ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Research Article

Preliminary Phytochemical Screening And Evaluation Of Free Radical Scavenging Activity Of *Luffa Acutangula* Var *Amara* Fruit

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The present study was aimed to screen and quantify the phytoconstituents of *Luffa acutangula* var *amara* fruit water extract and also evaluated the *in vitro* antioxidant activity in three models *viz.* DPPH (1,1- diphenyl - 2 - picrylhydrazyl) free radical screening activity, superoxide radical scavenging activity and reducing power assay. The total polyphenol and total flavanoids were quantitatively estimated in fruit of *Luffa acutangula*. The greater amount of polyphenol and flavanoids compounds leads to more powerful free radical scavenging effect as shown by water extract of *Luffa acutangula* var *amara* fruit.

Keywords: Antioxidant, DPPH, Superoxide radicals, Reducing power assay, *Luffa acutangula*

INTRODUCTION

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. [1] Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism.[2] The most common reactive oxygen species (ROS) include superoxide (0_2^-) anion, hydrogen peroxide (H_20_2), peroxyl (ROO^-) radicals and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO^-) and peroxynitrite anion ($ONOO^-$). ROS have been implicated in over a hundreds of disease states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome.[3] In treatment of these diseases, antioxidant therapy has gained an immense importance. Worldover the medicinal properties of plants have been investigated in the recent scientific developments, due to their potent antioxidant activities

The *Luffa acutangula* Linn. Var. *amara* Roxb. is a fairly large climber found in western, central and southern India, and regarded as wild variety of cultivated species.. It is commonly in Marathi as Kadudodak, Ranturai, in Sanskrit Katukoshataki, while in Hindi karviturai. All parts of plant are exceedingly bitter. A crystalline bitter principle identical with cucurbitacin B, luffin, and colocynthin is present.[4] While seeds shows presence of saturated and unsaturated fatty acid

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palmatic, stearic, oleic, linoleic and traces of lignoceric acid. The plant possesses laxative and purgative property. Fruit shows presence of cucurbetacin B and E and oleanalic acid.[5] Plant possesses laxative and purgative property. It is tonic to intestine, cures vata, kapha, anemia, asthma, jaundice, leucoderma and tumors, also useful as diuretic and in splenic enlargement. The dried fruit powder of fruit used in the form of snuff in jaundice. The seeds also possess emetic, expectorant, and demulcent property.[6] To understand the mechanisms of its pharmacological actions, *in vitro* antioxidant activity of *Luffa acutangula* was investigated.

MATERIALS AND METHODS:

Plant Material

The fruits of *Luffa acutangula* were collected from Sabarkantha and Gandhinagar districts of North part of Gujarat state, India. Their identity has been confirmed at the Department of Botany, School of science by Dr. M. K. Prajapati, Ahmedabad. Fruits were separated, cleaned, dried under shade and powdered to 60#.

Preparation of Plant extract

The dried powdered fruit of *Luffa acutangula* was allowed to pass through ss sieve (20 mesh).Macerated with water for 24 hours after that mixture is filter through the cotton bag and then using filter assembly (or) fixed standard Whatman filter paper size No.1.Then filtrate was evaporated on hot plate until it reaches the concentrated quantity.

Preliminary phytochemical screening

Five hundred milligrams of the dried water extract was reconstituted in 10ml of methanol and it was subjected to preliminary phytochemical testing for the presence of different chemicals groups of compounds by standard methods[7]

Quantification of polyphenol and total flavonoids

Preparation of stock solution for quantification of polyphenol and flavanoids

Stock solution of water extract and hydroalcoholic extract of *Luffa acutangula* var *amara* fruit 1mg/ml was prepared in methanol and used for the estimation of polyphenol and flavanoids.

