidant activities of *L. interrupta* extracts were determined according to the method of Braca *et al*. (16) based on hydrogen donating and radical scavenging abilities using the stable radical DPPH. Extract aliquots were taken in triplicate and made up to 100 μL using methanol. Approximately 3 mL of a 0.004% DPPH solution in methanol was added to all the tubes containing samples and standards. A negative control was prepared by adding 100 μL of methanol to 3 mL of a DPPH solution. The tubes were allowed to stand for 30 min at room temperature. The absorbance of the sample was measured at 517 nm against the blank (methanol). The radical scavenging activity of the samples was expressed as an IC_{50} value, which is the concentration of the sample required to inhibit a 50% DPPH$^{\bullet}$ concentration.

***ABTS$^{\bullet+}$ scavenging activity*:** An ABTS radical cation de-colorization assay was performed according to the method of Re *et al*. (17). ABTS$^{\bullet+}$ was produced by reacting a 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to the assay, this solution was diluted with ethanol (approximately 1:89 v/v) and equilibrated at 30°C to produce an absorbance value of 0.700±0.02 at 734 nm. After addition of 1 mL of diluted ABTS solution to sample aliquots or Trolox (an analog of vitamin E), the absorbance was measured 30 min after the initial mixing. Triplicate determinations were made and de-colorization was read at 734 nm against a blank (ethanol). A unit of antioxidant activity was calculated as the concentration of Trolox having an equivalent antioxidant activity expressed as μM/g of sample extract.

***Ferric reducing antioxidant power (FRAP) assay*:** The antioxidant capacities of different sample extracts were estimated according to the procedure described by Pulido *et al*. (18). Freshly prepared FRAP reagent (900 μL) was incubated at 37°C and mixed with 90 μL of distilled water

and 30 μL of a test sample or methanol (blank). Test tubes were incubated at 37ºC for 30 min in a water bath. The FRAP reagent was prepared by mixing 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$. $6H_2O$, and 25 mL of 0.3 M acetate buffer (pH 3.6). At the end of the incubation, absorbance readings were immediately taken at 593 nm against the reagent blank. Methanol solutions of a known Fe(II) concentration, ranging from 100 to 2,000 μM $FeSO_4 \cdot 7H_2O$ were used for preparation of a calibration curve. Equivalent concentrations were calculated as the concentration of antioxidant producing an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mM concentration of a Fe(II) solution.

***In vivo* studies**

***Animals and ethics*:** Swiss albino mice (20-30 g) and Wistar albino rats (200-250 g) were used for animal studies, which were carried out at Nandha College of Pharmacy, Erode, Tamil Nadu, India. Animals were maintained under standard conditions of temperature (24±1ºC), relative humidity (55±1%), light/dark cycle (12/12 h), and fed with a standard diet and water *ad libitum*. All animals were acclimatized for at least one week and were fasted for 12 h (free access to water but not to food) before the beginning of the experiments. All animal experimental protocols were approved by the Institutional Animal Ethical Committee (688/2/C-CPCSEA/2011/03).

***Acute oral toxicity*:** Acute oral toxicity studies were performed using a fixed dose procedure according to the Organization for Economic Co-operation and Development (OECD) guideline -420 with slight modification (19). Since female mice exhibit more behavioral changes than male mice due to hormonal actions, healthy female Swiss albino mice weighing between 20-30 g were selected using a random sampling technique for both sighting and main studies. Mice were housed in separate plastic cages and maintained at room temperature with a photoperiod of 12 h and frequent air changes. Mice had free access to tap water only for a short fasting period before treatment with plant extracts. A sighting study helps in selection of an appropriate starting dose for a main study. The test substance was administered to a single animal in a sequential manner and the animal was observed for at least 24 h. The starting dose for the sighting study was selected from fixed dose levels of 5, 50, 300, and 2,000 mg/kg as a dose expected to produce evident toxicity based on *in vitro* and *in vivo* data for the same extract and for related phytochemicals. From sighting study, the starting dose of *L. interrupta* extracts for main study was determined to be 2,000 mg/kg.

