International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1913-1918 www.ijprajournal.com ISSN: 2249-7781

Invitro Aticoagulant Activity of Leucas Aspera

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Submitted: 05-02-2023 Accepted: 20-02-2023

ABSTRACT: Leucasasperacommonly known as 'Thumbai' is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity. Further studies reveal the presence of various phytochemical constituents mainly alkaloids, saponins, glycosides, tannins, flavonoidsand carbohydrates.The current study has been taken up to evaluate the ethanol extract and aqueous extract ofleucasasperaleaves and stems for their role in the blood coagulation cascade. This study reveals that leucasasperais a source of medicinally active compounds and has various pharmacological effects; hence, this drug encourages finding its new therapeutic uses.

Keywords: Invitro, Lamiaceae,Leucasaspera, Extraction, Anticoagulant activity.

I. INTRODUCTION:

Blood clot (thrombus) developed in the circulatory system due to the failure of homeostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic disease[1-3]. Cardiovascular diseases (CVD)such as ischemia and myocardial infarction is one of the leading causes of death in developing countries. CVDs are caused duo to the blockage of arteries and veins with thrombi or blood clots. Thrombus formation is caused by the activation of blood coagulation factors during vascular injuries or imbalance in endogenous anticoagulants. The major causative agents for the aggregation of prothrombotic molecules in blood are oral contraceptives, heavy smoking, surgery, trauma, infection and other chronic inflammatory disorders [4-6]. Generally, the intrinsic/contact phase pathway of blood coagulation is triggered by the exposure of tissue factor during vascular injury. Factor VIIa binds to the tissue factor and thereby activates factor X and factor IX. Factor Xa converts the inactive prothrombin (factor II) to active thrombin (IIa). Thrombin activates both the platelet-mediated primary hemostasis and clotting factor-mediated secondary hemostasis. The activated platelets also activate the secondary hemostasis leading to the formation of a blood clot or thrombus. Anticoagulant drugs are used for prophylaxis and to treat venous thromboembolism (VTE), pulmonary embolism (PE), atrial fibrillation (AF), and other thrombolytic disorders. Anticoagulants directly or indirectly interact with the coagulation factor to inhibit thrombogenesis. The currently used anticoagulants in pharmacological and medicinal application are unfractionated heparin (UFH), low molecular weight heparin (LMWH), fondaparinux, hirudin, and argatroban[7-10]. The limitation of the existing anticoagulants is spontaneous hemorrhage and osteoporosis. There is a need to identify an anticoagulant molecule with fewer or no side effects. Medicinal plants were mainly used to treat different ailments by traditional medicinal practitioners[11,12]. Currently, these plants were studied for the presence of therapeutically important molecules. The current investigation shows leaves and stems parts of Leucasaspera have potent anticoagulant activity further study is needed to explore its full potential.

II. MATERIALS AND METHODS:

Collection of materials:

The leaves and stem parts of Leucasaspera(Willd.) Link was collected from surrounding areas of Jambai, Erode district, Tamil Nadu, India. The herbarium of these plants was identified and authenticated by Dr.B.Elayaraj.,M.Sc.,M.Phil.,B.Ed.,Ph.d., PG assistant in biology, A.R.R.Govt.Hr.Sec.School, Karanaiperichanur, Villupuram District – 605755.

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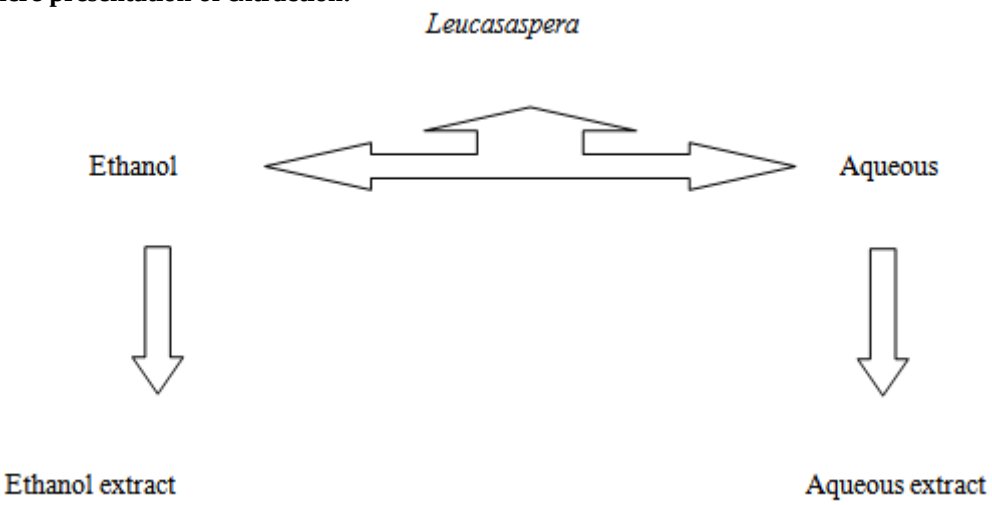
Sample collection:

The plant leaves and stems were collected, separated, and washed under the running tap water to remove dust and sand particles and later rinsed with distilled water. Then they were shade dried for two to three days at room temperature. The dried leaves and stems were ground into a course powder and used for extraction.