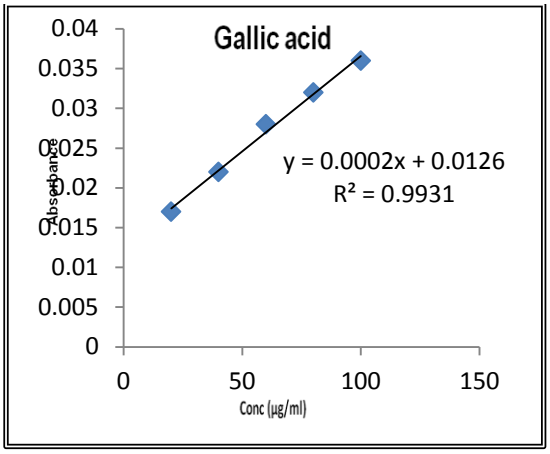
Quantification of polyphenol content

Total phenol content in plant extract was generally determined according to folin ciocalteu method. [8,9.10]

Principle: This colorimetric method was based on the reduction of a Phosphotungstate phosphomolybdate complex by phenolic to blue color product in alkaline condition.

Procedure: Each of 0.5 ml of stock solution of sample was taken in 25 ml volumetric Folin ciocalteau reagent were

added. The mixture was then kept for 5 min. And 4 ml of 20% w/v sodium carbonate solution was added. The volume was made up to 25 ml with double distill water. The mixture was kept for 30 min until blue color developed. The samples were then observed at 365 nm in UV-visible spectrometer Shimadzu, UV-1601, Japan.



Values are expressed as mean ± SD

Fig 1: Calibration curve for standard Gallic acid (Concentration Vs absorbance)

The % total phenol content was calculated from calibration curve of gallic acid plotted under similar procedure.

Quantification of total flavonoids

Aluminum Chloride colorimetric method was used for flavanoid determination. [11] The method is based on the quantification of yellow color produced by interaction of flavanoids with $AlCl_3$ reagent. 1 ml of sample from stock solution was mixed with 3 ml methanol, 0.2 ml of 10% $AlCl_3$, 0.2 ml of 1 M Potassium acetate and 5.6 ml distilled water. The solution was maintained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV-Visible spectrophotometer.

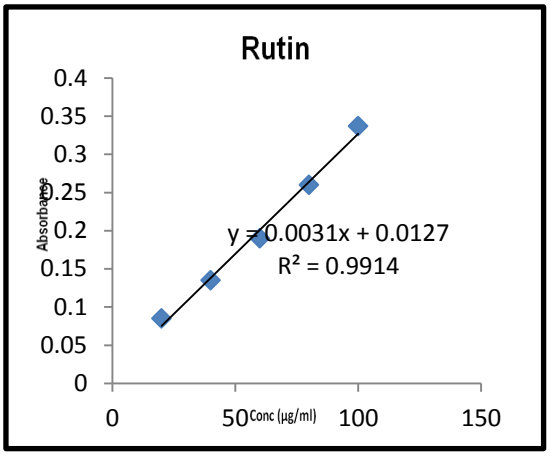


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Figure 2: Calibration curve for standard Rutin (Concentration Vs absorbance)

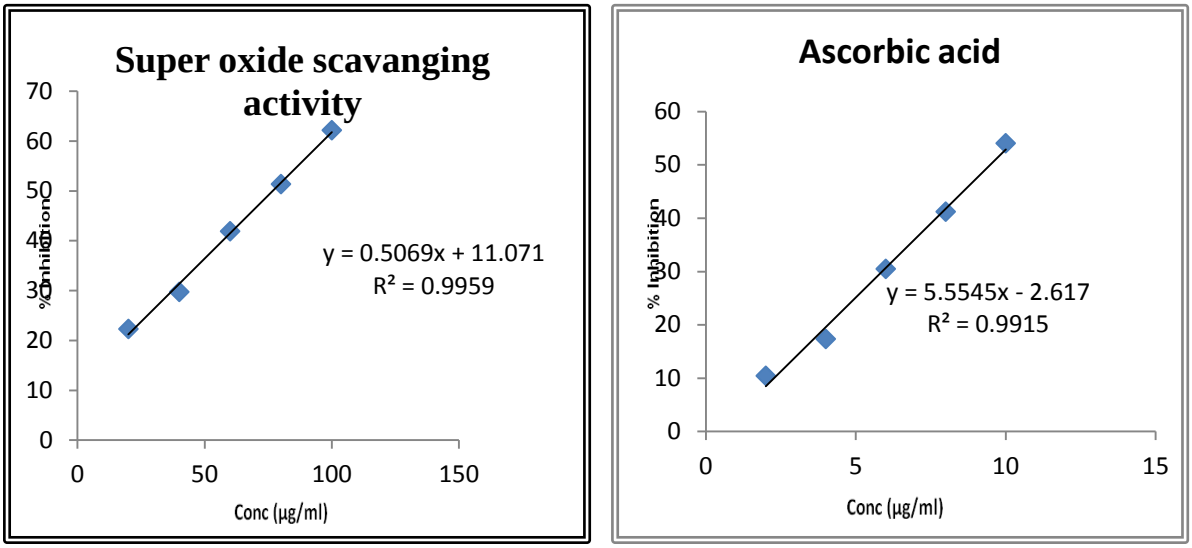
The % of total flavanoid was calculated from calibration curve of standard flavanoid (Rutin) plotted under similar procedure.