For the main study, mice were divided into 4 groups of 5 animals each and each group received leaf, stem, root, and flower extracts at a dose of 2,000 mg/kg. Animals were observed for general behavioral changes and signs of toxicity and mortality, including alertness, grooming, touch response, pain response, tremors, convulsions, righting replex, gripping strength, pinna replex, corneal replex, pupils, urination, salivation, skin color, lacrimation, and hyperactivity continuously for 1 h after treatment, then intermittently for 4 h, and thereafter over a period of 24 h. This method can be used to determine a dose for simple animal studies, rather than finding a lethal dose (LD_{50}) since it requires only a short time period.

***Antipyretic activity- Brewer's yeast-induced pyrexia in rats*:** Antipyretic activity was measured using the slightly modified method of Adams *et al*. (20). Prior to experiments, Wistar albino rats weighing 200-250 g were maintained in separate cages for 7 days and animals with approximately constant rectal temperatures were selected for the study. Pyrexia was induced by subcutaneously injecting a 15%(w/v) brewer's yeast suspension (10 mL/kg) into the dorsum region. Approximately 18 h after injection, the rectal temperature of each rat was measured using a digital Tele thermometer (461; Sunshine Instruments, Coimbatore, India). Only rats that showed an increase in temperature greater than or equal to 0.7ºC were used for subsequent experiments. Rats were divided into 10 groups of 6 animals each. The first group was the control group, receiving only the vehicle, carboxymethyl cellulose (10 mL/kg, p.o.). The second group (standard group) was administered the antipyretic drug paracetamol at a dose of 150 mg/kg for a comparative pharmacological assessment. Members of the remaining groups were administered leaf, stem, root, and flower ethanol extracts orally at doses of 200 and 400 mg/kg, p.o. The rectal temperature of all rats was measured 1, 2, 3, 4, and 5 h after extract administration and was compared with the control and standard groups. The relative potency of the extracts under investigation was calculated based upon the percentage inhibition of pyrexia as:

$$\% \text{ pyrexia reduction} = [(\text{temp. at } 0 \text{ h} - \text{temp. at } 1,2,3,4,5 \text{ h}) / (\text{temp. at } 0 \text{ h} - \text{temp. at } -18 \text{ h})] \times 100$$

Statistical analysis Results are expressed as mean± standard deviation (SD)/standard error of the mean (SEM). For studies in which a standard calibration curve was used, a regression equation and the coefficient of determination were also determined. Data were statistically analyzed by a one way ANOVA (Analysis of Variance) using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was determined by Duncan's test for antioxidant studies ($p<0.05$), and by Dunnet's *t*-test for antipyretic study ($p<0.05$, $p<0.01$, and $p<0.001$).

Results and Discussion

Nutritional evaluation The results of a proximate composition analysis of *L. interrupta* leaf extracts are summarized in Table 1. Among primary metabolites, the protein content was higher (31.3 g/100 g of dried powder) than the carbohydrate and free amino acid contents (19.80 and 3.27 g/100 g of dried powder, respectively). Amino acid profiling revealed that *L. interrupta* leaf extracts contained the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) required dietary amino acids in higher amount (Table 2). Glutamic acid and leucine were abundant in leaf extracts (10.32 and 7.56 g/100 g of protein, respectively). Similarly, the essential amino acids valine (5.75 g/100 g of protein), phenyl alanine (5.19 g/100 g of protein), arginine (4.57 g/100 g of protein), isoleucine (4.37 g/100 g of protein), and lysine (3.69 g/100 g of protein) were also found in leaf extracts. The total amino acid content at 80.44 g/100 g of protein was higher than the FAO/WHO/UNU requirements. Quantification of macro and micronutrients in the leaves of *L. interrupta* and nutrient proportions are shown in Table 3. Mineral analysis revealed that leaf extracts of *L. interrupta* are an especially rich source of boron (30,100 ppm) and also nitrogen (18,000 ppm), calcium (17,000 ppm), copper (13,000 ppm), and iron (1,810 ppm).

