IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Pharmacological and Phytochemical Screening of *Luffa-Acutangula* for Analgesic and Anti-Inflammatory Activity

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Abstract : Plants are one of the important sources of medicine. In India. Herbal medicines are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes. Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents ultimately aiming to perform the dual function of limiting damage and promoting tissue repair. Analgesic drug and Non–steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for their wide range of activity. However its side effects are high. Natural products from medicinal plants have more pharmacological significance with improved efficacy and there no side effects. The entire plant of *Luffa acutangula L.* is medicinally important and is used extensively in Indian traditional system of medicines. Chemical constituent of *luffa acutangula (L)* various bioactive compounds such as carbohydrates, carotene, fat, protein, phytin, aminoacid, alanine, arginine, cystine etc. *Luffa acutangula (L)* have various chemical constituents which are beneficial in pain and inflammation so we are trying to explore the effect and potency of *Luffa acutangula* (L) in pain and inflammation.

Keyword: Inflammation, Analgesic, *Luffa acutangula* (L), Pain.

INTRODUCTION

Plants are one of the important sources of medicine. In India, the Ayurvedic system of medicine has been in use for over three thousand years. Hippocrates, the 'Father of Medicine' was the first to give a scientific explanation of diseases. Indian system's of medicine includes Ayurveda, Siddha, Unani, Tibetan and Naturopathy. Herbal therapy provides rational means for the treatment of many internal diseases which are considered to be obstinate and incurable in other systems of medicine. It aims at both the prevention and cure of diseases. About 60 percent of the world's population use herbal medicines. Herbal medicines are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy.[1]

Pain

Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes. Pain can affect all areas of a person's life including sleep, thought, emotion, and daily activities. Unrelieved acute pain can cause chronic pain and long standing pain can cause anatomical and even genetic changes in the nervous system. Pain is warning signal, primarily protective in nature, but causes discomfort and suffering. Excessive pain may produce other effects such as sinking sensation, apprehension, sweating, nausea, palpitation, and rise or fall in BP, tachypnoea. Hence, these tests only measure the power of a drug to increase the minimal stimulus requiredto elicit pain or nociceptive response.[2]

Inflammation

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair. Inflammation is an important cellular response triggered by various mechanical, chemical or immunological stress factors and it is regulated by a delicate balance between local factors that finally determine the outcome of the disease process. The effective candidate drug in invitro test is later tested in whole animal models of acute, subacute and chronic inflammation.

PLANT PROFILE

Luffa acutangula L. belongs to the family Cucurbitaceae, is commonly known as ridge gourd and it is used as vegetable in Asian countries. The entire plant of *Luffa acutangula L*. is medicinally important and is used extensively in Indian traditional system of medicines. *Luffa acutangula (L.)* has been used in the folkloric Indian medicinal system to treat numerous health conditions. The fruits of the plant have been used in dysentery, jaundice, diabetes, hemorrhoids, leprosy, and ringworm infection.[3]

SCIENTIFIC CLASSIFICATION

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitace
Sub Family	Cucurbitoideae
Tribe	Benincaseae
Sub tribe	Luffinae
Genus	Luffa

CHEMICAL CONSTITUENTS

Chemical constituents of *luffa acutangula (L)* various bioactive compounds such as carbohydrates, carotene, fat, protein, phytin, aminoacid, alanine, arginine, cystine, and glutamicacid, hydroxyproline, leucine, serine and also presence of alkaloids and terpenoid, flavonoids, tannins, luffangulin, sapogenin, oleanolic acid, cucurbetacin B,E and anthraquinones. Leaves are a healthy food and contains good amount of fiber, differents types of vitamins such as Vitamin B2, Vitamin C, Calcium, phosphorus, iron and small quantities of iodine and fluorine. Seeds show presence of saturated and unsaturated fatty acid palmatic, stearic, oleic, linoleic and traces of lignoceric acid. Plant shows presence of oleanane type triterpene saponins- acutoside A, B, C, D, E, F and G.[4]

