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QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES OF *LUFFA ACUTANGULA* ROOT

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ABSTRACT

The *Luffa acutangula* Linn is medicinal climber found in western, central and southern India, the fruits used in vata, kaphaanemia, asthma, leucoderma, tumors, also useful as diuretic and in splenic enlargement. Scientifically it is proved as CNS depressant. The bioactive compounds that are produced by plants are collectively called as Phytochemicals. The phytochemical ingredients are plant derived compounds which protect the plants from environmental stresses, including insects, bacteria and fungus and weather changes. Though phytochemicals are not considered essential nutrients, it has become apparent that they offer many health benefits to the plants. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. There are more than thousand known phytochemicals and they offer protection to many chronic diseases such as diabetes, cancer, heart disease and alzheimer's. The aim of the present study is to examine *L. Acutangula*roots for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics, saponins, tannins and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic, flavonoids and saponins was carried out by FolinsCiocalteau reagent method, aluminium chloride method and vanillin-sulphuric acid colorimetric reaction methods respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants will be very useful in the curing of various diseases when taken along with our food.

K**eywords:** *Luffa acutangula*, Phytochemical screening, Flavonoids, Terpenoids, Tannin.

INTRODUCTION:

India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world [1]. Since ancient times plants have been traditionally used in therapeutic practices for the treatment of different types of ailments [2-5]. There are a number of crude drugs where the plant source has not yet been scientifically identified. A phytochemical is a natural bioactive compound found in plants foods that works with nutrients and dietary fibre to protect against diseases. Many researchers suggest that, phytochemical working together with nutrients found in fruits, vegetables and nuts. They can have complementary and overlapping mechanism of action in the body including antioxidant effect. *Luffa acutangula* (L.) Roxb (ridge gourd) belongs to Cucurbitaceae family. It is widely growing vegetative climber and found throughout India. The fruits usually are taken as vegetables. The plant has

been reported to have various medicinal properties such as treatment of jaundice, splenic enlargement and laxative. It is also proved as CNS depressant used traditionally in insect bites [6.] The plant also possesses potent α-glucosidase inhibitory effect [7]. The present study was designed to investigate the presence of various phytochemicals constituents in *L. acutangula* roots. Extensive effort have now been channelled towards screening of plants for more active and effective new drugs to eliminate diseases which have strains of pathogenic organism that resist the effect of drug in use today[8]. Based on the many ethnomedicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition.

MATERIALS AND METHODS

Plant materials

The root of plant of *Luffa acutangula* was collected from local area of Bhopal (M.P.).

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction Procedure

The powdered plant samples (50g/250 ml) were extracted successively with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus at 55-85°C for 8-10 h in order to extract the polar and non-polar compounds. For each solvent extraction, the powdered pack material was air dried and then used. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [9.]

Qualitative Phytochemical Analysis of Plant Extract

The*L. acutangula*roots extract obtained was subjected to the preliminary phytochemical analysis following standard methods byKhandelwal and Kokate [10, 11]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC, TFC tannins and saponins are determined. Extracts obtained from roots

of *L. acutangula* plant material of subjected to estimate the presence of TPC tannins, saponins and TFC by standard procedure

Total phenolics content

The total phenolics content of *L. acutangula* was estimated using Folin-Ciocalteau reagent by the method of Olufunmiso et al [12]. About 20 µg of root extracts were taken separately and it was made up to 1 ml with distilled water. Then 500 µl of diluted Folins-phenol reagent (1:1 ratio with water) and 2.5 ml of sodium carbonate Na_2CO_3 (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of colour. After incubation, the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/ml. The total phenolics content in the plant extracts were expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

Total flavonoids content

The total flavonoids content was estimated using the procedure described by Olufunmiso et al [12]. A total of 1 ml of plant extracts were diluted with 200 µl of distilled water separately followed by the addition of 150 µl of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150 µl of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 ml of sodium hydroxide (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

Determination of alkaloids

A total of 200 ml of 20% acetic acid was added to 5 g of root powders taken in a separate 250 ml beaker and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The percentage of total alkaloid content was calculated as:

$$\text{Percentage of total alkaloids (\%)} = \text{Weight of residue} \times 100 / \text{Weight of sample taken}$$

