PHYTOCHEMICAL SCREENING OF *LEUCAS ASPERA* AND THEIR ANTI-INFLAMMATORY ACTIVITY.

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ABSTRACT

*Leucasaspera*commonly known as'thumbai' distributed throughout India from the Himalayas down to Ceylon.The plant is used asantibacterial and anti-inflammatory treatment *etc*. Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. Phytochemical tests ofPetroleum ether and chloroform extracts showed the positive tests for steroids, the ethanol extract showed positive tests for alkaloids, steroids, carbohydrates, flavones, glycosides and saponins, and the distilled water extract showed positive tests for amino acids, proteins, glycosides and saponins. The active moieties have been isolated and further tested for their anti-inflammatory activity in various animal models. Various herbal medicines reported for the anti-inflammatory activity are listed in The Present Study.Hence, concluded that plant extracts showed anti-inflammatory and antibacterial properties due to the presence some phytochemical compounds.

Key words:*Leucasaspera*,Phytochemical Screening, Anti-inflammatory activity,

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INTRODUCTION

*Leucasaspera*Spreng (Labiatae) is an annual herb found throughout India as a weed in cultivated fields, waste lands and roadsides. Its leaves, roots, stalks, young shoots and flowers have been used for wide range of medicinal properties (Kirtikar and Basu, 1991).*Leucasaspera*commonly known as'thumbai' distributed throughout India from the Himalayas down to Ceylon (Prajapathi, M.S et al., 2010). The plant is usedas an insecticide and in traditional medicine forcoughs, colds, painful swellings and chronic skineruptions (Chopra, R.N *et al*.,2002). Traditionally, the decoction of thewhole plant is taken orally for analgesic antipyretic, antirheumatic, anti-inflammatory and antibacterial treatment *etc.* and its paste as an ointment is applied topically to inflamed areas (Gani,k A. (1998) The aim of this present study is to identify the phytochemical components of *Leucasaspera*and to determine the antimicrobial effects of the plant .

*Leucasaspera*Spreng whole plant

- **VERNACULAR NAMES :**
- Sanskrit: Dronapushpi, Chitrapathrika, Chitrakshup
- Punjabi: Guldor
- Bengali: Darunaphula, Hulkasha
- Gujarati: Kulnnphul
- Hindi : Gomamadhupati
- Sindhi : Kubo
- Maharashtra: Bahuphul
- Bombay : Tumba
- Telugu : Thummichittu.

SOURCE:R. Srinivasan, *et al.*,2011

Botanical Explanationof*Leucasaspera:*

*Leucasaspera*is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches.

LEAVES: Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long.

FLOWERS: Flowers are white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs

CALYX: Calyx variable, tubular, 8-13 mm long; tube curved, contracted above the nutlets, the lower half usually glabrous and membranous, the upper half ribbed and hispid; mouth small, very oblique, not villous, the upper part produced forward; teeth small, triangular, bristle-tipped, ciliate, the upper tooth being the largest.

COROLLA: Corolla 1 cm long; tube 5 mm long and pubescent above, annulate in the middle; upper lip 3 mm long, densely white-woolly; lower lip about twice as long, the middle lobe obviate, rounded, the lateral lobes small, subacute.

FRUIT: Fruit nutlets, 2.5 mm long, oblong, brown, smooth, inner face angular and outer face rounded. (Kirtikar KR, Basu BD (1975); Hooker JD.(1984).

Literature survey revealed that ethanol extract of leaves of *Leucasaspera* has been found to possess antibacterial activity (Shirazi, 1947), whereas chloroform and petroleum ether extracts possess fungistatic as well as fungicidal activities (Thakur *et al.*, 1987). The plant is fragrant and used as potential herb, it is eaten in times of scarcity. This plant has long been used as an antipyretic remedies in south India, the leaves are said to be useful in chronic rheumatism. The juice is used as an external application for psoriasis, chronic skin eruptions and painful swellings, flowers are given with honey for cough and cold in children in North Bengal(Kirtikar and Basu, 1991).