The ash content of leaf extracts provides an indication of the significant mineral content (21). The amino acids leucine, isoleucine, alanine, and valine present at high levels in the leaves of *L. interrupta* can enhance muscular energy production and stimulate metabolic signals. Some of these amino acids are involved in major metabolic functions, including regulation of protein turnover and signal transduction, transport of nitrogen and carbon in organs, and neurotransmission (22). People who are dependent on vegetable sources of protein should be able to use the protein rich leaves of *L. interrupta* for dietary improvement. The mineral necessities of the body are supplied by the diet and leafy vegetables make an important contribution to the dietary mineral intake of most people. Mineral elements play essential roles in many significant processes in the body, including enzyme systems and skeletal structures (calcium and phosphorus), as important physiological compounds (iodine in thyroxine, sulphur in cysteine and methionine), and in the blood (calcium is needed for neuromuscular irritability and blood clotting) (21). Nitrogen, phosphorous, and potassium are required for proper cell and organ function. These results support the use of *L. interrupta* as a leafy vegetable.

Table 1. A proximate compositional analysis of *L. interrupta* leaf powder

Parameter	Amount (g/100 g of dried powder)
Moisture	34.8
Ash	20.4
Crude lipid	0.86
Total carbohydrates[1)]	19.80
Total proteins[2)]	31.30
Starch[3)]	15.40
Total free amino acids[4)]	3.27

[1)]Regression equation: y=0.006x–0.008; R^2=0.996
[2)]BSAE, bovine serum albumin equivalents; regression equation: y=0.002x+0.006; R^2=0.998
[3)]Regression equation: y=0.013x+0.009; R^2=0.998
[4)]LE, leucine equivalents; regression equation: y=0.019x+0.016; R^2=0.997

Table 2. A leaf amino acid profile of *L. interrupta*

Amino acid	Amount (g/100 g of protein)	FAO/WHO/UNU (2007) requirement pattern (g/100 g of protein)
Aspartic acid	9.30	--
Threonine	4.16	2.3
Serine	3.76	--
Glutamic acid	10.32	--
Proline	4.07	--
Glycine	5.04	--
Alanine	5.29	--
Cysteine	1.21	0.6
Valine	5.75	3.9
Methionine	1.52	1.6
Isoleucine	4.37	3.0
Leucine	7.56	5.9
Tyrosine	3.14	3.8
Phenyl alanine	5.19	(Tyr+Phe)
Histidine	1.46	1.5
Lysine	3.69	4.5
Arginine	4.57	--
Total	80.44	27.7

Table 3. A leaf mineral profile of *L. interrupta*

Parameter	Amount (ppm)
Nitrogen	18,000
Potassium	428
Sodium	320
Calcium	17,000
Boron	30,100
Copper	13,000
Iron	1,810
Magnesium	Absent
Zinc	Absent
Iodine	Absent
Phosphorous	ND[1)]
Molybdenum	ND

[1)]ND, not detected

Table 4. Extract yields and total phenolic and flavonoid contents of *L. interrupta*

Parameter	Solvent	Leaf	Stem	Root	Flower
Extract yield[1)]	Petroleum ether	0.86	0.60	0.36	1.92
	Chloroform	0.73	0.48	0.38	0.70
	Ethanol	3.51	5.48	1.13	1.08
	Hot water	2.01	3.90	1.80	1.60
Total phenolics[2)]	Petroleum ether	12.88±0.58^{f}	6.86±0.40	4.10±0.56	4.62±0.38
	Chloroform	10.00±0.38	8.65±0.58	14.36±0.68^{e}	6.03±0.29
	Ethanol	36.22±0.22^{c}	23.65±0.19^{d}	37.56±0.48^{b}	46.35±0.19^{a}
	Hot water	11.03±0.40	12.56±0.11^{f}	5.06±0.48	10.13±0.22
Flavonoid[3)]	Petroleum ether	7.39±1.07	6.33±1.11	6.94±1.15	9.46±1.26
	Chloroform	12.18±1.29	13.78±1.75	9.81±1.26	12.39±1.50
	Ethanol	80.00±2.65^{c}	65.33±1.76^{d}	96.67±2.25^{a}	90.33±1.53^{b}
	Hot water	17.67±2.36^{f}	26.00±0.87^{e}	14.33±3.06	17.33±0.76^{f}

