

Chemical Constituents of *Luffa acutangula* (L.) Roxb Fruit

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Abstract. The phytochemical screening conducted on ethanol extract of *Luffa acutangula* (L.) Roxb's fruit revealed the presence of alkaloids, saponins, carotenoids and terpenoids and the absence of flavonoids, tannins and anthraquinones. The GC-MS of the analysis *L. acutangula* (L.) Roxb's fraction resulted in the identification of six compounds. The compounds that could be identified were 2,3-dihydro,3,5-dihydroxy-6-methyl-(4H)-pyran-4-one; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; (3 β , 20R)-cholest-5-en-3-ol; *n*-hexadecanoic acid; 9, 12, 15-octadecatrienoic acid methyl ester and citronellyl tiglate. The present study provides evidence that *L. acutangula*'s fruit contains medicinally important bioactive compounds and this justifies the possibly use of these fruits as traditional medicine for treatment of various diseases.

1. Introduction

Phytochemical compounds are known to have beneficial importance in industrial and medicinal sciences. Although phytochemical tests are helpful in discovering bioactive compounds for therapeutic agents, food additives, agrochemicals and industrial chemicals, only a small number of plant has been investigated phytochemically. Plant based natural constituents can be derived from different parts of the plant like bark, leaves, flowers, roots, fruits and seeds [1-5].

The phytochemical screening have been conducted in Cucurbitaceae family members, such as *Trichosanthes anguina* L. and *Sechium edule* Jacq. Swartz [6-7]. Alkaloids, tannins, polifenol, saponin, cardenolin/bufadienol and flavonoid have been found to be present in the ethanol extract of *T. anguina* L.'s fruits, while anthraquinones have been found to be absent [6]. The phytochemical analysis of ethanol extract of *S. edule* Jacq Swartz's fruits revealed the presence of alkaloids, saponin, cardenolin/bufadienol and flavonoid and the absence of tannin, phenolics and anthraquinones [7].

Luffa acutangula (L.) Roxb (ridge gourd) belongs to Cucurbitaceae family. It is widely growing vegetative climber and found throughout Indonesia. 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 [8]. The plant also possesses potent α -glucosidase inhibitory effect [9]. The present study was designed to investigate the presence of various phytochemicals constituents in *L. acutangula* (L.) Roxb's fruits.

2. Experimental

2.1. Materials and Instruments

Fresh fruits of *L. acutangula* (L.) Roxb were purchased from local market in Surakarta, Indonesia. All the chemicals used were of analytical grade, purchased from Merck Chemical Company (Merck, Germany).



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The chemical compositions of the sample was studied by GC Hewlett Pacard 5890 series II and GC-MS Shimadzu QP-5000. Components were separated on CD sil 5 CB non polar column. The oven temperature was programmed at 120-300°C at a rate of 10°C/min. Sample injection volume was 0.3 µL with a split ratio 1:10 and pressure at the column inlet 10 kPa with helium carrier gas at 1 ml/min flowrate. Compounds were identified by comparison of mass spectra with those in the Wiley and NIST Libraries.

2.2. Preparation of Fruit Extract

The fruit samples (2.5 kg) were cleaned, dried in the oven at 80°C for 4 h and ground. The powdered material (42 g) was transferred into a Soxhlet apparatus and extracted in the Soxhlet extractor using 350 mL petroleum ether for 6 h. The residue was sequentially macerated with ethanol and allowed to stand for 24 h and then filtered. The extract then was concentrated using a rotary evaporator and dried under vacuum dessicator to yield 30 g (32 mL) of brownish black liquid. This ethanol extract was used for phytochemical test.

2.3. Screening of Phytochemical Components for the Ethanol Extract

The plant extract was subjected to qualitative tests adopting standard procedure for the identification of the phyto constituents [10-15].

