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EXTRACTION, PHYTOCHEMICAL AND GC-MS ANALYSIS OF *Luffa acutangula* L (ROXB)

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ABSTRACT

Luffa acutangula L (Common name: Ridge gourd, Family: Cucurbitaceae) is a popular vegetable in India and other Asian countries. Various phytochemicals reported in L. acutangula. Chemical constituents of L. acutangula mainly include carbohydrates, carotenoids, fat, protein, phytin, amino acids (alanine, arginine, cystine, glutamic acid, glycine, hydroxyproline, leucine, serine,tryptophan), pipecolic acid, flavonoids and saponins. In order to study the compositions of the extracted constituents were analyzed by GC-MS method. The chemical compositions of leaf extracts were investigated using Perkin – Elmer GC-MS while the mass spectra of the compounds found in the leaf extracts was matched with the National Institute of Standards and Technology library. Results revealed the presence of biologically active compounds.

Keywords: Luffa acutangula, Cucurbitaceae, GC-MS, Alanine.

INTRODUCTION

Luffa acutangula L (Common name: Ridge gourd, Family: Cucurbitaceae) is a popular vegetable in India and other Asian countries [1]. It is a healthy food and contains good amount of fiber, vitamins and minerals including Vitamin B2, Vitamin C, carotene, niacin, calcium, phosphorus, iron and small quantities of iodine and fluorine. It is reported to contain many phytochemicals such as flavonoids, saponins, luffangulin, sapogenin, oleanolic acid and Cucurbitacin B. L. acutangula has been used extensively in Indian traditional system of medicines as diuretic, expectorant, laxative, purgative, hypoglycemic agent and bitter tonic. The ethnobotanical survey revealed its use to protect jaundice, insect bites, swollen hemorrhoids, dysentery and headache [2]. Various biological activities of this plant were reported including its use in weight loss, jaundice, blood purification, hypoglycemia, and constipation, skin care,immune system booster, wound healing, eye problems, stomach worms and asthma.

Various phytochemicals reported in L. acutangula. Chemical constituents of L. acutangula mainly include carbohydrates, carotenoids, fat, protein, phytin, amino acids (alanine, arginine, cystine, glutamic acid, glycine, hydroxyproline, leucine, serine,tryptophan), pipecolic acid, flavonoids and saponins. Luffangulin, a novel N-terminal ribosome inactivating peptide was isolated from the seeds of L. acutangula. Presence of sapogenin, oleanolic acid and a bitter principle, Cucurbitacin B were also identified from the seeds of L. acutangula.

MATERIALS AND METHODS

Preparation of *Luffa acutangula* extracts

Successive Solvent Extraction

About 500 g of dried powder was extracted with solvents of different polarity in succession, starting with a highly non-polar solvent [Petroleum Ether (40-60°C)], followed by comparatively less non-polar solvents (Diethyl Ether), then with intermediate polar solvents (Chloroform) and finally with a more polar solvent (Ethanol and Water).

Aqueous extract was prepared by macerating the dried drug powder in double distilled water. The extract was concentrated in water bath and stored in desiccators [3-5].

Phytochemical Studies

For detection of the existence carbohydrates, proteins, amino acids, glycosides, steroids, flavonoids, vitamins and alkaloids were qualitatively analyzed.

- Alkaloid Dragendroff's reagent: Mix 2mL of reagent with 2mL filtrate of plant drug extract – Reddish brown precipitate

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- Flavonids Shinoda test: Add magnesium powder and a few drops of con. H_2SO_4 to 2mL of sample solution - orange pink, red or , purple colour
- Tannins : Ferric chloride test: Mix 2mL of test solution with 5% ferric chloride solution - Blue, blue-black or blue green colour
- Saponins: Foam test: Shake aqueous solution of sample which produce foam stable for 15 second 5.Phenol: Shake 1 mol of sample with 20% sodium carbonate solution and folios ciocalteau - Greenish blue colour
- Glycosides: Add 2mL picric acid to the test solution - Yellow precipitate formed
- Carbohydrates: Mix 2Ml of Benedict'' reagent with 2mL of test solution. Boil in water bath – Formation of red, yellow, or green color depending on concentration.

Determination of total phenolic content in *Luffa acutangula*

Standard preparation

Take 10 mg Gallic Acid 10mL standard flask. Make up to volume with dist.water to get 1000ppm. 1,2,3,4 ppm were prepared by serial dilution. From this 1.0mL standard+4mL sodium carbonate solution +1mL phenol reagent and make up to 25 mL with distilled water. Sonicate for 10 minute. Kept in dark for 30 minute

Sample preparation

Take 0.5 g sample and add 25mL water sonicate for 20 minute. From this 1mL sample+4mL sodium carbonate solution +1mL phenol reagent and make up to 25 mL. sonicate for 10 minute kept in dark for 30 minute. Read the absorbance of both standard and sample at 760 nm [6].