[1)]g/100 g of dried powder
[2)]mg gallic acid equivalents (GAE)/g of extract; regression equation: y=0.028x–0.032; R^2=0.996
[3)]mg rutin equivalents (RE)/g of extract; regression equation: y=0.002x–0.013; R^2=0.995; Values are expressed as mean (n=3)±standard deviation (SD); Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}>^{e}>^{f}$ among each parameter.

Extract yield The percentage extract yields for leaves, stems, roots, and flowers of *L. interrupta* using non-polar to polar organic solvents are shown in Table 4. Higher yields were obtained using ethanol and hot water extraction for stems (5.48 and 3.90 g/100 g of dried powder, respectively). For leaves, a higher yield was obtained using ethanol (3.51 g/100 g of dried powder). The higher percentage of recovery in ethanol and hot water indicates that the plant probably contains more polar compounds than non-polar compounds (23).

Quantification of total phenolics and flavonoids Results for total phenolic and flavonoid contents of different extracts of *L. interrupta* are shown in Table 4. Flower extracts obtained using ethanol (46.35 mg GAE/g of extract) showed a higher phenolic content. Moreover, ethanol was better for extraction of phenolics from all plant parts, compared to other solvents. The total phenolic content of ethanol extracts of other parts follows, in decreasing order, root (37.58 mg GAE/g of extract)>Leaf (36.22 mg GAE/g of extract)>stem (23.65 mg GAE/g of extract). On the other hand, the flavonoid content was higher in ethanol extracts of roots (96.67 mg RE/g of extract) followed by flowers (90.33 mg RE/g of extract), leaves (80 mg RE/g of extract), and stems (65.33 mg RE/g of extract).

The arrangement and multiplicity of hydroxyl groups in the chemical structure of polyphenols make them ideal for free radical scavenging reactions and as metal chelating agents. The higher amounts of phenolics obtained using ethanol extraction could be due to a higher solubility for phenolics and other aroma compounds. Cells probably respond to polyphenols through direct interactions with receptors or enzymes involved in signal transduction, which may result in a modification of the redox status of the cell and may trigger a series of redox-dependent reactions (24). One of the most diverse and important groups of natural phenolics are flavonoids. Since redox potentials ($E7$) of flavonoids are low ($0.23<E7<0.75$ V), they are thermodynamically able to reduce highly oxidizing free radicals (superoxides, peroxyls, alkoxyls, and hydroxyl radicals) with redox potentials in the range 2.13-1.0 V, by hydrogen atom donation (25). Dietary flavonoids probably form the first antioxidant defense in the digestive tract and limit ROS formation by scavenging them. Anti-inflammatory, antioxidant, antiallergenic, hepato-protective, antithrombotic, antiviral, anticancer, antidiabetic, and antiaging properties have been ascribed to flavonoids, along with the ability to prevent cardiovascular diseases. Therefore, large amounts of phenolics and flavonoids in ethanol extracts of *L. interrupta* impart a radical scavenging ability, either by electron or hydrogen ion transfer.

***In vitro* antioxidant assays**

***DPPH˙ scavenging activity*:** The DPPH radical scavenging abilities *L. interrupta* extracts was good enough to be considered a functional food (Fig. 1). Root extracts showed the highest activity compared to other extracts. The significant results ($p<0.05$) for root and leaf extracts were supported by low IC_{50} values of 32.34 and 41.17 μg/mL, respectively. The IC_{50} values of crude plant extracts were also comparable with values of pure natural and synthetic antioxidants.