Phytochemical studies reveals that,The whole plant powder of *Leucasaspera* has been reported to have insecticidal properties and Methyl-*O*-acetyloleolanolate, Methyl-*O*-acetyl ursolate and *β*-sitosterol acetate compounds were isolated (Chaudhry and Ghosh, 1969). The glycosides containing galactose and D-fructose have been isolated from aqueous extract of the plant (Chatterjee and Patil, 1994). Mixture of the sterols having β-sitosterol and other uncharacterised compounds have been isolated from the benzene extract of the whole plant of *Leucasaspera*. This mixture has shown antibacterial activity against *micrococcus pyogenes* and *Escherichia coli* (Chatterjee and Patil, 1994; Khaleque, 1970). The mixture of essential

oils which has antifungal activity against various pathogenic fungi was isolated (NarasimhaRao*et al.,* 1972). A triterpenoidleucolactone and two long chain compounds, I-hydroxy tetratriacontan-4-one, and 32-methyl-tetratriacontan-8-ol, were isolated from root and shoot part of *Leucasaspera,* respectively (Pradman, 1990). Two new aliphatic enols were isolated from the shoots of the same plant 28-Hydroxy pentatriacontan-7-one and 7-hydroxy dotriacontan-2-one has been also isolated together with 5-acetoxytriacontan and β-sitosterol (Mishra *et al.,* 1992).

The plant *Leucasaspera* has been screened for its antifertility activity. The various extracts of the same plant have been studied for their fungistatic and fungicidal activities. The smoke of the leaves prevents filarial vector mosquito (Pandian, 1994).

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent (Robbins and Cotran, 1995).

Tissue injury caused by introduction of a foreign antigen, trauma or local exposure to certain chemical triggers a complex process of inflammation. This may consist of a fluid stasis as well as accumulation of several cellular and non-cellular elements of immune response. The prominent cellular elements invading the injury sites are lymphocytes, monocytes, macrophages and the various granulocytes (Conner and Grisham, 1996).

A complex phenomenon of inflammation, characterized by erythrema, oedema, pain etc., involves endogenous chemical mediators such as histamine, serotonin, various chemothetic factors, bradykinin, leukotrienes and prostaglandins etc (Robbins and Cotran, 1995).

Phases of inflammation

Inflammation is commonly divided into three phases.

(a) Acute

(b) Sub-acute and

(c) Chronic inflammation

a.Acute inflammation

Acute inflammation is the initial response to tissue injury; it is mediated by the release of autocoids and usually precedes the development of the immune response. Acute inflammation is characterized by a sudden onset and has short course (a few minutes to several hours). The microscopic picture comprises of exudation of fluid, plasma and leukocytic emigration (Katzung, 1999; Rang *et al.,* 1999).

b. Sub-acute inflammation

It is said to last for one to six weeks (or more) and is usually seen in tubular structures like an appendix or fallopian tube. It is characterized by vascular exudative changes of acute inflammation and proliferative changes of chronic inflammation.Here, the exudative consists chiefly of eosinophils, lymphocytes, plasma cells, histocytes and fibroblasts (Dey, 1995).

c. Chronic inflammation

In this case, inflammation persists for months or years and is characterized by proliferation of connective tissue and blood vessels, with the presence of lymphocytes, plasma cells and histocytes but the absence of polymorphs (Dey, 1995).

Process of inflammation

It is clearly known that the inflammation involves various mechanisms that are continuously operating during the process.The histopathological and biochemical studies of inflammation indicate that it develops in two distinct events.

(a) Vascular events and

(b) Cellular events (leukocytic infiltration)

(a) Vascular events

Two sets of vascular events take place in acute inflammation.

i. Change in vascular flow

It consists of; (a) transient vasoconstriction of arteriols (b) persistent vasodilation and (c) slowing of the blood flow and stasis.

Following an injury there may be transient vasoconstriction of the arteriols, possibly due to neurogenic mechanisms or biochemical mediators (Spector and Willoughby, 1963). The persistent vasodilation is the fundamental event of inflammatory reaction and takes place immediately after vasoconstriction of arteriols. The progressive dilation of the blood vessels which involves mainly the arteriols but also to a less extent the venules and capillaries, develop within 30 minutes it appears to be due to kinins (Lewis, 1987) and prostaglandins (Smith *et al.,* 1974).

