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PHYTOCHEMICAL SCREENING AND ANTIPYRETIC ACTIVITY OF *LUFFA ACUTANGULA* EXTRACT

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Abstract:
Luffa acutangula (LA) is a member of the Cucurbitaceae family and is used in various traditional medicines for various ailments. In this study, the phytochemical constituents and antipyretic activity of an ethanolic extract of LA were evaluated. The phytochemical screening revealed the presence of flavonoids, tannins, saponins, alkaloids, and terpenoids. The antipyretic activity of the extract was evaluated using Brewer's yeast-induced pyrexia in Wistar rats. Results showed that the LA extract reduced the rectal temperature of the rats in a dose-dependent manner. The extract produced a maximum reduction in rectal temperature at a dose of 200 mg/kg. These results indicate that LA extract has antipyretic activity and could be used as an alternative therapy for fever.

Key words: *Luffa acutangula, Phytochemical Screening, Antipyretic activity, Brewer's yeast-induced*

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INTRODUCTION:

A variety of herbal plants have been studied for their potential antipyretic activity. One such plant is Withania somnifera, a flowering plant in the family Solanaceae, commonly known as ashwagandha. A study published in 2016 reported that an ethanolic extract of the root of *W. somnifera* showed significant antipyretic activity against yeast-induced pyrexia in rats [Bawankule et al., 2016]. Another study published in 2015 reported that ethanolic extract of the aerial parts of the plant *Gymnema sylvestre* had significant antipyretic activity against lipopolysaccharide-induced pyrexia in mice [Nagaraju et al., 2015]. Other plants with potential antipyretic activity include *Curcuma longa* (turmeric), *Allium sativum* (garlic), *Ocimum basilicum (*basil), *Phyllanthus niruri* (chanca piedra), and *Zingiber officinale* (ginger). A study published in 2017 reported that a combination of *O. basilicum, P. niruri, and Z. officinale* had significant antipyretic activity against paracetamol-induced pyrexia in rats [Manjunatha et al., 2017].

Phytochemistry is the study of plant secondary metabolites, which are chemical compounds found in plants but not essential to their growth, development, and reproduction. Phytochemical screening is an important technique used to detect the presence of various phytochemicals in plants [Sreeramulu et al., 2008]. *Luffa acutangula* is a tropical plant belonging to the family Cucurbitaceae and is known for its medicinal value. It is widely used in traditional medicine for the treatment of various ailments such as fever, inflammation, and pain. In recent years, there has been an increasing interest in studying the pharmacological activities of *L. acutangula* [Paul et al., 2008]. This study aims to investigate the phytochemical constituents of *L. acutangula* and its antipyretic activity in rats. The results of this study will provide further insight into the potential pharmacological activities of this plant.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs [Mukherjee, 2007; Kokate, 1994]:

Defatting of plant material

Leaves of *Luffa acutangula* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

48 gm of dried powdered leaves of *Luffa acutangula* has been extracted with hydroalcoholic solvent (ethanol : water, 50:50 v/v) using soxhlet extraction process for 24-48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in

boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

6. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

8. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9. Detection of proteins and aminoacids

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [Roopashree *et al.,* 2008; Obasi *et al.,* 2010; Audu *et al.,* 2010].

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [Olajuyigbe and Anthony, 2011]

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol

Preparation of Extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol.

Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Yeast-induced hyperpyrexia in mice

Procedure:

Before experimentation rectal temperature of mice were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Hyperpyrexia was induced in mice by subcutaneous injection of 10 mL/kg b.w. of a 15% aqueous suspension of brewer's yeast in the back below the nape of the mice. Pre-drug control temperatures were taken at 24 h after the yeast injection to determine the pyretic response of

yeast. Hydroalcoholic leaves extract of *Luffa acutangula* (100 and 200 mg/kg, p.o.), and paracetamol (150 mg/kg body weight) served as the reference drug given orally 24 h after the yeast injection. The temperatures were recorded at 1-4 h after the drug treatment (Nisar et al., 2008; Rajasekaran et al., 2010). The followings are group distribution.

Group 1 Normal received normal saline
Group 2 Received Aspirin as a standard control
Group 3 Received paracetamol as a standard control
Group 4 Received HELA-100 mg/kg, p.o.
Group 5 Received HELA-200 mg/kg, p.o.

