Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2041-2046
Received: 07-03-2019
Accepted: 09-04-2019

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Extraction isolation and analytical characterization of phytoconstituent of fruit of *Luffa echinata* Roxb

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Abstract
The herbal system of medicine has been practiced since historical times and traces its roots to ancient civilizations. Almost 70% of the populations of the third world countries including India, China, Bangladesh and Pakistan are dependent upon their indigenous systems of medicines, based mainly on herbage formulations. Due to the prime importance of plants as a source of medicine, work began to focus on the isolation of chemical constituents responsible for their activity. The present study deals with the extraction, identification and analytical characterization of phytoconstituents of fruit of *Luffa echinata* Roxb from sponified matters of petroleum ether extract. The structure methyl esters of fatty acids were isolated and characterized by GC-MS analysis. The fruit of *Luffa echinata* Roxb contains important fatty acid.

Keywords: *Luffa echinata* Roxb, methyl ester, unsponified matter, sponified matter, fatty acid

Introduction
The herbal system of medicine has been practiced since historical times and traces its roots to ancient civilizations. Although, we define alternative systems of healing as subject that are not taught in medical schools, it is worthwhile to mention that before the availability of synthetic drugs, plant-based remedies formed the basis of primary healthcare system. Herbal infusion, decoction and tincture were house-hold remedies for common ailments [1]. Due to the prime importance of plants as a source of medicine, work began to focus on the isolation of chemical constituents responsible for their activity. Most of the phytochemical constituents are potent bioactive compounds found in medicinal plant parts, which are precursors for the synthesis of useful drugs [2]. The Cucurbitaceae or cucurbit family is the gourd family of flowering plants, belonging to the order Cucurbitales and containing 118 genera and 845 species of food, ornamental and medium-sized plant family, primarily found in the warmer regions of the world [3]. *Luffa echinata (L. echinata)* has a place with family Cucurbitaceae. The genus Luffa comprises more than eight species, three of which are found in India viz. *Luffa acutangula* Roxb., *Luffa aegyptiaca* Mill. and *Luffa echinata* Roxb [4]. Preliminary review of literature suggested the plant *Luffa echinata* Roxb. (Cucurbitaceae) have hepetoprotective activity and promising other pharmacological action. As limited work is reported on this plant. It was decided to carry out research work on this plant after exhaustive literature review.

Material and methods
Procurement and authentication of plant material
Whole plants of *Luffa echinata* Roxb. Was authenticated by Dr. H. B Singh, Raw Materials Herbarium and Museum (RHMD) of NISCAIR, New Delhi.

Collection and processing of plant material
Luffa echinata Roxb collected from local area of Bamorkalan Dist Shivpuri M. P. The collected plant material was naturally dried under shade and subjected to size reduction using hand grinder. The powder so obtained was passed through sieve and then used for further extraction process.

Preparation of extract by successive solvent extraction method
Luffa echinata Roxb fruit was extracted by soxhlet extraction method using petroleum ether. Then store in containers for further use.

Sepration of saponifiable and unsaponifiable fraction from petroleum ether extract of fruit of *Luffa echinata* Roxb

The pet-ether extract (10g) was taken in flask and 40 ml of 20% Methanolic KOH was added and the mixture was refluxed for 6 hrs and then cooled at room temperature. Then twice of its volume of distilled water was added and extracted with ether. The combined ethereal extract was washed with distilled water till neutral to litmus paper and dried. After this the ether was evaporated to obtain unsaponifiable fraction (fraction A).The aqueous portion left after ether extraction was named as saponifiable fraction. It was acidified with 5N H2SO4 and the aqueous layer was extracted 3-4 times with ether and ethereal layer was washed with distilled water evapourated and dried over anhydrous Na2SO4, obtain fatty acid portion (fraction-B). This portion used for preparation of methyl esters of fatty acids [5].

Preparation of methyl esters of fatty acid fraction B of fruit and aerial part *of Luffa echinata* Roxb

An accurately weighed portion of (fraction B) fatty acid was taken into flask and refluxed on water bath for 3 hrs with solution of 5% methanolic Conc.H2SO4. Mixture was cool and diluted with (40ml) water and extracted with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness, then subjected to GC/MS analysis for fatty acid methyl esters.

Characterization of methyl ester by GC-MS chromatography [6]

The technique of gas chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time. For this purpose, the fatty acid components of lipids are converted to the simplest convenient volatile derivative, usually methyl esters, although other esters may be preferred for specific purposes.