During slowing of the blood flow and stasis; the concomitant changes take place in vascular permeability factors, leading to the movement of fluid protein into the tissues (exudates).The escape of plasma into the tissue causes haem-concentration and consequently rises in the viscosity of the blood leading to increased resistance to flow in the venules with a corresponding rise in hydrostatic pressure in the capillary bed. The latter effect assists the expulsion of more plasma leading to further increase in viscosity, which leads to the stasis of blood flow.

ii. Change in vascular permeability

Increased permeability at the time of inflammation which leads to leakage of protein in fluid through endothelium has been recognized to occur in three phases: (a) the immediate transient response (Hurley *et al.,* 1967), (b) the immediate sustained reaction (Cotran*et al.,* 1994) and (c) the delayed prolonged leakage (Hurley *et al.,* 1967).

The chemical mediators involved are referred to as permeability factors and they are; Histamine, 5-hydroxytryptamine (5-HT), bradykinin, kallikrein, plasmin, prostaglandins particularly E_1 and E_2 and leukotriens.

b. Cellular events (Leukocytic infiltration)

The histopathologic feature of inflammation is the infilteration of leukocytes. In the early stage of inflammation specifically in bacterial infection, the predominant cell is the neutrophil while during resolution the predominant cell is the mononuclear phagocyte.

The main morphological features of emigration of leukocytes are well established. Escape of cells occurs from venules to veins of moderate size. It is seen that the leukocytes emigrate by acute amoeboid movement. In addition to leukocytes, some erythrocytes escape and in general the more severe damaging stimulus, the greater the number of erythrocytes that escape. The escape of erythrocytes is entirely passive, the cells being seen to be pushed by intravascular pressure through tiny breaches in the vascular wall.

In contrast with other type of leukocytes, lymphocytes were believed to migrate through the cytoplasm of endothelial cells (Wiener *et al.,* 1967) but, later it is proved that all leukocytes cross the blood vessels at inter-endothelial junction only (Schoefl, 1972).

The main mechanism involved in leukocyte migration at the site of inflammation is chemotaxion. The unidirectional migration of cells towards an attractant, or more simply, locomotion oriented along a chemical gradient is called as chemotaxis (Synderman and Mergehhagen, 1976).

The inert precursor serine esterase contained in neutrophil is converted to active enzyme after exposure of neutrophil to chemotactic factor. Inhibition of this active enzyme inhibits chemotaxis (Becker, 1977). Bacterial products and components of complement system were reported as significant chemotactic agents for neutrophil (Ward, 1974).

Mediators and events occurring in acute inflammation

Acute Inflammation

Mediators

Cellular changes

1) Margination

2) Adhesion

3) Migration / Chemotaxis

4) Recognition and Attachment

5) Killing / Degradation

Fever

1) Cell derived

- Histamine
- Serotonin
- Lysosomal enzymes
  - a) Acid proteases
  - b) Neutral proteases

Newly syntehsised

- Cytokines (TNFα, IL6)
- Nitric oxide
- Eicosanoids

a. Prostaglandins

b. Leukotrienes

c. Platelet activity factor

- Lymphocyte products

Lymph node permeability factor

- Miscellaneous

a. C-amp

b. Pyrogens

c. Leukocyte products-LTB41
LTC41
LTD4

- Oxygenmetabolites

2) Plasma derived

a) Complement activation

- C3A
- C5A
- C5B1-9

Vascular changes

Vasodilation

Altered vascular Permeability(Oedema)

1) Acute transient phase (early phase)

2) Immediate Sustained (Intermediate phase)

3) Delayed Prolonged (Late Phase)

Tissue Damage

Leukocyte activation

	b) Hageman activation	Factory
Pain	•Kinin system • Coagulation or Fibrinolytic product a) Fibrinopeptides b) Fibrin-Degradation products	

MATERIALS AND METHODS:

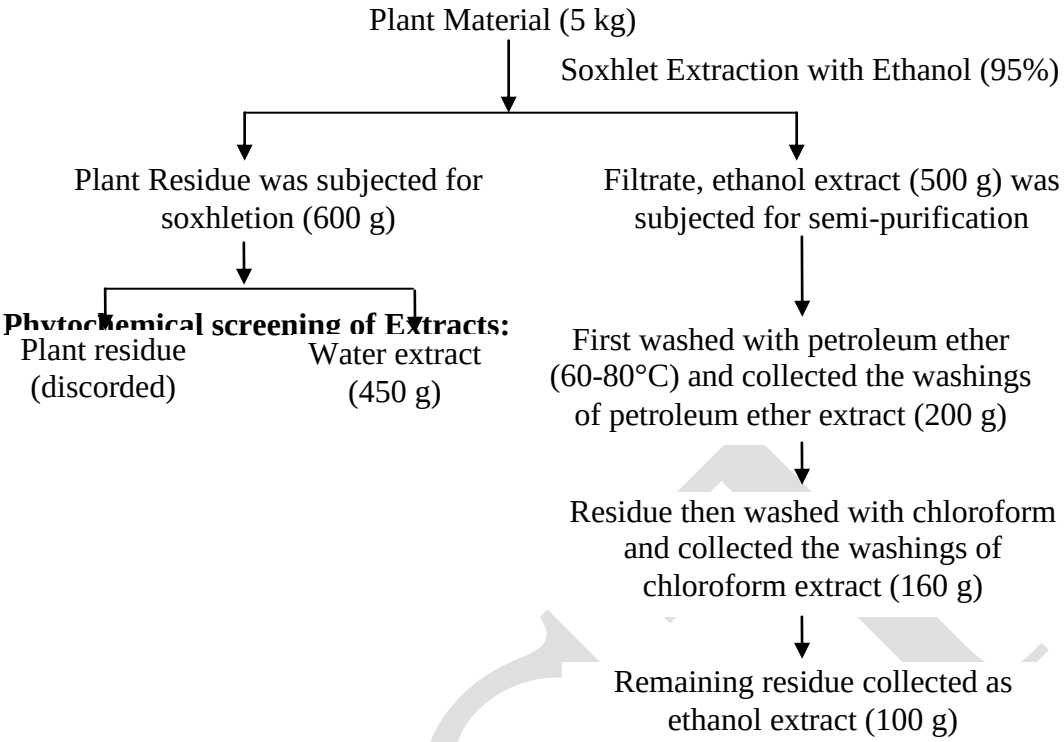
Collection, extraction and phytochemical screening of *L. aspera:*

The fresh whole plant of *Leucasaspera* was collected in the month of July and August in and around Sri Venkateswara University campus and authenticated at the Herbarium (Voucher No. 210), Department of Botany, S.V.University, Tirupati. Collected plants were immediately sprayed with alcohol to cease the enzymatic degradation of secondary metabolites. They were chopped into small fragments; thick stem, roots, twigs and other woody parts were chopped into 1" to 2" pieces and split longitudinally into several sections to enable early drying under shade. Chopping fresh plant materials at once after collection hastens drying and advantages on reduction in bulk and drying speed. The plant was kept under shade for drying for about 7 days.

Extraction of whole plant

The shade dried whole plant material (5 kg) was powdered and extracted with ethanol (95%). The ethanol extract (500 g) was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (40-50°C). The ethanol extract was purified as detailed below:

Scheme of extraction:



Phytochemical screening of Extracts:

The standard methods by were used to test for the presence of Phytochemical in the test samples.(Odebiyi, O.O. and E.A. Sofowora ;1978).Phytochemical screening of the extracts of the plant was carried out in order to know the class of organic compounds present in the different extracts of the plant selected for the study, which further facilitates for the identification of active constituents and their isolation.

Preparation of reagents for phytochemical study:

Following reagents and solutions, which are required for the phytochemical study, were prepared (Harborne, 1984).

Molisch's reagent: 10 g of α-naphthol dissolved in 100 ml 95% ethanol.

Anthrone reagent: 0.2 g of anthrone dissolved in 100 ml of concentrated H_2SO_4 and shaken (the solution should be freshly prepared).

Biuret reagent:1.5 g of $CuSO_4$, 6g of sodium potassium tartarate were dissolved in 500 ml of water, 300 ml of freshly prepared NaOH solution was added to this solution and solution made upto the volume of one litre.

Ninhydrin reagent: 0.1 % Ninhydrin dissolved in 100 ml of acetone.

Dragendroff's reagent: 50 g of tartaric acid was dissolved in 200 ml of distilled water. 4.5 g of basic bismuth nitrate was added and solution was shaken for two hours. 100 ml of 40%

potassium iodide was then added and solution was shaken vigorously and allowed to stand for 24 hours and filtered.