AIM AND OBJECTIVE

Medicinal plants play an important role in the development of potent therapeutic agents. Pain can result from an injury, such as a broken bone, a burn or a sprain from overuse of muscles from infections, such as sinus infections or meningitis or from natural events such as childbirth. Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair. Analgesic drug and Non–steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for their wide range of activity. However its side effects are high.[5] Natural products from medicinal plants have more pharmacological significance with improved efficacy and there no side effects.The entire plant of *Luffa acutangula L*. is medicinally important and is used extensively in Indian traditional system of medicines. Chemical constituent of *luffa acutangula (L)* various bioactive compounds such as carbohydrates, carotene, fat, protein, phytin, aminoacid, alanine, arginine, cystine etc. Leaves are a healthy food and contains good amount of fiber, differents types of vitamins such as Vitamin B2, Vitamin C, Calcium, phosphorus, iron and small quantities of iodine and fluorine.[6]

Luffa acutangula (L) have various chemical constituents which are beneficial in pain and inflammation so we are trying to explore the effect and potency of Luffa acutangula (L) in pain and inflammation.

- Collection of plant material and its authentication.
- Preparation of *luffa acutangula* extract.
- Phytochemical analysis of *Luffa acutangula* extract
- Pharmacological studies.

REASERCH METHODLOGY

The plant material was collected, authenticated and then the leaves were washed to remove the dust Particles and allowed to air dry in a shade for complete drying. Then the dried leaves without moisture were powdered in a mixer grinder.

Preparation of extract

The coarse powder was packed tightly in the soxhlet apparatus and extracted with ethanol for 72 hours with occasional shacking maintained at60ºc throught out the extraction process. The extract was concentrated to ofits original volume by evaporation. The resulting ethanolic extract of the *Luffa acutangula (L.) Roxb.* was subjected to phytochemical study.

Phytochemical analysis[7]

The ethanolic extract of *Luffa acutangula (L.) Roxb.* were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, and free aminoacids and triterpenoids.

Test for carbohydrate

Small quantity of extract was dissolved in 5ml of water and filtered.

Molisch test

The filtrate was treated with a few drops of α- napthol (20% in ethyl alcohol). Then 1 ml of concentrated H_2SO_4 was added along the sidesof inclined test tube and observed for formation of violet coloured ring at the interface.

Test for glycosides and anthroquinones

1. Borntrager's test

A small amount of ethanolic extract was hydrolysed withhydrochloric acid for few hours on water bath and the hydrosylate was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

2. Legal test

The extract was dissolved in pyridine and made alkaline with few drops of 10% NaOH and freshly prepared sodium nitroprusside was added and observed for formation of blue colour.

Test for flavonoids

Ammonia test

Filter paper strips were dipped in the dilute solution of the extract, ammoniated and observed for colour change from white to yellow

Test for Proteins and Amino acids

Small amount of extract was dissolved in distilled water and filtered

1. Biuret's test

To the ammoniated alkaline filtrate add 2-3 drops of 0.002% copper sulphate and observed for appearance of red or violet colour.

2. Millon's test

To 2 ml of filtrate 5-6 drops of millons reagent (1 g of mercury + 9 ml of fuming nitric acid solution) was added and observed for red precipitates.

3. Ninhydrin test

To the filtrate lead acetate solution was added to precipitate tanninsand filtered. The filtrate was spotted on paper chromatogram and sprayed with ninhydrin reagent and heated at 110ºC for five minutes and observed for red or violet colour.

4. Xanthoprotein test

To the filtrate a few drops concentrated nitric acid was added bythe side of test tube and observed for appearance of yellow colour.

Test for sterols and tri terpenes

The extract was refluxed with alcoholic potassium hydroxide until the completion of saponification. Then the mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to the following tests.

Salkowski's reaction

To the ether soluble residue 2 ml of concentrated sulphuric acidwas added and observed for the formation of yellow ring at the junction which turns red after one minute.

