Abstract

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere between natural product organic chemistry and plant biotechnology and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with chemical structures of these substances; their biosynthesis; turnover and metabolism; their natural distribution and biological functions. Atherothrombosis is a major cause of global life threatening heart and cerebral diseases. Atherothrombosis characterized by atherosclerotic lesion disruption with superimposed thrombus formation is the major cause of acute coronary syndromes (ACS) and cardiovascular death. It is the leading cause of mortality in the industrialized world. Considering this, present study was designed to investigate thrombolytic activity of ethanolic extract of *Luffa cylindrica*. The ethanolic extract was found to have significant thrombolytic activity (45%) compared to the effect of Streptokinase (57%) used as a positive control and water (0%) used as a negative control. Preliminary phytochemical screening of the extract showed the presence of carbohydrates, proteins, steroids, flavonoids, terpenoids and tannins in fruits of *Luffa cylindrica* Linn. one of which has thrombolytic properties.

**Keywords:** Thrombolytic activity, streptokinase, phytochemical screening, *Luffa cylindrica*, ethanol.

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INTRODUCTION

History says that, natural products are nothing new as the agents for treating various diseases. Naturally plants not only provide us food, shelter but also they provide remedies for many years. Different chemical constituents contained in plant exhibit different activities in alleviation of abnormal health condition of human beings or animals. In case of traditional medicine, the practitioners are appreciated to use different parts of plant because of having several chemical constituents in them which fulfill their wants [1]. Herbal medicines are the synthesis of therapeutic experiences of generations of practising physicians of indigenous systems of medicine for over hundreds of years while nutraceuticals are nutritionally or medicinally enhanced foods with health benefits of recent origin and marketed in developed countries. The marketing of the former under the category of the latter is unethical. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available [2]. India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a dismal share of the world market due to export of crude extracts and drugs. WHO too has not systematically evaluated. Traditional medicines despite the fact that it is used for primary health care by about 80% of the world population. However, in 1991 WHO developed guidelines for the assessment of herbal medicine. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world [3]. In Germany and France, many herbs and herbal extracts are used as prescription drugs and their sales in the countries of European Union were around $ 6 billion in 1991 and may be over $ 20 billion now. In USA, herbal drugs are currently sold in health food stores with a turnover of about $ 4 billion in 1996 which is anticipated to double by the turn of the century in India, the herbal drug market is about $ one billion and the export of plant-based crude drugs is around $ 80 million. Herbal medicines also find market as nutraceuticals (health foods) whose current market is estimated at about $ 80–250 billion in USA and also in Europe herbal remedies. Herbal medicine is triumph of popular therapeutics diversity [4]. Nature always stands as a golden mark to exemplify the outstanding phenomenon The World Health Organization (WHO) has recently defined traditional
medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today [5]. In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions of herbs or to confirm the claim made about them in the official book of Ayurveda. The emerging importance of biologically active medicinal plants and their constituents has become a subject of active scientific investigation. It is likely that in future safe and effective medicines will be developed from medicinal plants to treat various degenerative diseases. So, Medicinal plants are source of effective cardioprotective, nephroprotective, hepatoprotective, anti diabetic, antimicrobial agent etc and its derived played an important role in toxicity treatment. Several medicinal plants have been screened based on the integrative approaches on drug development from Ayurveda and Unani traditional system of medicines. Although the fruits of *Luffa cylindrica* were used for several biological activities; the possible protective activities of these plants have not been reported so far [6,7]. *Luffa cylindrica Linn.* fruit is coined as sponge gourd, vegetable sponge etc. It belongs to the family Cucurbitaceae. It is generally 2-3 inches in diameter and 15-18 inches in length. The exterior is green, sometimes molted and smooth. *Luffa* is a sub-tropical plant which requires warm summer temperatures and a long frost-free growing season when grown in temperate regions. *Luffa cylindrica* is a large climbing vine, with a thin but very tough light green, succulent stem, attaining a length of 10-30 feet. *Luffa* is a subtropical plant which requires warm summer temperatures and a long frost-free growing season when grown in temperate regions. The fruit is elliptical ovate, fleshy and dehiscent with a green epidermis, longitudinally marked with black ridges varying from 10-15 in a number; under each of these ridges is found a tough woody fiber [8-10]. Therefore, in the present investigation, we aimed to evaluate the thrombolytic effect of the ethanolic extract of dried fruits of *Luffa cylindrica Linn.*

**Plant Profile:**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td>Curcubitales</td>
</tr>
<tr>
<td>Family</td>
<td>Cucurbitaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Luffa</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>L. cylindrica</em></td>
</tr>
</tbody>
</table>

Biological name: *Luffa cylindrica Linn.*
MATERIALS AND METHODS

Selection of plant

The plant *Luffa cylindrica* is selected on the basis of information by folk claims and their literature survey. The plant is having potent thrombolytic property which is beneficial in treating heart attacks and pulmonary embolism and Atherothrombosis. The complied plant is therefore subjected to identification and their literature survey confirms that fruit extract of *Luffa cylindrica* has not been previously investigated for their *in vitro* thrombolytic activity. Thus, the plant has been choosen to explore its potential activity.