Estimation of tannins content

Tannins content of *L. acutangula* was estimated by the method of Siddhuraju and Manian [13]. A total of 500 µl of the extracts were taken in test tube separately and treated with 100 mg of polyvinyl

polypyrrolidone and 500 µl of distilled water. This solution was incubated at 4 °C for 4 h. Then the sample was centrifuged at 5000 rpm/min for 5 min and 20 µl of the supernatant was taken. This supernatant has only simple phenolics free of tannins (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannins content of the extract was calculated as follows:

Tannins (mg GAE/g extract)=Total phenolics (mg GAE/g extract)-Free phenolics (mg GAE/g extract)

Estimation of total tannins content

Estimation of total saponins content was determined by the method described by Makkar et al. based on vanillin-sulphuric acid colorimetric reaction with some modifications [14]. About 50 µl of plant extract was added with 250 µl of distilled water. To this, about 250 µl of vanillin reagent (800 mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

Results and Discussions

The crude extracts so obtained after the soxhlet process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using petroleum ether, chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

Table 1 Result of percentage yield of extracts

S. No.	Solvents	% Yield
1	Petroleum ether	2.8
2	Chloroform	2.8
3	Ethyl acetate	1.6
4	Methanol	5.6
5	Water	3.58

Preliminary phytochemical screening of *L. acutangula* root extracts revealed the presence of various components such as phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids,

fats or fixed oils, protein and amino acid and tannins among which phenols and flavones were the most prominent ones and the results are summarized in Table 2.

Table 2 Result of phytochemical screening of roots extracts of *L. acutangula*

Plant constituents	Petroleum ether	Chloroform	Ethyl acetat	Methanol	Water
Alkaloids	-	+++	-	++	-
Cardiac glycosides	-	+++	+++	+++	-
Flavonoids	-	-	+++	++	+++
Glycosides	-	-	+	+++	-
Phenols	-	-	-	+++	-
Resins	-	+	-	+	-
Saponins	-	-	+++	+	-
Steroids	+++	++	+++	+++	++
Tannins	-	-	++	++	-
Terpenoids	++	++	+++	+++	++
Triterpenoids	++	+++	+	+	-

+++: highly present, ++: moderately present, +: Low, -: absent.

Total phenolics content of various extracts of root parts of *L. acutangula*was varying widely between 0.44 to 5.04 mg GAE/100mg extract (Table 3). Methanolic extract of root parts were demonstrating higher total phenolics content 5.04 mg GAE/100mg than that of the other solvent extracts. The total flavonoids content was high in ethyl acetate and chloroform root extracts (14.31 and 14.28 mg RE/100 g extract respectively). Among the solvents used the methanolic root extracts were found high amount of tannins, 1.61 mg GAE/100mg. The ethyl acetate extract of root parts depicted high content of saponins 16.69 mg DE/100mg.

Table 3 Estimation of total phenolic, Tannins, saponins and flavonoids content of *L. acutangula*

Sample	Total phenolics	Total flavonoids	Tannins	Saponins
	R	R	R	R
PE	0.46 ± 0.01^{a}	-	0.06 ± 0.02^{b}	13.02 ± 0.06^{b}
CH	0.71 ± 0.02^{a}	14.31 ± 0.03^{a}	0.29 ± 0.03^{b}	12.86 ± 0.02^{a}
EA	0.44 ± 0.05^{bc}	14.28 ± 0.06^{b}	0.18 ± 0.01^{a}	16.69 ± 0.02^{c}
ME	5.04 ± 0.03^{b}	6.36 ± 0.07^{bc}	1.61 ± 0.09^{c}	16.25 ± 0.02^{c}
WA	0.70 ± 0.01^{a}	03.61 ± 0.05^{b}	0.18 ± 0.03^{b}	13.40 ± 0.03^{b}

Values were performed in triplicates and represented as mean±SD. PE: petroleum ether, CH: chloroform, EA: ethyl acetate, ME: methanol, WA: water, R: root, -: not detected. [1]: mg GAE/100 g

extract, [2]: mg RE/100 g extract, [3]: mg AE/100 g extract, [4]: mg DE/100 g extract. Mean values followed by different superscript in a column are significantly different (P<0.05).

CONCLUSION

L. acutangula roots have potential to act as a functional food and a source of useful drugs because of the presence of various phytochemical components. Methanolic extracts shows good results regarding presence of phytoconstituents hence these plants may directly use in medicine preparation or for the development of novel agents for various pathological disorders. Further research on the health benefits of phytochemicals in this plant may be warranted.

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