Result and discussions

The present research work aimed to Extraction Isolation and analytical characterization of phytoconstituent of fruit of *Luffa echinata* Roxb. The plant was selected carefully on the basis of extensive literature review on reported pharmacognostical and phytochemical profile. The plant material was collected and authenticated.. The active constituents was also estimated qualitatively as well as quantitatively. The amount of extractive, a drug yield to a specific solvent is often an approximate measure of the amount of a certain constituent present in the sample. Extractive values indicate the nature and quantity of the constituents present in a crude drug. petroleum ether- soluble extractive values for plant was determined 4.21% in fruit of *Luffa echinata* Roxb.(Table No.-1). The separation of saponified and unsaponified fractions from petroleum ether extract of fruit of *Luffa echinata* Roxb. Was done and % yield w/w of the unsponified and saponified fraction was found 8.7,72.9 respectively results shown in Table No.2.The sponified fraction of the extracts of fruit of *Luffa echinata* Roxb. Was subjected to esterification to get fatty acids of methyl esters and the identification of fatty acids by GC/MS analysis gave the total 8 and 10 retention peaks of different compounds results shown in Table No.3. The 5 mass spectra of those compounds whose abundance appeared more than 90% was identified by comparison with library search and literature review successfully identify as Methyl 4,8,12-tridecanoate (I - *), Benzoacetic acid, α-oxo-methyl ester (I I-**), Methyl docosanoate, (III - *****), Methyl-13-docosenoate, (IV- ***), Methyl tricosanoate (V- ****) in fruit of the plant. (Figure no-1-6). The results obtained in the present study indicate that the selected plant may possess high therapeutic value. They can be exploited for discovery or development of new therapeutic agents. We also conclude that these finding will contribute to the new source of economically important material with high pharmacological activity of phytoconstituents present and evaluated in this plant selected for present study.

Acknowledgment

The authors are thankful to Dr. H. B Singh, Raw Materials Herbarium and Museum (RHMD) of NISCAIR, New Delhi India and Director, Sophisticated Instrumentation Centre for Applied Research and Testing – SICART, Sponsored by Department of Science and Technology, Govt. of India, New Delhi) Gujrat for GC-MS, providing us facilities and also for useful discussions and collaboration.

Table 1: Successive solvent extractive value of plant under investigation

Pl Plant	Solvent Used	Color and Consistency	Average Extractive Values in %w/w on Dry Weight Basis
Luffa echinata fruit	Petroleum ether	dark green oily mass	4.21%

Table 2: Sponified and unsaponified fraction value of *Luffa echinata* Roxb fruit

S. No	Plant part	Fraction	%Yield w/w
1	Fruit	Unsaponified fraction(A)	8.7
		Saponified fraction(B)	72.9

Table No 3: Area percent report of chromatogram of fatty acids methyl esters of petroleum ether extract of fruit of *Luffa echinata* Roxb

S. No	Name	RT	Area	Height	BL	Cone	Areal Cone	m/z	Area %
1		7.227	147.6	951	bb	0	0	TIC	2.43
2		7.392	95.4	735	bb	0	0	TIC	1.57
3	(I - *)	8.694	398.8	2,531	bb	0	0	TIC	6.57
4	(II - **)	9.537	401	2,421	bb	0	0	TIC	6.6
5		10.6	148.8	990	MM	0	0	TIC	2.45
6	(III - *****)	22.371	3,332.80	25,896	bb	0	0	TIC	54.88
7	(IV - ***)	26.496	531.7	3528	MM	0	0	TIC	8.75
8	(V- ****)	26.973	1,017.00	7,625	bb	0	0	TIC	16.75

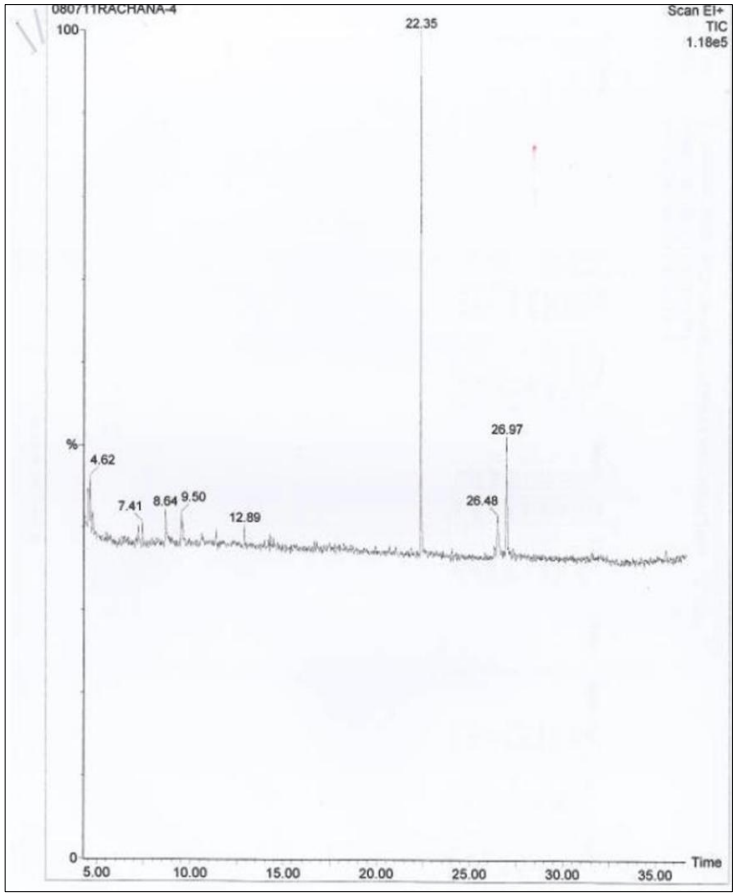

Fig 1: GC-MS Chromatogram of fatty acids (identified as methyl esters of sponified matter) of the petroleum ether extract of fruit of *Luffa echinata* Roxb