***In vitro* Antioxidant activity**

A) DPPH Free radical scavenging activity

4.3mg of DPPH (1, 1- Diphenyl-2-picrylhydrazyl) was dissolved in 3.3ml methanol; it was protected from light by covering the last test tube with aluminum foil. 150 µl DPPH solutions were added to 3 ml methanol and absorbance was taken immediately at 516 nm for control sample. Different concentrations of methanolic

ISSN 2249-3875



Values are expressed as mean ± SD.

Figure 4: Effect of Water extract *Luffa acutangula* and ascorbic acid On Superoxide Free radical scavenging activity

extract of plant were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3 ml and to each 150 µl DPPH was added. Absorbance was taken after 15 minutes at 516 nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601. Japan. IC-50 value for extract was calculated. [12,]

B) Super Oxide radical scavenging activity

100 µl Riboflavin solution, 200 µl EDTA solution, 200 µl methanol & 100 µl NBT (nitro blue tetrazolium) solution were mixed in test tube & reaction mixture was idiluted up to 3 ml with phosphate buffer. The absorbance of solution was measured at 590 nm using phosphate buffer as blank after illumination for 5 min. This was control. Different concentration of ethanolic extract of plant were taken & diluted up to 100 µl with methanol to each of this, 100 µl Riboflavin, 200 µl EDTA, 200 µl methanol & 100 µl NBT were mixed in test tube & further diluted up to 3 ml with phosphate buffer. Absorbance was measured after illumination for 5 min. at 590 nm on UV visible spectrometer SIMADZU, UV-1601-Japan.IC_{50} value for extract was calculated. [13,14]

C) Reducing power Assay

Different concentrations of methanolic extract dissolved in methanol (1 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) & 2.5 ml of 1% potassium ferricyanide. After incubation for 20 min at 50^0c, 2.5 ml of 10% trichloro acetic acid

ISSN 2249-3875

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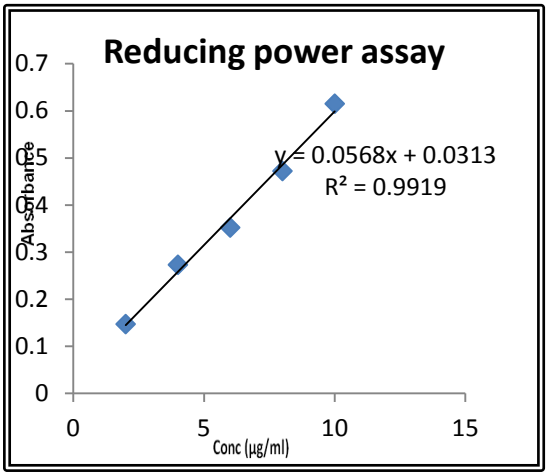

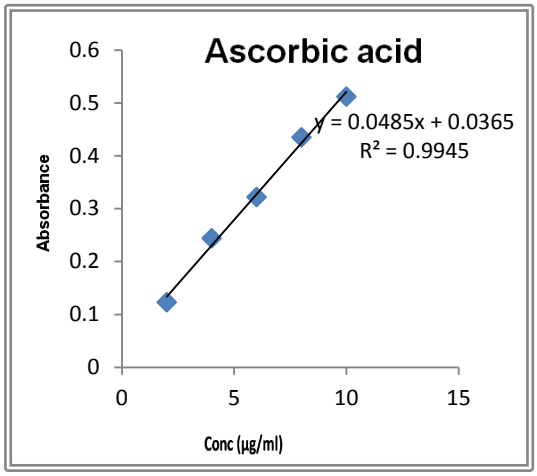

Values are expressed as mean ± SD.
Reducing power increases as concentration increases

