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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *LUFFA ACUTANGULA* PEEL EXTRACT

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Abstract

Luffa acutangula is a common plant grown in villages of Tamilnadu. It is generally known as Perkangai in Tamil and Ridge gourd in English. In the present study, *Luffa acutangula* peel was extracted with water and subjected to qualitative phytochemical screening. The preliminary phytochemical screening of aqueous extract of Luffa acutangula peel showed the presence of Flavanoids, Carbohydrates, Amino acids, Proteins, Phenols, Quinones, Terpenoids, Coumarins, Saponins, Tannins, and Leucoanthocyanidins' study was performed on the antioxidant activity of the methanol extract of *Luffa acutangula* (L.) Roxb (ridge gourd) fruit (LAM) and its derived fractions, such as n-hexane (LAH), chloroform (LAC), ethyl acetate (LAE), n-butanol (LAB) and residual aqueous fraction (LAA) and their correlation to the total phenolics and flavonoids contents.

Keywords: *Luffa acutangula*, Peel, Aqueous extract, DPPH assay and Antioxidant activity

Introduction

Luffa acutangula is a cucurbitaceous vine that is commercially grown for its unripe fruits as a vegetable. Mature fruits are used as natural cleaning sponges. Its fruit slightly resembles a cucumber or zucchini with ridges. It ranges from central and eastern Asia to southeastern Asia. It is also grown as a houseplant in places with colder climates. English common names include angled *luffa,* Chinese okra, dish cloth gourd, ridged gourd, sponge gourd, vegetable gourd, strainer vine, ribbed loofah, silky gourd, silk gourd and sinkwa towel sponge [1-4]. The family *Cucurbitaceae* comprises members that were cultivated throughout the world as a source of food, fiber and indigenous medicines. *Luffa acutangula* (L.) Roxb commonly known as Ridge gourd belonging to family *Cucurbitaceae* is an annual herb found in parts of India, especially along the coastal lines. It is dark green in color and has a tapering end to it. The pulp of this vegetable is white in color and consumed after peeling off the skin. *Luffa acutangula* is a medicinal plant, usually referred as a ridge gourd. It is prevalent in subtropical region of Asia. India is considered as a primary center of origin. The plant is widely cultivated in India, Southeast Asia, China, Japan, Egypt, and other parts of Africa. *Luffa acutangula*, commonly known as ridge gourd, angled loofah, or Chinese okra, is a domesticated vegetable of the Cucurbitaceae originating from India [6-9].

Medicinal compounds from plant sources play a key role in prevention and treatment of disease since ancient time. Some of these compounds are toxic to plant predators, but have the beneficial effects in the treatment of human diseases. The purpose of the present study is to analyze the traditional uses, phytochemical activity, antioxidant activity of *Luffa acutangula*

peel extract. The present study aims to analyze the qualitative analysis of phytochemical constituents present in aqueous extract of *Luffa acutangula* peel extract. Various experimental studies were critically assessed to provide justification for traditional and medicinal uses of *Luffa acutangula* [10-15].

Materials and Methods

Phytochemical activity of *Luffa Acutangula* Peel Extract

Phytochemical screening of *Luffa acutangula* peel extracts:

All the chemicals used in the phytochemical analysis by Harbone, (1998), chemicals were procured from Hi media, India, of analytical grade with high purity [16].

Collection and Standardization of Plant sample:

The *Luffa acutangula* was collected from the fields of Tirupattur, Tirupattur district, Tamilnadu, India and used for the study.

Preparation of plant sample:

Luffa acutangula obtained were washed thoroughly with double distilled water to devoid of any impurities and minerals. The skin of cleaned *Luffa acutangula* was peeled off carefully with a skin peeler. The peeled skin was shade dried for dehydration for about a week. The completely dried skin was made into a fine powder using electric mixing grinder. The ground powder was sieved, stored in an air tight container and used whenever it was needed.