Fig 2: Mass spectra of peak no. 3 (RT-8.694) of chromatogram

Fig 3: Mass spectra of peak no.4 (RT-9.537) of chromatogram

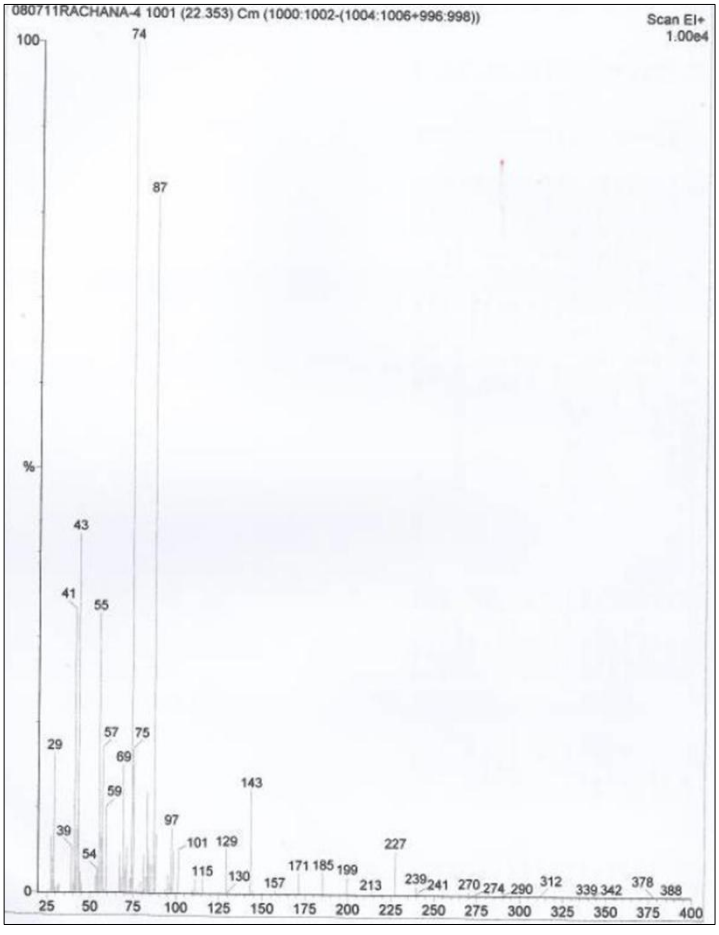

Fig 4: Mass spectra of peak no.6 (RT-22.371) of chromatogram

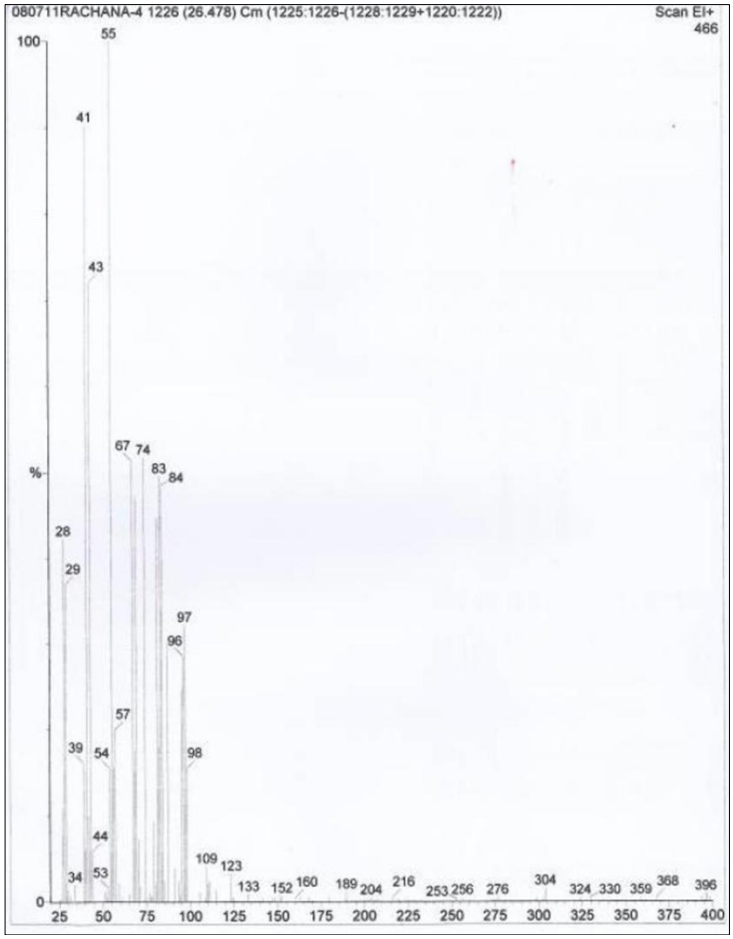

Fig 5: Mass spectra of peak no 7(RT-26.496) of chromatogram

Fig 6: Mass spectra of peak no. 8(26.973) of chromatogram

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