2.3.1. Test for Alkaloids

Ethanol extract (0.25 g) was stirred with 3 mL of 1% hydrochloric acid on a steam bath. The filtrate (1 ml) was treated with few drops of Dragendoff's reagent, after which it was observed whether the alkaloids were present or absent. Alkaloids, if present in the solution of sample, will react with Dragendorff's reagent and produce an orange or orange red precipitate formation.

2.3.2. Test for Flavonoids

Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

2.3.3. Test for Tannins

Ethanol extract (0.25 g) was dissolved in 10 ml distilled water and filtered. A few drops of 0.1% ferric chloride were added to the filtrate. The appearance of intense brownish-green or a blue-black colour indicated the presence of tannins in the test samples.

2.3.4. Test for Terpenoids

Ethanol extract (0.25 g) was mixed with 1.5 ml of chloroform (CHCl₃) and concentrated H₂SO₄ (1.5 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

2.3.5. Test for Saponins

Ethanol extract (0.25 g) was boiled together with 10 mL of distilled water in a water bath and filtered. Ten mL of the filtered sample is mixed with 1 mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

2.3.6. Test for Anthraquinones

Ethanol extract (0.25 g) was taken into a dry test tube and 3 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone.

2.3.7. Test for Carotenoids

Ethanol extract (0.25 g) was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

2.4. Column Chromatography

Modification of the method of Selowa *et al.* [16] was used to elute the fractions during column chromatography. A suspension of silica gel 60 and n-hexane was poured into a glass column (50 cm long \times 1.5 cm diameter) up to a height of 30 cm being careful to prevent formation of gaps and bubbles and equilibrated with 100% n-hexane. A mixture of 2 g ethanol extract and 2 g of the silica gel 60 in 100% n-hexane was loaded onto the column and eluted with 100% n-hexane first and then with the solvent combination of n-hexane and ethanol with increasing volume of ethanol (polarity). The fractions of 2 mL of eluents were collected on the basis of solvent polarity and/or colour separation. Thin layer chromatography (TLC) was carried out and the fractions with similar profiles could be pooled together. The fractions were dried in a rotary evaporator after which they were dried to a constant weight in a fume cupboard.

3. Results and Discussion

3.1. Phytochemical Screening of the Ethanol Extract

The phytochemical active compounds of *L. acutangula* (L.) Roxb's fruits were qualitatively analyzed and the results are presented in table 1. Based on the presence or absence of colour change indicate positive and negative results are indicative. In the screening process of *L. acutangula* (L.) Roxb's fruit extract, alkaloids, terpenoids, carotenoids and saponins gave positive results whereas flavonoids, tannins and anthraquinones gave negative results. Tupe *et al.* (2013) reported that the phytochemical analysis of aqueous leaf extract of *L. acutangula* (L.) Roxb showed the presence of alkaloids, tannins, carbohydrates, saponin and terpenoids and the absence of flavonoids, anthocyanin and steroids [17]. In the present study, we have found that the biologically active phytochemicals were present in the ethanol extract of the *L. acutangula* (L.) Roxb's fruits. The medicinal properties of *L. acutangula* (L.) Roxb's fruits may be due to the presence of above mentioned phytochemicals.

Table 1. Phytochemical analysis of the ethanol extract of *L. acutangula* (L.) Roxb's fruits.

Phytochemical Components	Test	Results
Alkaloids	Draggendorff Test	+
Flavonoids	Alkaline Reagent Test	-
Tannins	Ferric Chloride Test	-
Terpenoids	Salkowski Test	+
Saponins	Foam Test	+
Anthraquinones	Borntragers test	-
Carotenoids	Sulphuric Acid test	+

+ = Presence; - = Absence

3.2. GC-MS Analysis

The GC-MS analysis of *L. acutangula* (L.) Roxb was carried out for three fractions, however only one fraction could detect the peaks of the compounds. Nineteen (19) of bioactive phytochemical compounds were shown in the chromatogram (figure 1). Tables 2 show the compounds found in the *L. acutangula* (L.) Roxb fraction. The compounds that could be identified were 2,3-dihydro,3,5-dihydroxy-6-methyl-(4H)-pyran-4-one; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; (3 β , 20R)-cholest-5-en-3-ol; *n*-hexadecanoic acid; 9, 12, 15-octadecatrienoic acid methyl ester and citronellyl tiglate. Some compounds

remained unidentified because of a lack of library spectra of the corresponding compounds. Cecheovska *et al.* (2011) reported that compound 2,3-dihydro,3,5-dihydroxy-6-methyl-(4H)-pyran-4-one found in prunes has antioxidant activity [18].