Preparation of Peel extract:

Ten grams of *Luffa acutangula* peel powder was weighed and added to 100ml of double distilled water in the conical flask. This was kept in boiling water bath at 100°C for 60 minutes. The conical flask was removed after that period and allowed to cool to room temperature. Then the contents were filtered through Whatman No: 1 filter paper. The filtrate can be used for further studies by storing in refrigerator at 4°C not more than a week.

Antioxidant Activity of *Luffa Acutangula* Peel Extract

DPPH Scavenging Activity

Total free radical scavenging activity of the extracts from *Luffa acutangula* peel sample were estimated according to the previously reported method (Brand-Williams) with slight modification [5].

The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenging activity (\%)} = \frac{A0 - At}{A0} \times 100$$

Where A_0 is the initial absorbance and A_t is the absorbance at 60 min. A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Ascorbic acid).

Ferric reducing antioxidant power

Ferric reducing antioxidant power of the extracts from *Luffa acutangula* peel sample were estimated according to the previously reported method (Nilima S. et al., 2011) [32].

Hydrogen peroxide scavenging activity

The ability of the *Luffa acutangula* sample extract to scavenge hydrogen peroxide (H2O2) was determined according to the method of Ruch et al (1989) [39].

The ability of the extracts to scavenge the H2O2 was calculated using the following equation:

$$\text{H2O2 scavenging activity (\%)} = \frac{A0 - A1}{A0} \times 100$$

where: A_0 = Absorbance of control, A_1 = Absorbance of sample.

Reducing power assay

The reducing power was determined according to the Oyaizu et al. method with some modifications (1986) [34].

Antibacterial activity evaluation of luffa acutangula

The ethanolic extract of *Luffa acutangula* was tested for its antibacterial potential. The antibacterial activity was determined against bacterial species including *Vibrio cholera, Escherichia coli*. The antibacterial activities of the *Luffa acutangula* were determined by the agar well diffusion method [35].

Results and Discussion

Phytochemical Activity of *Luffa Acutangula* Peel Extract

Table -1 Phytochemical screening of *Luffa acutangula* peel aqueous extract

S. No	Phytochemical	Test	Result
1	Flavonoids	Lead Acetate test	+
		Decolorization test	+
		Ammonia test	+
2.	Carbohydrates	Molisch test	+
		Fehling's test	+
		Cellulose test	+
		Iodine test	+
3.	Amino Acids and Proteins	Millon test	+
		Birut Test	+
		Bradford test	+
		Ninhydrin test	+
4.	Phenols	Ferric Chloride test	+
		Phospho Molybdic test	+
5.	Quinones	Chloroform – Ammonia test	-
6.	Terpenoids	Salkowski test	-
7.	Saponins	Froth test	-
8.	Tannin	Braemer's test	+
9.	Leucoanthocyanidines	Leucoanthocyanidine test	-
10.	Steroids	Libermann-Buchard test	+
11.	Volatile oils	Precipitation test	-

12.	Alkaloids	Mayer's test	+
		Picric acid test	+
13	Glycosides	KellarKillani	-

Positive (+),Negative (-).

Antioxidant Activity of *Luffa Acutangula* Peel Extract

DPPH scavenging activity

Antioxidant properties, especially radical scavenging activities, are very important due to sathe deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance. In this study, the antioxidant activities of *Luffa acutangula* and standard antioxidant such as Ascorbic acid were determined using a DPPH method.

DPPH free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical was used in this work. The method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. In the DPPH assay, the anti-oxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH by the reaction. Ascorbic acid was used as reference radical scavengers. The DPPH radical scavenging activity results are shown in Figure -1 and Table -1 as comparable with known antioxidant Ascorbic acid 31%, 44%, 57%, 69% and 82%. From the analysis of Figure-1, we can conclude that the scavenging effects of *Luffa acutangula* leaves extracts on DPPH radicals were excellent 27%, 31%, 43%, 55% and 67%. The free radical scavenging activity of these samples also increased with increasing concentration [37-39].