Collection plant material

The fruits of *Luffa cylindrica* were collected from the Uppal area in the month of March 2017, Medchal, Telangana.

Preparation of powder

The fruits of *Luffa cylindrica* was dried in shade and then powdered with a mechanical grinder. The powder was stored in a labelled air tight container for further studies.

Extraction of plant material

The powdered plant fruit material was subjected to maceration using ethanol. The extract obtained was later kept for evaporation to remove ethanol. These extracts were stored in a cool dry place for the analysis for the presence of preliminary phytochemical analysis and *in vitro* thrombolytic activity.
Preliminary phytochemical screening [11-22]

The chemical complexity of a crude drug could be efficiently understood with the aid of qualitative chemical test for detection of different plant constituents. This approach of qualitative evaluation to reveal chemical nature of both major and minor phytoconstituents and where certainly useful in laying down chemical quality control parameters for crude drugs and extracts derived from fruits of *Luffa cylindrica* were subjected to qualitative chemical tests for identification of various plant constituents.

A. Test for alkaloids:

i) Dragendorff's Test: In a test tube containing 1ml of extract, few drops of Dragendorff’s reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

ii) Wagner's Test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

iii) Mayer's Test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's Test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.
B. Test for terpenoids:

i) Salkowski Test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink colour indicates the presence of terpenoids.

ii) Hirshonn reaction: When the substance was heated with trichloroacetic acid, red to purple colour was observed.

C. Test for steroids:

i) Liebermann Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

D. Test for coumarins:

i) To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

E. Test for tannins:

i) Ferric Chloride Test: To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

ii) Lead Acetate Test: The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

F. Test for saponins:

i) Foam Test: To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

G. Test for flavones:

i) Shinoda Test: To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red colour showed the presence of flavones.

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow colour shows the presence of flavones.

H. Test for quinones:

i) To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.

I. Test for flavanones:

i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.
ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

**J. Test for anthocyanins:**

i) To the extract, 10% sodium hydroxide was added, and the blue color shows the presence of anthocyanins.

ii) To the extract, conc. sulphuric acid was added, and the yellowish orange colour confirms the presence of anthocyanins.

**K. Test for anthraquinones:**

i) Borntrager's test: The extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet colour in the aqueous layer after shaking indicates the presence of anthraquinones.

**L. Test for phenols:**

i) Ferric chloride test: To the extract, few drops of 10% aqueous ferric chloride were added. Appearance of blue or green colour indicates the presence of phenols.

**M. Test for proteins:**

i) Biuret Test: To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

ii) Xanthoprotein Test: To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed; it is then boiled and cooled. Then 20% sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

iii) Tannic Acid Test: To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

**N. Test for carbohydrates:**

i) Molisch's Test: To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

ii) Fehling's Test: To the extract, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

iii) Benedict's Test: To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates.
O. Test for amino acids:
  i) Ninhydrin test: Two drops of ninhydrin solution were added to the extract, a characteristic purple colour indicates the presence of amino acids.

P. Test for Fixed Oils and Fats:
  i) Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

**In-vitro thrombolytic study**

The thrombolytic activity of all extractives was evaluated by using streptokinase as a standard.

**Streptokinase Solution Preparation** [23-28]

Commercially available lyophilized Altepase (Streptokinase) vial of 15,00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μl (30,000 I.U) was used for *in vitro* thrombolysis.

**Specimen**

Whole blood (5 ml) was drawn from healthy human volunteers without a history of oral contraceptive or anti coagulant therapy. 500 μl of blood was transferred to each of the three previously weighed alpine tubes to form clots. Three micro centrifuge tubes were taken, sterilized, weighed, (Let n= 1), 3 ml blood was drawn from volunteer. The blood was distributed in 3 different pre weighed (W₁) micro centrifuge tubes, each tube 1ml. The blood specimen was centrifuged at 2500rpm for 5 minutes. All the tubes were incubated at 37°C for 45 minutes and observed for clot lysis. After clot formation i.e. incubation, the serum was completely removed by decantation, capillary absorption and by removing the serum from the inner surface of the tube carefully by cotton bar or by use of cotton bound at top of a glass rod without disrupting the clot and ensure complete removal of serum, or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of serum and then removed the liquids of the tube surface by the cotton rod. Each tube was weighed (W₂) again. Weight of colt was found as,

\[
\text{Weight of clot} = \text{weight of tube containing clot (W}_2\text{)} - \text{weight of empty tube (W}_1\text{)}
\]