Determination of total flavanoid content in *Luffa acutangula*

Procedure Standard preparation Take 10 mg Quercetin standard in 10mL standard flask. Make up to volume with methanol to get 1000ppm. 1,2,3,4 ppm were prepared by serial dilution. From this 0.5mL standard+1.5mL ethanol+0.1ml 10% Aluminium chloride+0.1mL sodium acetate +2.8mL dist.water. Sample preparation Take 0.5 g sample and add 25mL methanol sonicate for 20 minute. From this 0.5mL sample+1.5mL ethanol+0.1ml 10% Aluminium chloride+0.1mL sodium acetate +2.8mL dist.water. Incubate both standard and sample 30 minute at room temperature. Read the absorbance of both standard and sample at 415 nm[8].

$$\text{Flavanoid \%} = \frac{\text{Observed concentration x Purity of standard}}{\text{Sample concentration}}$$

GC-MS Analysis

GC-MS was analyzed at the CARe Keralam, Koratty. For the identification of phytoconstituents which was present in the extract was done by GC- MS method [9-10]. These criteria were selected to achieve improved signal to noise ratio, better sensitivity and mass spectral integrity. The operation was carried out in electron impact (EI) mode.

Instrument Model- 7890 A GC with 5975C with triple axis detector

Column -DB 5MS 30 m x 0.250mm Diameter x 0.25 Micro Meter Thickness

Sample Preparation

Weigh 5gm sample and keep overnight for maceration with 20ml methanol in a stoppered flask for 24 hrs. Filter and take 100 microliter of the filtrate and add 900 microliter methanol and injected to GCMS.

Injection Volume	3 µL
INJECTOR TEMP	280 °C
Pressure	7.0699 psi
Flow	1 mL/min
Carrier Gas	Helium
Injection Mode	Split
Library	NIST 08 Spectral Data
Ionization Temperature	80eV
Sample storage	4°C

Interpretation of the mass spectrum of the ethanolic extracts was conducted using the database of the National Institute of Standard and Technology (NIST) library, having more than 62,000 spectral patterns. The spectra of the compounds were compared with the spectra of the National Institute of Standard and Technology (NIST) library database

RESULTS AND DISCUSSION

PHYTOCHEMICAL STUDIES

Extraction

On successive exhaustive solvent extraction of dried powder of corms of *Luffa acutangula* with solvents viz., Petroleum ether, Chloroform, Ethanol (95%) and Water, the percentage of yield was found to be very less in nonpolar solvents Petroleum ether and Chloroform (0.51%, 0.36% respectively), while Aqueous extract showed relatively high yield (6.56%) (Table 1).

Phytochemical analysis of various extract

The phytochemical investigation revealed the presence of various constituents as mentioned in Table 2. Based on the results ethanolic and aqueous extracts were selected for studying pharmacological activities.

Estimation of total Phenolic and flavonoid

Phenolic and flavonoid content is the essential groups of medicinally active constituents of the plant having different effects like antioxidants, antimicrobial, anticancer, anti-inflammatory and hepatoprotective activity. The phenolic and flavonoids contents of ethanolic and aqueous extracts of *Luffa acutangula* were measured using standard methods and are reported in the following table 4.

GC-MS Analysis of ethanolic leaf extract of *Luffa acutangula*

The active principles with their retention time (RT) molecular formula, molecular weight (MW) and molecular structure in the ethanol leaf extract of *Luffa acutangula* revealed 11 compounds namely (i) Gallic acid (ii) Ribitol, 1,3:2,4-di-O-benzylidene: (iii) Ethyl ethoxy(3-methoxy-4-[(trimethylsilyl)oxy]phenyl)acetate (iv) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol: (v) 16-Octadecenoic acid, methyl ester: (vi) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (vii) Octadecanoic acid, methyl ester (viii) Methyl 20-methyl-heneicosanoate: (ix) Tetracosanoic acid, methyl ester (x) β-Sitosterol acetate: (xi) Stigmastan-3,5-diene is presented in Table 4 and chromatogram is showed in Figure 1.