Mayer's reagent: 1.3 g of mercuric chloride and 5 g of potassium iodide were dissolved separately in 60 ml and 10 ml of distilled water respectively. Both were mixed thoroughly and diluted upto 100 ml with distilled water.

Phenolphthalein reagent: 1 g of phenolphthalein dissolved in 100 ml of ethanol.

Lead acetate (10%) solution: 10 g of lead acetate dissolved in 100 ml of methanol.

$FeCl_3$ (5%) solution: 5 g of $FeCl_3$ dissolved in 100 ml of ethanol.

Alcoholic KOH (0.1 N): 5.6 g of KOH dissolved in 100 ml of ethanol.

Gelatin solution containing 10% NaCl: 1 g of gelatin dissolved in 100 ml of 10% NaCl.

HCl solution: 50 ml of concentrated HCl dissolved in 50 ml of water.

NaOH solution (10%): 10 g of NaOH dissolved in 100 ml of distilled water.

$CuSO_4$ solution: 24.7 g of copper sulphate dissolved in 100 ml of distilled water.

Iodine in KI; Dissolve 20 g of KI and 12.7 g of iodine in 30 ml water diluted to one litre.

RESULTS AND DISCUSSION:

1.PLANTS SHOWING ANTI-INFLAMMATORY ACTIVITY

The plant kingdom is a rich source of biologically active agents revealing various types of pharmacological activities. The search for plant based agents for the control of human diseases is as old as is recorded history and was in practice in prehistoric times. India has a rich heritage of uses of medicinal plants for anti-inflammatory activity. Since last few decades plants have been systematically screened for anti-inflammatory activity.

The active moieties have been isolated and further tested for their anti-inflammatory activity in various animal models. Various herbal medicines reported for the anti-inflammatory activity are listed in Table.1

Ayurvedic medicine from the Indian subcontinent is suggesting important leads for researchers in this field. Ancient Indian literature contains information on large number of plants reported to have anti-inflammatory properties. The methanol extract of the leaves of *Chasmantheradependens* (OlungbengMorebise and Awe, 2000) and hydrosoluble fraction of Euphorbia royleana latex (Bani*et al.*,2000) have shown dose dependent inhibition of anti-inflammatory effects in different models in rats and mice.

Biochanin-A (5, 7-dihydroxy-4-methxoy isoflavone) isolated from the flowers of *Dalbergiasissoides* have shown their anti-inflammatory activity against PGE, bradykinin, 5-

HT and histamine induced rat hind paw oedema. It showed dose dependent inhibition on inflammation (Karimova*et al.,* 1966).

A bioflavonoid gossypin isolated from *Hibiscus vitifolius* Linn. (Parmar and Ghosh, 1978) was found to be significant in reduction of paw oedema and the increased vascular permeability induced by various phlogistic agents. It produced significant inhibition of the accumulation of pouch fluid and granulosa tissue formation in the carrageenan induced granulosa pouch in rats which would be attributed to the decreased formation of collagen tissue.

Jigrine (Karunakar*et al.,* 1997) a polypharmaceutical herbal formulation containing 14 medicinal plants had shown significant effect only on acute phase of inflammation caused by carrageenan.

Dried rhizome of *Zingiberofficinale*, dried stem of *Tinosporacordifolia* and dried leaves of *Vitexnegundo* has shown anti-inflammatory effect on carrageenan induced hind paw oedema and cotton pellet granulose in rats (Telang*et al.,* 1999).

Most species belonging to the *Scrophularia* genus have been used as anti-inflammatory drugs by folk medicine. Iridoids and phenyl propanoids are considered to be the active principle of these drugs (Garcia *et al.,* 1996; Fernandez *et al.,* 1998).

The roots of *Scrophularianingpoensis* have been used for the treatment of inflammation in Chinese traditional medicine (Kajimoto*et al.,* 1989). From this species were isolated harpagide, harpagoside, aucuboside, 6-*O*-methyl catalpol, ningpogenin, ningpogosideA, ningpogoside B, verbascoside, angoroside A and angoroside B (Kajimoto*et al.,* 1989; Zhang *et al.,* 1994; Qian*et al.,* 1992). Pharmacological investigations are necessary for evaluating their potential as anti-inflammatory agents. Alcohol extract of *Scrophulariaauriculata* showed marked effects on both acute and chronic models of inflammation (Cuellar *et al.,*1969).