Finally weighed very carefully, because result varies for inappropriate weighing, checked the balance before weighing. To each micro centrifuge tube containing pre weighed clot, 100 μl of ethanolic extract of ‘n’ plant/herb (*Luffa cylindrica*) was added separately. As a positive control, 100 μl of streptokinase was added to clot of tube no.5 (Standard). As a negative control 100 μl water was added to clot of tube no.4 (Blank). All the tubes were incubated at 37°C for 90 minutes and observed if clot lysis has occurred. After 90 minutes of incubation, the released fluid was
completely removed by decanted colt containing liquid from the inner surface of the tube carefully by cotton bar or by use of cotton tightly bound at top of a glass rod without disrupting the clot. The tubes were then weighed again. And ensured complete removal of released fluid or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of released clot and then removed the liquids of the tube surface by the cotton rod. Weigh the tubes (W₃) very carefully, because result varies for inappropriate weighing. The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis using the following formula:

\[
\text{% of clot lysis} = \left( \frac{\text{wt. of released clot}}{\text{wt. of clot}} \right) \times 100 = \left( \frac{W₂ - W₃}{W₂ - W₁} \right) \times 100
\]
RESULTS AND DISCUSSION

As the chemical constituents present in a plant are directly responsible for its therapeutic and other pharmacological properties, the constituents of the plant which were reported and detected during this investigation should have some direct relationship with local medicinal uses. The result showed that the ethanolic extract of fruits of *Luffa cylindrica* Linn. contains carbohydrates, proteins, tannins, steroids, saponins, glycosides and flavonoids [29-31].

Table 1: Preliminary phytochemical screening of *Luffa cylindrica* Linn.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Fixed oils and fats</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
</tbody>
</table>

(+): Present, (-): Absent

Table 2: % Clot lysis activity of ethanolic extract of *Luffa cylindrica*

<table>
<thead>
<tr>
<th>Observation</th>
<th>Weight of empty tubes (W₁) in gm</th>
<th>Weight of clot containing tube (W₂) in gm</th>
<th>Weight of clot (W₂-W₁) in gm</th>
<th>Weight of tube after incubation (W₃) in gm</th>
<th>% of clot lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>1.04</td>
<td>1.88</td>
<td>0.84</td>
<td>1.50</td>
<td>45%</td>
</tr>
<tr>
<td>Standard</td>
<td>1.04</td>
<td>2.01</td>
<td>0.97</td>
<td>1.45</td>
<td>57%</td>
</tr>
<tr>
<td>Blank</td>
<td>1.04</td>
<td>1.55</td>
<td>0.41</td>
<td>1.55</td>
<td>0%</td>
</tr>
</tbody>
</table>

Fig. 6: Comparative % *in vitro* thrombolytic effect of formulation, streptokinase and water (negative control)
Plant-based drugs have a long history of utilization for the prevention and treatment of human illnesses. Today, numerous pharmaceuticals at present sanction by the Food and Drug Administration (FDA) have inceptions to plant sources. The observed thrombolytic percentage shows quite good effect of thrombolysis of the ethanolic extract of *Luffa cylindrica* fruits. It possesses a significant percent of thrombolysis comparing with streptokinase and makes a difference from negative control (water). A huge numbers of thrombolytic drugs are obtained from different sources and then further modified, using recombinant technology. This is performed to make them more site-specific and to reduce their side-effects. Sometime patient may dies due to bleeding or internal hemorrhage, so direct use of herbal thrombolytic drug is not expected. Their further modification is required. There is a requirement for more goal and scientific approaches to authenticate individual herbs to identify chemical constituents, detect adulteration or contamination of herbs, and screen the quality of herbs and herbal medicines.

**CONCLUSION**

The ethanolic extract of fruits of *Luffa cylindrica* Linn. (Family-Cucurbitaceae) herb was studied for its preliminary phytochemical screening and *in vitro* thrombolytic activity. To get preliminary idea about the active constituents present in the plant extracts, different qualitative chemical tests were performed and showed the presence of reducing carbohydrates, flavonoids, tannins, saponins, and glycosides and it possesses a significant percent of thrombolysis comparing with streptokinase as standard drug. Further studies needed to determine the active compounds responsible for these activities and this development effort will help to identify the active constituents, structures and their action mechanism responsible for the activity. This is only a preliminary study and the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potential.

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**Conflict of interest**

There is no conflict of interest.
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2. Sarangadhara samhitha, Madhyamakhandha, Adhyaya 6, 93-94.