Figure 5: Effect of Water extract *Luffa acutangula* and ascorbic acid on reducing power assay

was added to the mixtures. After centrifugation, 2.5 ml of the upper layer were diluted with distilled water & 0.5 ml of 0.1% Ferric chloride was added. The absorbance was measured at 700 nm. Increase in absorbance of the reaction indicated the reducing power of polyphenol test samples.[15]

RESULTS AND DISCUSSION

Preliminary phytochemical screening seeds showed presence of Alkaloid, protein and amino acid, tannins, flavonoids, saponins. Estimation of polyphenols and total flavonoids were done by taking Gallic acid and Rutin as Standard. Plants containing flavonoids and polyphenols are known to possess strong antioxidant properties.[16]

Significant amount of polyphenol showed in (Figure-1)(4.05 % w/w of Gallic acid)(Table-1) and flavonoids (0.19 %w/w of Rutin)showed in (Figure-2)(Table-2)were present in Fruit.

Table 1: Quantification of polyphenol in Water extract of *Luffa acutangula* fruit

Conc. of extract	Absorbance ± SD (n=3)	µg gallic acid/ml	% w/w of gallic acid
100 µg	0.023 ± 0.01	52	4.05

Due to presence of significant amount of polyphenols, *in vitro* antioxidant activity of water extract of fruit was performed.

In vitro antioxidant activity of extract was investigated by DPPH (1, 1- diphenyl-2-picrylhydrazyl) scavenging activity, superoxide free radical scavenging activity and by reducing power assay.

Table 2: Quantification of total flavonoid in Water extract of *Luffa acutangula* fruit.

µg of sample solution	Absorbance ± SD (n=3)	µg of rutin/ml	% w/w of rutin
1000	0.091	25.25	0.19

ISSN 2249-3875

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To elevate the antiradical activity of water extract of *Luffa acutangula* fruit exhibited DPPH radical scavenging activity in concentration dependant manner.

Table 3: IC_{50} value of *Luffa acutangula* and ascorbic acid of on DPPH free radical scavenging activity.

	IC_{50} value
Luffa acutangula	38.68
Ascorbic acid	8.87

This method is based on the reduction of methanolic DPPH solution in the presence of hydrogen donating antioxidant (AH) due to the formation of non- radical form DPPH – H by the reaction DPPH+ AH → DPPH – H + A. The remaining DPPH measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant[17]. The sensitivity of the method is determined by the strong absorption of DPPH. In the present study provides information on the reactivity of test

Table 4: IC_{50} value of *Luffa acutangula* and ascorbic acid on Superoxide free radical scavenging activity

	IC_{50} value
Luffa acutangula	76.79
Ascorbic acid	9.47

compounds with a stable free radical since add electron of DPPH give strong absorption band at 517 nm and when it is quenched by the extract there is a decreased in absorbance. Water extract of *Luffa acutangula* fruit showed good antiradical activity in scavenging DPPH radical with IC_{50} (µg/ml) 38.68. is compared with standard ascorbic acid IC_{50} value 8.87 µg/ml showed in (Table-3,Figure-3)

The *in vitro* superoxide radical scavenging activity was also measured by riboflavin/light/NBT (Nitroblue tetrazoline) system reduction.The method is based on generation of superoxide radicals by auto oxidation of riboflavin in the presence of light. The superoxide radical reduces NBT to a blue colored formazone that can be measured at 560 nm[18]. Water extract of fruit showed (Table-4,figure-4) Significant superoxide free radical scavenging activity with IC_{50} value 76.79 µg/ml is compared with standard ascorbic acid IC_{50} value 9.47 µg/ml. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[19] For the measurement of the reducing ability, the Fe^{3+} to Fe^{2+} transformation was also investigated in the presence of methanolic extract of seed. Extract showed dose dependent increase in absorbance so reducing power of extract was dose dependent (2-10 µg/ml) showed in is compared with Standard Ascorbic acid (Figure-5).

Conclusion

To conclude, the above experiments

clearly indicate that water extract of *Luffa acutangula* showed effective free radical scavenging activity which can be attributed to the presence of tannins and phenolics along with other compounds

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