Two catalpol derivatives 6-*O*-α-L-(2″-*O*-acetyl-3″-*O*-di-*p*-methoxycinnamoyl)-hamnopyranosylcatalpol and 6-*O*-α-L-(4″-acetyl-2″3″-di-*p*-methoxycinnamoyl)-rhamnopyronosylcatalpol, two saponinsverbascosaponin A and verbascosaponin B, two iridoidsscropolioside A and scrolevlentinoside isolated from *S. auriculata* species assayed with various acute and chronic experimental models showed anti-inflammatory activity against all the inducers with the exception of arachidonic acid (Giner*et al.,* 1991; 2000), *p*-coumaric, caffeic, ferulic, gentisic, protocatechuic, syringic and isovanillic acids isolated from *Scrophulariafrutescens* are moderate systemic anti-inflammatory agents but have a

strong anti-inflammatory effect when applied locally at the site of inflammation (Fernandez *et al.,* 1998).

Buddle jasaponine I, a biologically active compound from *Scrophulariascorodonia* Linn.exerts potent *in vivo* anti-inflammatory effects on mouse ear oedema. The screening for *in vitro* effects of this on cellular systems generating cyclooxygenase (COX) and lipoxygenase (LOX) metabolites showed a significant effect (Bermejo Benito *et al.,* 1998).

Seven iridoid glycosides isolated from different extracts of *Scrophulariascorodonial* namely, bartisioside, qucuboside, harpagide, harpagoside, 8-*O*-acetylharpagide, scorodioside and seropolioside B, have been evaluated for their *in vitro* anti-inflammatory activity in cellular systems generating cyclooxygenase (COX) and lipoxygenase (LOX) metabolites (Bermejo Benito *et al.,* 2000).

Table-.1: Plants showing anti-inflammatory activity

Name of the plant	Part used	Animal model	References
*Leucasasperas*Spreng	Leaves*	Rat	Srinivas*et al.,* (2000)
*Helitropiumindicum*Linn.	Leaves	Rat	Srinivas*et al.,* (2000)
Chasmantheradependens	Leaves	Rat/ Mice	OlugbengMorebise and Awe (2001)
Swertiachirata	Aerial	Rat	Shivaji Banerjee *et al.,* (2000)
Madhukalongifolia	Flower	Rat	Dineshchandra (2001)
*Euphorbia royleana*Latex	Whole plant	Rat / Mice	Bani*et al.,* (2000)
*Hibiscus vitifolins*Linn.	Flower	Rat	Parmar and Ghosh (1978)
*VitexNegundo*Linn.	Leaves	Mice	Telang*et al.,* (1999)
Zingiberofficinale	Rhizome	Rat	Utpalendu Jana *et al.,* (1999)
Tinosporacordifolia	Stem	Rat	Utpalendu Jana *et al.,* (1999)
Jigrine	Whole Plant	Rat	Karunakar*et al.,* (1997)
Raphanussativus	Leaves	Rat	Srinivas and Prakash (2001)
Pongamiapinnata	Seeds	Rat	Singh and Ghosh (1978)
*Sidarhombifolia*Linn.	Leaves	Rat	Kavimani*et al.,* (1997)
Dalbergiasissoo	Leaves	Mice	Singh and Ghosh, (1978)

Aloe vera Linn.	Whole plant	Rat	Tandon*et al.*, (1997)
Venegar	Whole plant	Rat	Tandon*et al.*, (1997)
MoringapterygospermaGaertn	Root	Rat	Raoand Mishra (1998)
Jasminofficinale	Whole plant	Rat	Adekile*et al.*, (1983)
Hodychiumapicatum	Leaves	Rat	Tandon*et al.*, (1997)
PlerygospermaGaertn	Roots	Rat	Rao and Mishra, (1998)
Rubiacardifolia	Leaves	Rat	Latha*et al.*, (1999)
Hemidesmusindicus	Leaves	Rat	Shah (1989)
Scrophularianingpoensis	Root	Rat	Kajimoto*et al.*, (1989) Qian *et al.*, (1992)
S. auriculata	Whole plant	Mouse	Cuellar *et al.*, (1998) Giner*et al.*, (1991)
S. frutescens	Whole plant	Rat	Garcia *et al.*, (1996) Fernandez *et al.*, (1996)
S. scorozonia	Whole plant	Mouse	Bermejo Benito *et al.*, (1998)