PHARMACOLOGICAL SCREENING

Animals

Albino rats weighing 150- 200 gm were used for this study. The animals were obtained from animal house, Vedic Institute of Pharmaceutical Education and Research Sagar (M.P). On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºc and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow.[8]

Animal approval

The study was conducted after obtaining from Committee for the Purpose of Control and Supervision Experiments on Animals (CCSEA) and Institutional Animal Ethics Committee (IAEC), proposal number (Vedic/CCSEA/2025/10).

ANALGESIC ACTIVITY

Hot Plate Method in Rat

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally medicated analgesic effects ofethanolic extract of *Luffa acutangula (L.) Roxb.*

Group I: Normal control (CMC)

Group II: Standard (Pentazocine 3 mg/kg

Group III: Test Drug I (Ethanolic leaf extract of *Luffa acutangula*)

Group IV: Test Drug II (Ethanolic leaf extract of *Luffa acutangula*)

Each animal was then individually placed gently on Eddy's hot plate at 55ºC. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined at 30, 60, 90 and 120 min after administration of the drugs or vehicle.[9]

Tail Immersion Test

The Albino rats were divided into four groups each consists of six animals.

Group I: Normal Control

Group II : Pentazocine (3 mg/kg)

Group III: Test Drug I (Ethanolic extract leaf *Luffa acutnagula*)

Group IV: Test Drug II (Ethanolic extract leaf *Luffa acutangula*)

The reaction times of the groups were taken at 0, 30, 60, 90 and 120min. The cut off time of the immersion was 15seconds. The reaction time was measured.[10]

ANTI-INFLAMMATORY ACTIVITY

Carrageenan-Induced Paw Edema In Rats

The Albino rats were divided into four groups each consists of six animals.

Group I: Normal Control (CMC)

Group II: Diclofenac Sodium (10mg/kg)

Group III: Test Drug I (Ethanolic extract leaf *Luffa acutanula*)

Group IV: Test Drug II (Ethanolic extract leaf *Luffa acutangula)*

Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw of the rats. The animals were pre treated with the drug 1hour before the administration of carrageenan. The paw thickness was measured at 1, 2, 3 and 4 h after carrageenan injection by using digital vernier callipers.[11]

Cotton Pellet Induced Granuloma Method In Rats

Cotton pellets, weighing 5mg each were sterilized. Under ether anaesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals. Starting from 30 min after the implantation of cotton pellet for all the rats.

Group I: Normal Control (CMC)

Group II: Diclofenac sodium (10 mg/kg)

Group III: Test Drug I (Ethanolic extract leaf *Luffa acultangula*)

Group IV: Test Drug II (Ethanolic extract leaf *Luffa acultangula*)

The test drugs were administered daily for 7days. On the 8th day, the animals were sacrificed with diethyl ether. The granulomas were removed and the weighed.[12]

DETERMINATION OF PERCENTAGE YIELD:

Coarse powder (100gm) of the *Luffa Acutangula* leaves was extracted by soxhlet extraction method. Firstly the crude drug was extracted with petroleum ether then successively extracted with ethanol as solvent. Extract thus obtained was concentrated dried, weighed and stored. After the extraction of crude drug, the yield of extracts was as follows.[13]

Table: 1 Percentage yield of extract of *Luffa Acutangula*

S. No.	Solvent	Colour & consistency	Percentage yield (W\W)
1.	Ethanol	Brown semisolid mass	**14.4**

Table: 2 Qualitative Phytochemical Evaluation of *Luffa acultagula.*[14]

S.No	Parameters	Value
1	**Alkaloid**	+
2	**Carbohydrates**	+
3	**Glycosides**	-
4	**Flavonoids**	++
5	**Tannins & Phenolic compounds**	+
6	**Proteins & Amino acids**	+
7	**Saponins**	+
8	**Sterols or Triterpenes**	+

PHARMACOLOGICAL EVALUATION (Analgesic activity)

Hot plate Method[15]