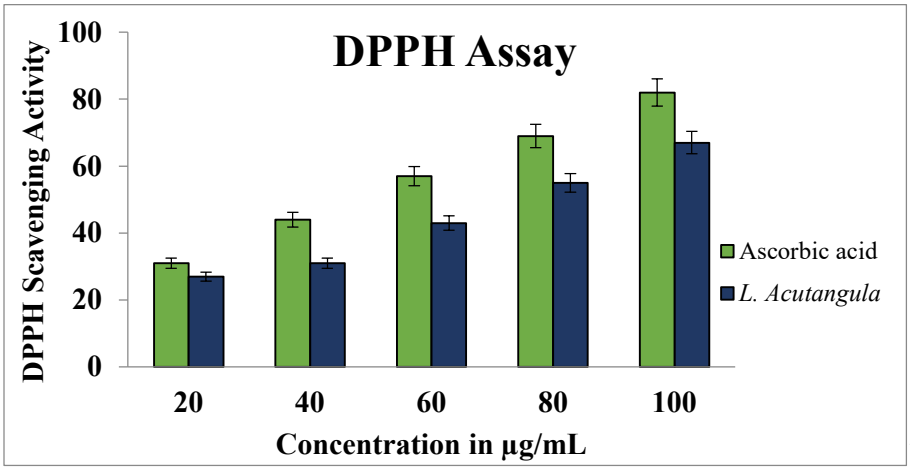


Figure 1: DPPH scavenging activity of *L. acutangula* leaf extract. Ascorbic acid used as standard. The value is representing as the mean ± S.E.M.

Table-2 DPPH scavenging activity of *L. acutangula* leaf extract. The value is representing as the mean ± S.E.M.

Concentration	DPPH scavenging activity (%)	
	Ascorbic acid	*L. acutangula*
20	31	27
40	44	31
60	57	43
80	69	55
100	82	67

Reducing power assay

Reducing power is also widely used in evaluating antioxidant activity of *L. acutangula* extracts. The reducing power is generally associated with the presence of reductants, which exert antioxidant action by breaking the free radical chains by donating a hydrogen atom. In this assay, the presence of reductants in the antioxidant sample reduces Fe^{3+}/ferricyanide complex to the Fe^{2+}/ ferrous form. Thus, the reducing power of the sample can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. In this study, the iron reducing capacity of the ethanolic extracts of *L. acutangula* was estimated from their ability to reduce the Fe^{3+}/ferricyanide complex to the ferrous form by donating an electron. All the extracts showed a good reducing power capacity, which was concentration-dependent (Table-2). The reducing power activity of *L. acutangula* plant extract is found to be 14%, 27%, 35%, 42% and 57%. The reducing power assay for ascorbic acid is found to be 20%, 34%, 55%, 61% and 76%. Here, we assume that the antioxidant activity and reducing power capacity of the extracts was likely due to the presence of polyphenols, which can act as free radicals' scavenger by donating an electron or hydrogen.