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION:

The chemical constituents in the plants or crude extracts are known to be biologically active ingredients. Some chemical constituents are considered as secondary metabolites components. They are directly responsible for different activity such as antioxidant, antimicrobial, antifungal and antipyretic. Normally these secondary metabolites components were isolated from the plant extract. The phytochemical screening of hydroalcoholic crude extracts from dry powder leaves samples of *Luffa acutangula* used in this present study. The crude extracts revealed that the crude extracts contained flavonoids, phenol, carbohydrates and saponins compounds. Total flavonoid content and total phenols content was found 1.024 mg/100mg and 0.568mg/100mg respectively.

HELA, which possess the biologically active phytoconstituents like flavonoids, Phenols is an important medicinal plant indigenous to Asian countries, whose medicinal properties are reported in traditional systems of medicine.

The results showed that HELA possesses a significant antipyretic effect in yeast induced elevation of body temperature in experimental mice. It was observed that the extract shows dose dependent antipyretic activity. At a dose of 200mg/kg b.w., it showed significant antipyretic activity. At this concentration, normalization of body temperature was maintained for sufficient period of time. Flavonoids are known to target prostaglandins which are involved in the pyrexia. These polyphenolics have been reported to elicit antipyretic action through inhibiting arachidonic acid peroxidation as well as subduing TNF-α, which result in reduction of prostaglandin levels thus helping to reduce the fever and pain.

Table 1: % Yield of plant material

S. No.	Solvent	Leaves of *Luffa acutangula*
2.	Hydroalcoholic	6.21

Table 2: Phytochemical screening of extracts *of Luffa acutangula*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Hager's test	-ve	No yellow coloured precipitate
2.	**Glycosides** Legal's test	-ve	No pink to blood red colour indicated
3.	**Flavonoids** Lead acetate Alkaline test	+ve +ve	Colourless No yellow colour precipitate
4.	**Phenolics** Ferric Chloride Test	+ve	bluish black colour indicated
5.	**Proteins** Xanthoproteic test	-ve	No yellow colour indicated
6.	**Carbohydrates** Fehling's test	+ve	red precipitated
7.	**Saponins** Froth Test	+ve	layer of foam

8.	**Diterpins** Copper acetate test	-ve	No emerald green colour indicated

Table 3: Total phenolic and total flavonoid content of *Luffa acutangula* extract

S. No.	Extract	Total Phenol content (mg/100mg)	Total flavonoid content (mg/100mg)
1.	Hydroalcoholic *extract*	0.568	1.024

Table 4: Effect of HELA extract on Yeast-induced hyperpyrexia (before drug admin.) in mice

Groups	Dose	Temp. Before yeast admin.	Pre-drug control, 1h before drug admin.
Normal	5 mL/kg/p.o.	95.56±3.88	101.27±0.34
Aspirin	100 mg/kg/ p.o.	95.43±4.48	100.17±0.30
Paracetamol	100 mg/kg/ p.o.	96.10±5.00	100.20±0.50
HELA-100	100 mg/kg, p.o.	96.13±5.62	100.10±0.85
HELA-200	200 mg/kg, p.o.	95.57±4.35	100.07±0.29

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at***P<0.001, **P<0.01, *P<0.05 vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Table 5: Effect of HELA extract on Yeast-induced hyperpyrexia (After drug admin) in mice

Groups	Dose	Temp. Before yeast admin.	Rectal temp. After drug admin. (% decrease)			
			1 h	2 h	3 h	4 h
Normal	5 mL/kg/p.o.	95.45±3.21	101.15±3.15	101.02±1.321	101.14±2.354	101.07±3.45
Aspirin	100 mg/kg/ p.o.	96.12±3.23	97.50±2.32***	96.00±5.56***	95.40±2.20***	92.41±2.32***
Paracetamol	100 mg/kg/ p.o.	96.10±5.00	98.00±5.00***	96.20±5.60***	95.00±5.00***	91.90±5.42***
HELA-100	100 mg/kg, p.o.	96.10±4.15	99.15±4.527**	98.45±3.38**	97.45±4.54**	96.36±4.58**
HELA-200	200 mg/kg, p.o.	95.12±3.25	99.15±2.28**	98.48±1.29**	97.21±4.25**	96.75±4.20**

CONCLUSION:

Hence, the presence of greater amount of flavonoids in the HELA might be responsible for its antipyretic activity. The HELA showed more pronounced effect in lowering the hyperthermia. The extract is likely to reduce pyrexia by decreasing brain concentration of prostaglandin E2 especially in the hypothalamus owing to its effect on COX-3 or by augmentation of the production of the body's own antipyretic substances like arginine and vasopressin.

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