*(dried powder in gum acacia)

2. PHYTOCHEMICAL TESTING RESULTS OF *LEUCAS ASPERA* SPRENG.

Plants are important source of potentially useful structures for the development of newchemotherapeutic agents (Tona, L *et al*.,1998). The Phytochemicalscreening and quantitative estimation of the crudeyields of chemical constituents of the plants showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. Theywere known to show medicinal activity as well as exhibiting physiological activity (Sofowara, A. 1993).Tannins havebeen reported to prevent the development of microorganisms by precipitating microbial proteinand making nutritional proteins unavailable forthem(. Sodipo, O.A *et al*., 1991).Classes of alkaloids are among the major powerful poisons known (Fluck, H. 1973). Apart from being poisonous, some alkaloids have also been proved to be useful in correcting renal disorders (Konkwara, J.O. 1976). The alkaloids of *Leucasaspera*may be a poison that can be tried on loweror higher organism. Glycosides are also found topossess antimicrobial activity. The secondary metabolites identified in the plant materials usedin this study could be responsible forantimicrobial activity exhibited by these plants. In the present study,the ethanol crude extract of *Leucasaspera*leafwas exhibited the highest antimicrobial

activity .The aqueous extract of*Leucasaspera*plants studied here canbe used as a potential source of useful drugs for the treatments of the various enteric pathogens.

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The Phytochemical analysis of the plants assayed in this study revealed that *Leucasaspera.*Recent studies shows that The preliminary phytochemical analysis of the extracts of *L. aspera*revealed the presence of alkaloids, tannins, carbohydrate, proteins, steroids, saponins, phenolic compound, and flavonoids.(Tambe Vijay D; 2016).

Phytochemical tests of petroleum ether, chloroform, ethanol and distilled water extracts of *Leucasaspera* were carried out. The results of the phytochemical tests are shown in Table-2.

Petroleum ether and chloroform extracts showed the positive tests for steroids, the ethanol extract showed positive tests for alkaloids, steroids, carbohydrates, flavones, glycosides and saponins, and the distilled water extract showed positive tests for amino acids, proteins, glycosides and saponins. (Table.2)

Table-2: Phytochemical test of various extracts of *Leucasaspera*Spreng.

Plant Secondary metabolites	Petroleum ether extract	Chloroform extract	Ethanol extract	Water extract
Alkaloids				
1. Mayer's reagent	-	-	+	-
2. Wagner's reagent	-	-	+	-
3. Dragendroffs reagent	-	-	+	-
Steroids				
1. Libermann's test	+	+	+	-
2. LibermannBurchard test	+	+	+	-
Carbohydrates				
1. Molisch's reagent	-	-	+	-
2. Anthrone	-	-	+	-
Flavones				
1. Aqueous NaOH	-	-	+	-
2. Conc. H_2SO_4	-	-	+	-
3. Mg-HCl	-	-	+	-

Aminoacids and Proteins				
Ninhydrin reagent	-	-	-	+
Millon's reagent	-	-	-	+
Glycosides				
1. Keller-Kiliani test	-	-	+	+
Saponins				
1. Saponification test	-	-	+	+
Phenolic compounds				
1. $FeCl_3$ solution (5%)	-	-	+	-
2. Lead acetate (10%)	-	-	+	-

'+' indicates present; '-' indicates absent

CONCLUSION

This plant has a great importance for the Ayurvedic and Modern systems of formulations in which some have been patented for their better acceptability and therapeutic profile. Possible Anti-inflammatory activity substances contained in the extractsincluded tannins, Alkaloids and glycosidesPhenolic compounds.Therefore Present study of the Phytochemical investigation may serve as new suggests that constituents of the plantextracts could serve as a source of drugs useful inthe chemotherapy of enteric diseases. The positive Antimicrobial activity shows that whole herb extract can be useful for the formulation development against the specific microbial infections. These studies reveal that *L. aspera*is a source of medicinally active compoundsand have various pharmacological effects. Hence, this drug encourage finding its newtherapeutic uses.

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