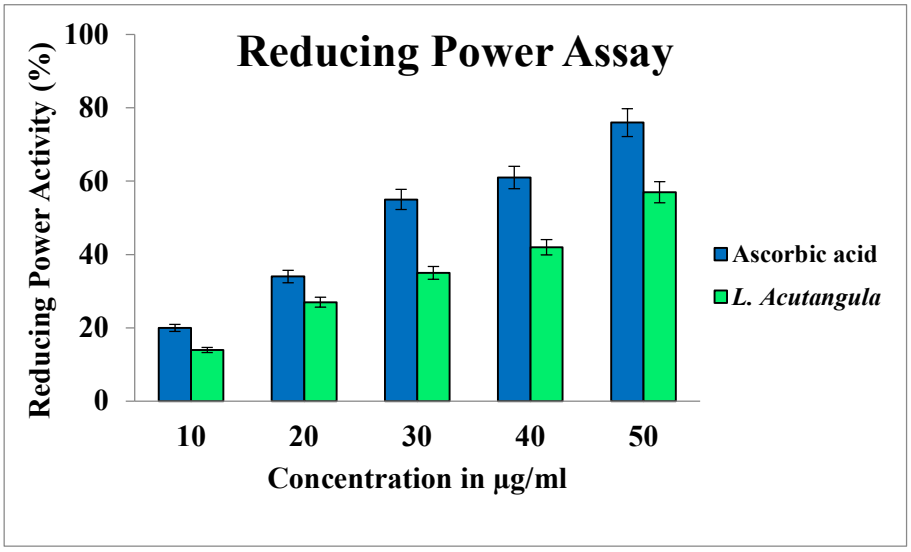


Figure 2: Reducing power assay of *L. acutangula* leaf extract. Ascorbic acid used as standard. The value is representing as the mean ± S.E.M.

Table-3: Reducing power assay of *L. acutangula* leaf extract. The value is representing as the mean ± S.E.M.

Concentration	**Reducing Power Assay (%)**	
	Ascorbic acid	***L. acutangula***
20	20	14
40	34	27
60	55	35
80	61	42
100	76	57

Hydrogen peroxide scavenging activity

Hydrogen peroxide can be formed in vivo by many oxidase enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. The ability of *L. acutangula* to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989) and is shown In Figure-3. compared with that of ascorbic acid as standard. The hydrogen peroxide scavenging activity of standard ascorbic acid is found to be 22%, 40%, 57%, 67%, and 79% had strong activity than *L. acutangula* Plant extract. The hydrogen peroxide scavenging activity of *L. acutangula* plant extract is found to be 14%, 31%, 53%, 64%, and 68%. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion-dependent OH radical mediated oxidative DNA damage. Levels of hydrogen peroxide at or below about 20–100 µg/ml seem to have limited cytotoxicity to many cell types. Thus, removing hydrogen peroxide is very important for protection of food systems [40-42].

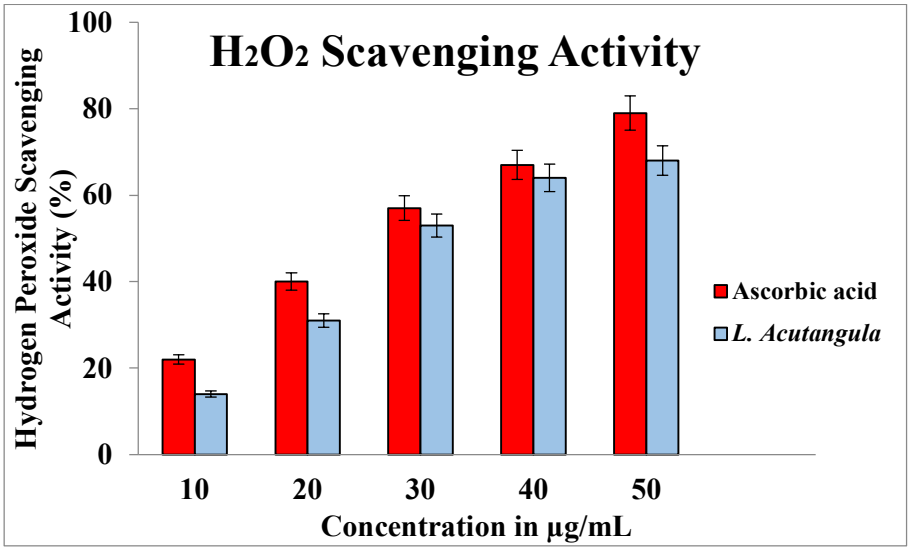


Figure.3 Hydrogen peroxide scavenging activity of *L. acutangula* leaf extract. Ascorbic acid used as standard. The value is representing as the mean ± S.E.