The DPPH assay has been widely used to determine the free radical scavenging activities of plants and pure compounds because of accurate and repeatable results. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (26). The antiradical scavenging activities of ethanol extracts of *L. interrupta* are related to the nature of phenolic

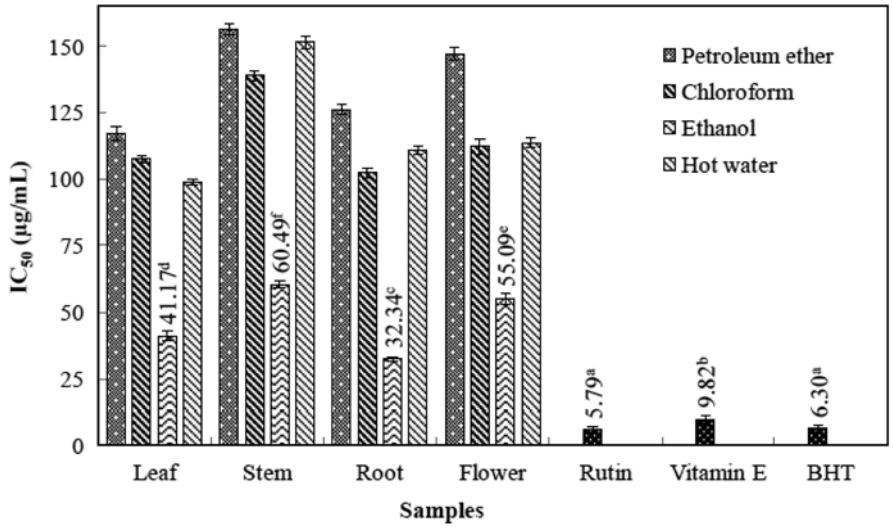

Fig. 1. The DPPH• scavenging activity of *L. interrupta*. Values are expressed as mean (n=3) ±standard deviation (SD). Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}>^{e}>^{f}$.

compounds and contribute to the electron transfer/hydrogen donating ability. Thus, DPPH assay results confirmed that *L. interrupta* contains large amounts of antioxidants that can be isolated and purified.

***ABTS•+ scavenging activity*:** The ABTS radical cation scavenging activities of *L. interrupta* extracts are shown in Table 5. Similar to stable free radical assay results, an ethanol root extract showed greater ABTS•+ dis-colorization with a value of 1,072.57 μM TE/g of extract. Hot water extracts also exhibited higher activities against hydrophilic radical cations. The ABTS•+ scavenging activities of hot water extracts of plant parts ranged from 492.75 to 893.69 μM TE/g of extract.

The ABTS radical is soluble in both aqueous and organic solvents, is not affected by ionic strength, and can be used to measure the antioxidant capacity of both hydrophilic and lipophilic compounds in test samples. This radical is suitable for evaluating the antioxidant capacities of phenolics due to comparatively lower redox potentials (0.68 V) (27). The total antioxidant activities of *L. interrupta* extracts were sufficient for use as a nutraceutical or antioxidant when ingested with nutrients. Moreover, high molecular weight phenolics have a greater ability to quench free radicals. Hence, the presence of phenolics can support the antioxidant activities of *L. interrupta* extracts.

***FRAP assay*:** The antioxidant potential of *L. interrupta* extracts was estimated from an ability to reduce the TPTZ-Fe(III) complex to TPTZ- Fe(II). Ethanol extracts showed significantly better ($p<0.05$) ferric reducing antioxidant activities, compared to other solvent extracts (Table 5). The reducing power ranged from 3,291.53 to 9,456.01 mM Fe(II)/mg of extract for ethanol and hot water extracts of all plant parts. The ethanol root extracts showed the highest ferric reducing power, followed by leaf, flower, and stem extracts. The ferric reducing power of hot water extracts of different plant parts was, in descending order, 4,395.76> 3,962.40>3,753.19>3,291.53 mM Fe(II)/mg of extract for flowers, leaves, roots, and stems, respectively.