The analgesic activity of ethanolic leaves extract of *Luffa acutangula (L) Roxb.* was assessed using hot plate method in Albino rats. The ethanolic leaves extract of *Luffa acutangula (L) Roxb*. Showed significant analgesic activity at 200 and 400 mg/kg. Analgesic activity was comparable with standard drug pentazocine. Among the two doses, 400 mg/kg showed maximum analgesic activity at reaction time 120 min (7.2±0.44) is slightly lower than the standard drug pentazocine (9.9±0.34) in this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally active drugs. In the present study, all extracts showed significant ($p<0.05$ and $p< 0.01$) analgesic activity but among the two doses, 400 mg/kg showed highest analgesic activity at reaction time120 min.

Table: 3 Analgesic effect of ethanolic extract of of Luffa acutangula (L) Roxb on hot plate test

GROUP	Paw licking or jumping in seconds			
	30min	**60min**	**90min**	**120min**
Group-I Control	2.2±0.22	2.6±0.12	2.9±0.21	2.8±0.10
Group-II Pentazocine (3mg/kg)	2.8±0.18	6.9±0.62**	9.8±0.64**	9.9±0.34**
Group-III EELA (200mg/kg)	2.7±0.20	3.7±0.15*	4.6±0.21**	4.1±0.41**
Group-IV EELA (400mg/kg)	2.8±0.14	5.8±0.37**	7.4±0.39**	7.2±0.44**

Values were mean ± SEM, (n=6), *P<0.05 **P<0.01 Vs control and data were analyzed by using One-way ANOVA followed by Dunnett's test.

Tail Immersion Method[16]

There was a significant reduction of pain full sensation due to tail immersion in warm water. The maximum inhibitory effect of *Luffa acutangula (L) Roxb*. Showed significant ($p< 0.01$) at 90 min post dose in400 mg/kg.

The maximum anti- nociceptive properties of the plant extract(3.5±0.04) were not as effective as that of pentazocine, 3 mg/kg (5.8±0.06)

Table: 4 Analgesic effect of ethanolic leaves extract of *Luffa acutangula (L)Roxb* on tail immersion method in rats

GROUP	Mean latency to tail immersion in min.				
	0 min	30min	60min	90min	120min
Group-I Control	1.5±0.04	1.4±0.02	1.6±0.01	1.6±0.03	1.7±0.04
Group II Pentazocine (3mg/kg)	1.8±0.06	2.6±0.04**	4.2±0.02**	5.8±0.06**	5.4±0.02**
Group III EELA (200mg/kg)	1.2±0.02	1.9±0.01*	2.1±0.04*	2.4±0.02	2.8±0.04*
Group IV EELA (400mg/kg)	1.4±0.01	2.0±0.04*	2.6±0.01**	3.5±0.04**	3.2±0.01**

Values were mean ± SEM, (n=6), *P<0.05 **P<0.01 Vs control and data were analyzed by using One-way ANOVA followed by Dunnett's test.

(Anti-Inflammatory Activity)

Carrageenan-Inuced Paw Edema in Rats[17]

The anti-inflammatory effect of the ethanolic leaves extract of*Luffa cautangula (L.) Roxb* on carrageenan – induced hind paw edema. The ethanolic leaves extract of *Luffa acutangula (L.) Roxb*. at doses 200 and 400 mg/kg produced a significant effect aganist carrageenan induced inflammatory effect . The dose of 400 mg/kg exhibited a significant inhibition of 48 % after 3 h, the effect increased after 3h (52%). Anti-inflammatory activity of ethanolic extract of *Luffa cautangula (L.) Roxb*. showed significant and similar to that of indomethacine (10 mg/kg).

Table: 5 Anti inflammatory activity of ethanolic extract of *Luffa acutangula (L)Roxb* on Carrageenan induced paw edema method in rats.