Table 4: Hydrogen peroxide scavenging activity of *L. acutangula* leaf extract. The value is representing as the mean ± S.E.M

Concentration	H_2O_2 scavenging activity (%)	
	Ascorbic acid	***L. acutangula***
10	22	14
20	40	31
30	57	53
40	67	64
50	79	68

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Ferric Reducing Antioxidant Power (FRAP) assay

The antioxidant activity measured by FRAP method was observed in *L. acutangula* plant extracts. (Figure-4) In general, values of antioxidant activity of extracts of fresh samples were higher with respect to the dried samples. It was observed that the antioxidant activity measured by FRAP was positively correlated to the concentration of the polyphenols. The FRAP value was obtained by plotting the graph of standard curve of FeSO4 at concentrations between 20 and 100 µM. The standard curve was found linear within this concentrations range as shown inFigure-4. The antioxidant capacity in FRAP value is presented in Figure-4 In this evaluation, the antioxidant activity is evaluated based on the capacity to reduce ferric (III) iron to ferrous (II) ion. The FRAP activity of ascorbic acid is found to be 35%, 46%, 58%, 70%, 89%. The FRAP activity of *L. acutangula* plant extract is found to be 30%, 34%, 45%, 61%, and 73%. The results indicated as µM ferrous ion equivalent/plant material. The results demonstrate that the *L. acutangula* leaf extract possess similar antioxidant capacity when comparable to standard ascorbic acid [43-46].

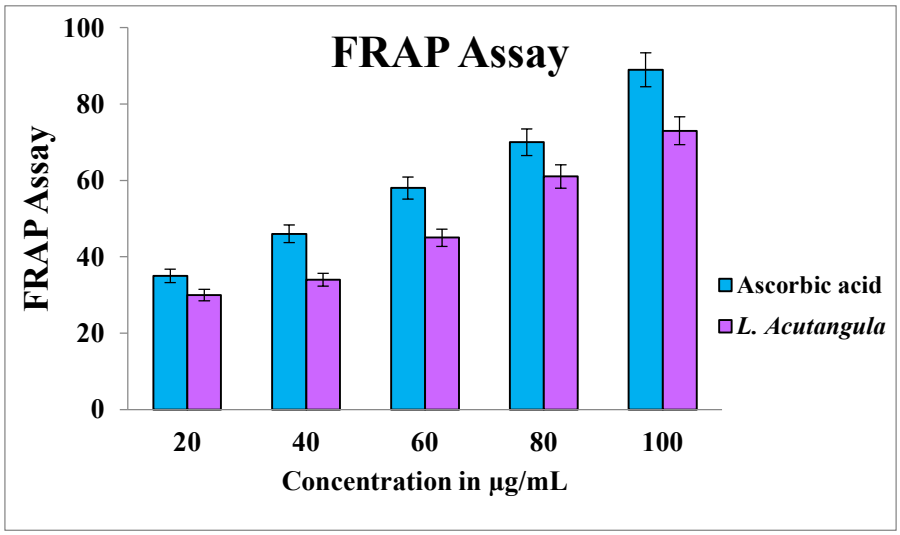


Figure-4. Ferric Reducing Antioxidant Power (FRAP) Assay (%) of *L. acutangula* leaf extract. Ascorbic acid used as standard. The value is representing as the mean ± S.E.M.

Table 5 Ferric Reducing Antioxidant Power (FRAP) Assay (%) of *L. acutangula* leaf extract. The value is representing as the mean ± S.E.M.

Concentration	**FRAP Assay (%)**	
	Ascorbic acid	***L. acutangula***
20	35	30
40	46	34
60	58	45
80	70	61
100	89	73

Conclusion

The study was conducted to evaluate the antimicrobial and phytochemical activities of the peel extracts of Luffa acutangula. The antimicrobial activity of aqueous, petroleum ether, acetone and chloroform peel extracts were tested against the selected bacterial and fungal isolates using agar well diffusion method. The results proved that the acetone extract exhibited maximum antimicrobial activity against the tested bacterial and fungal isolates when compared to chloramphenicol and fluconazole (standard antibiotics) respectively. Preliminary phytochemical screening was carried out in the acetone extract of L. acutangula which revealed the presence of secondary metabolites such as saponins, phenols, terpenoids, tannins, flavonoids, sterols, anthocyanin, carbohydrates and amino

acids. The FT-IR analysis revealed the presence of hydroxyl, amines and carboxylic acids as functional groups in L. acutangula

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