One of the essential factors in antiradical activity is the ability to chelate or deactivate transition metals that possess

Table 5. ABTS•+ scavenging activity and Ferric reducing antioxidant power of *L. interrupta*

Assay	Solvent	Leaf	Stem	Root	Flower
ABTS•+ scavenging assay[1)]	Petroleum ether	119.85±16.97	101.25±23.00	176.95±14.17	67.50±13.09
	Chloroform	474.52±20.28	606.82±13.92^{f}	416.47±19.32	407.02±17.29
	Ethanol	973.92±23.73^{b}	722.25±6.51^{d}	1072.57±10.39^{a}	508.95±22.82
	Hot water	647.32±17.84^{e}	893.69±19.27^{c}	723.60±20.40^{d}	492.75±14.37
FRAP assay[2)]	Petroleum ether	682.73±72.93	569.55±97.35	1358.53±71.74	781.31.9±115.05
	Chloroform	708.29±106.38	1595.47±105.25	3585.25±66.02^{f}	1383.72±56.21
	Ethanol	8313.25±121.97^{b}	6863.82±151.90^{c}	9456.01±239.05^{a}	7020.81±199.87^{c}
	Hot water	3962.40±144.02^{e}	3291.53±60.77	3753.19±104.10^{f}	4395.76±72.93^{d}

[1)]μM trolox equivalents (TE)/g of extract; regression equation: y=5.006x–0.005; R^2=0.996
[2)]mM Fe(II)/mg of extract; regression equation: y=0.465x–0.026; R^2=0.993; Values are expressed as mean (n=3)±standard deviation (SD); Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}>^{e}>^{f}$ among each assay.

Table 6. Pyrexia reduction by ethanol extracts of different parts of *L. interrupta*[1)]

Treatment	Dose (mg/kg)	Rectal temperature (°C)						
		−18 h	0 h	1 h	2 h	3 h	4 h	5h
Control	--	37.21±0.14	38.29±0.15	38.33±0.10	38.37±0.09	38.40±0.11	38.42±0.08	38.43±0.15
Paracetamol	150	37.23±0.05	38.55±0.09	38.20±0.07 (26.5)	38.13±0.14 (31.8)	37.96±0.15^{a,b,c} (44.7)	37.85±0.09^{a,b,c} (53.0)	37.75±0.10^{a,b,c} (60.6)
Leaf	200	37.67±0.09	38.63±0.13	38.54±0.10 (9.4)	38.48±0.16 (15.6)	38.42±0.07 (21.9)	38.36±0.13 (28.1)	38.28±0.10 (36.5)
	400	37.74±0.12	38.75±0.10	38.60±0.14 (14.9)	38.51±0.11 (23.8)	38.35±0.15 (39.6)	38.28±0.12 (46.5)	38.17±0.14^{a} (57.4)
Stem	200	37.42±0.10	38.49±0.09	38.44±0.08 (4.7)	38.39±0.16 (9.3)	38.32±0.07 (15.9)	38.28±0.10 (19.6)	38.25±0.11 (22.4)
	400	37.25±0.08	38.80±0.13	38.73±0.11 (4.5)	38.65±0.09 (9.7)	38.58±0.08 (14.2)	38.49±0.14 (20.0)	38.4±0.09 (25.8)
Root	200	37.19±0.07	38.36±0.10	38.18±0.13 (15.4)	38.10±0.0^{a} (22.2)	38.02±0.14^{a,b} (29.1)	37.95±0.15^{a,b,c} (35.0)	37.86±0.08^{a,b,c} (42.7)
	400	37.52±0.15	38.55±0.14	38.25±0.12 (29.1)	38.16±0.10 (37.9)	38.05±0.13^{a,b} (48.5)	37.98±0.05^{a,b,c} (55.3)	37.85±0.06^{a,b,c} (68.0)
Flower	200	37.37±0.11	38.52±0.06	38.44±0.09 (7.0)	38.41±0.05 (9.6)	38.34±0.08 (15.7)	38.30±0.11 (19.1)	38.22±0.07 (26.1)
	400	37.61±0.13	38.60±0.11	38.51±0.16 (9.1)	38.47±0.13 (13.1)	38.40±0.09 (20.2)	38.34±0.06 (26.3)	38.21±0.11 (39.4)