GROUP	Paw thickness in mm					% Inhibition at 3hr
	0 hr	1hr	2hr	3hr	4hr	
Group-I Carrageenan (control)	14±0.03	3.4±0.06	4.9±0.06	6.4±0.05	4.8±0.02	------
Group-II Diclofenac sodium (10mg/kg)	1.4±0.04	2.2±0.03**	2.9±0.04**	3.1±0.02**	2.2±0.04**	52
Group-III (200mg/kg)	1.2±0.02	3.0±0.04	4.2±0.03	4.7±0.01*	3.5±0.04**	27
Group-IV (400mg/kg)	1.1±0.01	2.7±0.04**	3.5±0.02*	3.3±0.06**	2.8±0.04**	48

Cotton Pellet-Induced Granuloma Method in Rats[18]

The anti-inflammatory effect of the ethanolic leaves extract of *Luffa acutangula (L.*) Roxb. assessed by using cotton pellet induced granuloma method in rats. The ethanolic leaves extract of *Luffa acutangula (L.) Roxb*. Showed significant anti-inflammatory activity at 200 and 400 mg/kg dose. After 7 days, the mean weight of granulomatous tissue surrounding the threads was significantly lower for the group treated with Luffa acutangula (L.) Roxb. extract as compared to the control group. Among the two doses 400 mg/kg showed maximum decreased formation of granuloma tissue. The results indicate that Luffa acutangula (L.) roxb. at dose level of 200mg/kg and 400 mg/kg produced a significant decrease in the weight of granuloma 38.16±0.04 (7.4% inhibition) and 34.58±0.04 (16.1% inhibition) respectively. Among the two dose 400 mg/kg showed the slightly lower reduced weight of granumola than standard drug diclofenac Sodium 28.92±0.04 (29.8% inhibition)

Table: 6 Anti inflammatory activity of ethanolic extract of *Luffa acutangula (L) Roxb* on cotton pellet induced granuloma model rats

GROUP	Granuloma weight (mg)	% Inhibition
Group-I Control	41.24±0.04	……
Group-II Diclofenac Sodium(10mg/kg)	28.92±0.04**	29.8
Group-III EELA 200mg/kg	38.16±0.04**	7.4
Group-IV EELA 400mg/kg	34.58±0.04**	16.1

DISCUSSION

The inflammation is complex process, which is frequently associated with pain and involves several events, such as the increase of muscular permeability, increase of granulocytes and mono nuclear cell migration, as well as the granulomatous tissue proliferation. Pain is subjective experience, which is difficult to define exactly even though weall experience it. Pain distinguished as two types, peripheral or neurogenic pain may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by different inputs pain sensation.

The hot plate model was selected to investigate central antinociceptive activity because it has several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibits prostaglandin synthesis. A number of phenolic compounds have been reported to produce analgesic activity. Other studies have demonstrated that various flavanoids such as rutin, quercetin, luteolin, biflavonoids and triterpenoids produced significant antinociceptive effect. As phytochemical test showed presence of flavonoids and tannins in ethanolic extract of *Luffa acutangula (L) Roxb*, they might suppress the formation of prostaglandin and bradykinins. The centrally acting analgesic activity of the extract was also corroborated in our study by tail immersion test results.[19] The fact that in thermal stimuli (hot plate & Tail immersion tests), the anti nociceptive effect should be shown by acting centrally on opioid receptors. Since the drugs had shown the analgesic activity in tail immersion test, it seems that the ethanolic extract can act centrally. Taking this in to consideration the ethanolic extract of *Luffa acutangula (L) Roxb*, posses peripheral and central analgesic properties.[20]

The ethanolic extract of *Luffa acutangula (L) Roxb* showed anti- inflammatory activity on an acute inflammatory process like in carrageenan induced paw edema in rats paw. It is well known that leukocytes migration to the injured tissues in an important aspect of the inflammatory process. These cells can be either spread or in granulomaform. The *Luffa acutangula (L) Roxb* extract showed significant anti- inflammtory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. It reflected its efficacy ininhibiting the increase in the number of fibroblasts and synthesis ofcollagen and mucopolysaccharide during granuloma tissue formation.[21]

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