Table 1: Percentage Yield and Physicochemical Characters of Various Extracts of *Luffa acutangula*

S.No	Extracts	Colour and consistence	Odour	yield (%w/w)
1	Petroleum Ether	Light yellowish mass	Characteristic	0.51
2	Chlorform	Light yellowish mass	Characteristic	0.36
3	Ethanol	Brownish semisolid	Pleasant	3.12
4	Water	Dark brown sticky mass	Pleasant	6.56

Table 2. Phytochemical Screening of Different Extract of *Luffa acutangula*

S.No	Phytoconstituients	Petroleum Ether	Chlorform	Ethanol	Aqueous
1	Alkaloids	-	-	+	+
2	Flavonoids	-	-	+	-
3	Saponin	-	-	-	-
4	Tanin& Phenolic compounds	+	+	+	+
5	Glycosides	-	-	+	-
6	Carbohydrates	-	-	+	+

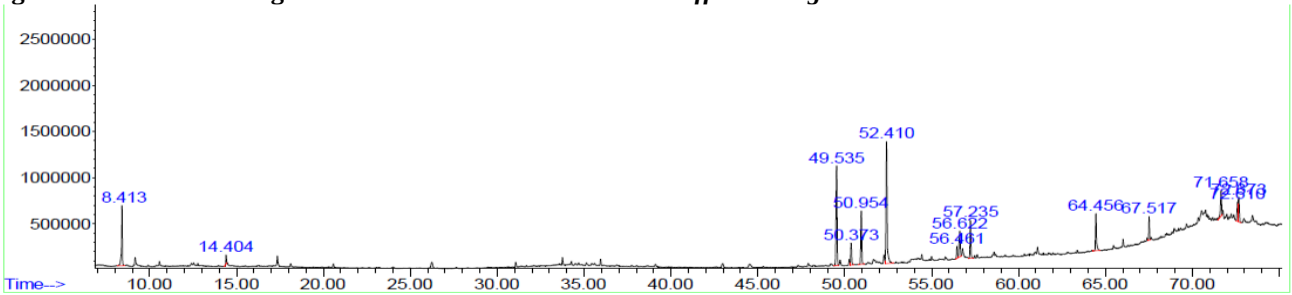
Table 3. TPC & TFC of *Luffa Acutangula* in Different Extracts

S.No	Extract	Total Phenolic Content	Total Flavonoid Content
1	Ethanolic Extract	1.05%	0.004%
2	Aqueous Extract	0.73%	0.002%

Table 4. Phytochemicals Identified in the Ethanolic Leaf Extract of *Luffa acutangula*

S.No	Retention Time	Name of the compound	Molecular Formula	Peak area (%)	Molecular Weight
01	8.409	Gallic acid	$C_4H_6O_5$	0.765	171
02	8.413	Ribitol, 1,3:2,4-di-O-benzylidene	$C_{19}H_{20}O_5$	8.646	328
03	14.404	Ethyl ethoxy(3-methoxy-4-[(trimethylsilyl)oxy]phenyl)acetate	$C_{16}H_{26}O_5Si$	0.665	326
04	49.535	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	18.292	296
05	52.410	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	23.669	270
06	56.622	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	5.522	294
07	57.235	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	5.495	298
08	64.456	Methyl 20-methyl-heneicosanoate	$C_{23}H_{46}O_2$	6.152	354
09	67.517	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	3.174	382
10	71.658	β-Sitosterol acetate	$C_{31}H_{52}O_2$	5.035	456
11	72.610	Stigmastan-3,5-diene	$C_{29}H_{48}$	2.992	396

Fig 1. GC-MS Chromatogram of methanolic leaf extract of *Luffa acutangula*



CONCLUSION

The identification of plant material, taxonomically and pharmacognostically is important to provide standards and to avoid adulteration of drugs. The detailed botanical and pharmacognostical studies help in evolving specific characters to fulfill this objective. Phytochemical analysis helps in formulating pharmacopoeial standards of the plant. The drug powder was extracted successively with solvents of different polarity in succession, starting with a highly non-polar solvent (Petroleum Ether), followed by comparatively less polar solvents (Chloroform) and finally with a more polar solvent (Ethanol and Water) in Soxhlet extractor. Preliminary phytochemical screening of various extracts had shown the presence of carbohydrates, proteins, steroids, flavonoids, terpenoids, tannins & phenolic compounds and fixed oils. The total phenolic content in ethanol extract was determined and was found to be 1.05%. Quantitative analysis was carried out to determine the potential bioactive components of leaf extracts of Luffa acutangula using Gas Chromatography – Mass Spectrometry analysis (GC-MS). The chemical compositions of leaf extracts were investigated using Perkin – Elmer GC-MS while the mass spectra of the compounds found in the leaf extracts was matched with the National Institute of Standards and Technology library. Results revealed the presence of biologically active compounds.

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