[1)]Values are expressed as mean (n=6)±standard error of the mean (SEM); Values within brackets indicate percentage pyrexia reduction; Statistically significant at $^{a}p<0.05$, $^{b}p<0.01$, and $^{c}p<0.001$ when compared to control group.

the ability to catalyze hydroperoxide decomposition and Fenton-type reactions (28). Chelating agents that form σ-bonds with a metal are effective as secondary antioxidants since they can reduce the redox potential of the oxidized form of the metal ion (29). Hence, the phenolic compounds and flavonoids present in *L. interrupta* may inhibit interactions between metals and lipids through formation of insoluble metal complexes with ferrous ions.

In vivo studies

***Acute oral toxicity*:** Leaf, stem, root, and flower ethanol extracts of *L. interrupta* were evaluated for acute oral toxicity using a fixed dose procedure in mice. Extracts did not show any sign of toxicity or mortality in terms of changes in general behavior, even at the highest dose of 2,000 mg/kg. Thus, the acute toxicity study revealed that extracts can be considered as broadly nontoxic. Dosages of 200 and 400 mg/kg are suggested for future *in vivo* studies of all the extracts.

***Antipyretic activity-Brewer's yeast induced pyrexia in rats*:** The effects of *L. interrupta* ethanol extracts against Brewer's yeast induced pyrexia in Wistar albino rats are shown in Table 6. From time 0, it is clear that yeast alters the rat system and causes an interleukin-induced increase in temperature. The standard (paracetamol) and root ethanol extracts significantly ($p<0.01$, $p<0.001$) decreased the degree of pyrexia at different times (3, 4, and 5 h). The ethanol root extract at a dosage 400 mg/kg showed the highest pyrexia reduction rate of 68.0% after 5 h. On the other hand, the ethanol leaf extract also caused a significant decrease in the rectal temperature from 38.75 to 38.17°C at a dosage of 400 mg/kg ($p<0.05$). All the ethanol extracts of plant parts exhibited dose and time dependent antipyretic activities.

The edible nature of leaves is visual clue that leaves are generally less toxic than the whole plant. Plants possessing significant antioxidant activities would also exhibit pharmacological properties that promote anti-inflammatory, analgesic, and antipyretic activities. Ethanol root extracts of *L. interrupta* possessed both antioxidant and antipyretic effects, comparable to the standard antipyretic drug paracetamol. In general, non-steroidal anti-inflammatory drugs produce antipyretic actions through inhibition of prostaglandin synthetase within the hypothalamus. This action takes place over time and is dependent on the dosage (23). Flavonoids are known to target prostaglandins that are involved in pyrexia (30). Hence, the presence of phenolics and flavonoids in ethanol extracts of *L. interrupta* probably cause inhibition of the prostaglandin synthesis in the hypothalamus that contributes to the antipyretic activity. Further studies of purified fractions and compounds are recommended to explore the exact action mechanism behind the fever reducing ability of *L. interrupta* extracts.

Leaves of *L. interrupta* can be used in a nutritious diet as they contain minerals, amino acids, carbohydrates, and proteins. *L. interrupta* possesses strong antioxidant and antipyretic activities due to high total phenolic and flavonoid contents. Ethnobotanical reports of this plant as a potent fever reliever are validated. Research on *L. interrupta* should continue and focus attention on other under-used nutritious medicinal plants in order to help address food and medicine supply concerns in terms of both quality and quantity.

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