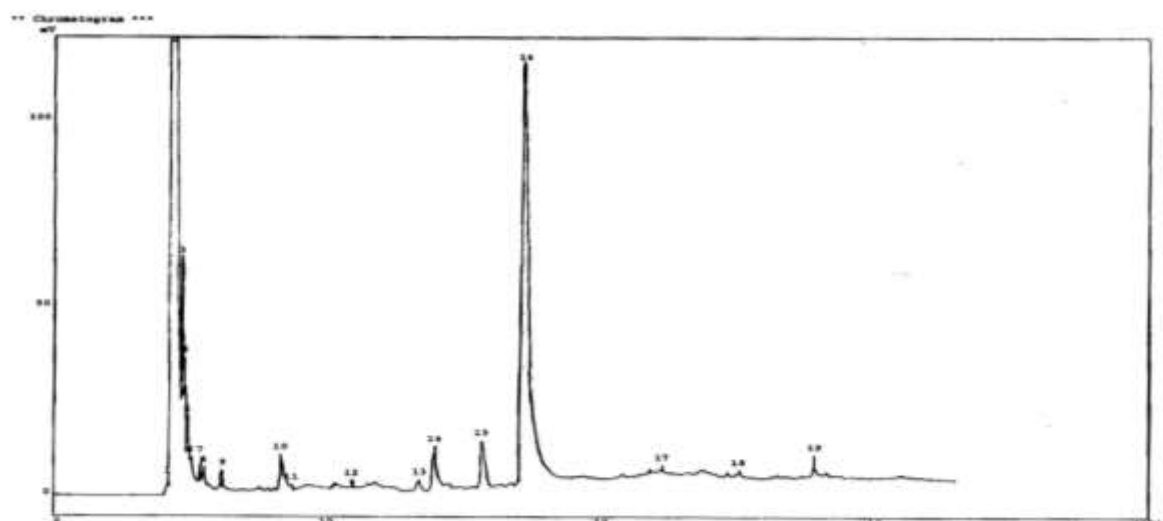


Figure 1. GC-MS chromatogram of the fraction of *L. acutangula* (L.) Roxb's fruits.

Table 2. Compounds that could be identified in the fraction of *L. acutangula* (L.) Roxb's fruits.

No.	Name	Molecular Formula	Molecular Weight
1.	2,3-dihydro,3,5-dihydroxy-6-methyl-(4H)-pyran-4-one	C ₆ H ₈ O ₄	144
2.	3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296
3.	(3β, 20R)-cholest-5-en-3-ol	C ₂₇ H ₄₆ O	386
4.	<i>n</i> -hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
5.	9, 12, 15-octadecatrienoic acid methyl ester	C ₁₉ H ₃₂ O ₂	292
6.	citronellyl tiglate	C ₁₅ H ₂₆ O ₂	238

4. Conclusion

L. acutangula (L.) Roxb's fruits have potential to act as a functional food and a source of useful drugs because of the presence of various phytochemical components. The ethanol extract of *L. acutangula* (L.) Roxb's fruit shows the presence of alkaloids, saponins, carotenoids, and terpenoids and the absence of flavonoids, tannins and anthraquinones. The compounds that could be identified by GC-MS analysis were 2,3-dihydro,3,5-dihydroxy-6-methyl-(4H)-pyran-4-one; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; (3β, 20R)-cholest-5-en-3-ol; *n*-hexadecanoic acid; 9, 12, 15-octadecatrienoic acid methyl ester and citronellyl tiglate.

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