Preparation of extract:

The course powdered Leucasaspera sample (50 g) was taken. And then the dried powdered material was extracted with Ethanol and Water by using soxhlet apparatus. The solvent was distilled off and the resulting semisolid mass was dried under shade in a desiccator to get a yield.

Schematicre presentation of extraction:



EthanolExtract of *LeucasAspera*

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WaterExtractof*LeucasAspera*

TESTING FOR ANTICOAGULANT ACTIVITY:

Bloodsamplecollectionandbloodanalysisstudypopulation:

Blood samples was obtained from 20 – 25 years old participants, were used to assess the anticoagulanteffectofLeucasAspera.Theyhadbeenchosenforthisstudyaccording to the following criteria: having normal prothrombin time, not suffering from anycardiovascular diseases (hypertension, congestive heart failure, coagulation disorder such as,hemophilia A or B) or diabetes, not recently using non-steroidal anti-inflammatory drugs, notobese or smokersandfreefromdyslipidemicdisorders.

Collectionofbloodsamples:

The blood samples were obtained from normal individuals by using sterile syringe,withdrawn from vein of left arm of each individual and placed separately in containerscontaining tri-sodium citrate to prevent the clotting process. Centrifugation (15 minutes at rate3000rpm)wascarried outto separatetheblood cellsfromplasmainordertoobtainpureplateletplasma (PPP) for prothrombin time test. The obtained plasma sample of each individual werepouredseparatelyin planecontainersusing automaticpipetteand storedat roomtemperature.

Apparatus:

Steriledisposableprickingneedle, stopwatch, dryglass capillarytube(narrowdiameter1top2mm10cmlong), cottonswabofabsorbentcotton, spiritwettedcottonswab.

Chemicals:

70% v/vethylalcoholor70%v/vdenatured spirit, Distilledwater.

Collectionofbloodandplasmare-calcification:

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of$CaCl_2$(25 mM) were added together in a clean fusion tube and incubated at 37°C in water bath.For control experiment extract solution as replaced by same volume of 0.9% saline water. Theclotting time was recorded with stopwatch by titling the test tubes every 5 seconds. This time iscalledtheprothrombintime.

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III. RESULTS:

PRELIMINARY PHYTOCHEMICAL SCREENING OF Leucasaspera

Sr.No	PHYTO CHEMICAL CONSTITUENTS	ETHANOL EXTRACT	WATER EXTRACT
1	Alkaloids	+	+
2	Saponins	+	+
3	Glycosides	+	+
4	Carbohydrate	+	+
5	Tannins	+	+
6	Flavonoids	+	+
7	Steroids	–	–
8	Proteins	–	+
9	Triterpenoids	–	–
10	Fixed oils & Fats	–	–
11	Gums & Mucilage	–	–

+PRESENT -ABSENT

The preliminary phytochemical studies were reported. The ethanol extract showed the presence of alkaloids, saponins, glycosides, carbohydrate, tannins, and flavonoids. The water Extract showed the presence of alkaloids, saponins, glycosides, carbohydrates, tannins, flavonoids, proteins.

INVITROANTICOAGULANTACTIVITYOF Leucasaspera

The scavenging the stable ethanol and water extract to evaluate the free radical scavenging ability of various sample including plant extract shows in the table.

EELA – Ethanol Extract of LeucasAspera
AELA – Aqueous Extract of LeucasAspera
EDTA – Ethylene Diamine Tetra Aceticacid

Name of the extract		Amount of plasma	Amount of extract	Amount of calcium chloride	Time of coagulation (minutes)
Control	-----	0.2 ml	-----	0.2 ml	0.57
EELA	100 µg/ml	0.2 ml	0.1 ml	0.2 ml	1.59
	200 µg/ml	0.2 ml	0.1 ml	0.2 ml	2.38
	300 µg/ml	0.2 ml	0.1 ml	0.2 ml	3.56
	400 µg/ml	0.2 ml	0.1 ml	0.2 ml	4.31

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	500 µg/ml	0.2 ml	0.1 ml	0.2 ml	5.56
WELA	100 µg/ml	0.2 ml	0.1 ml	0.2 ml	1.47
	200 µg/ml	0.2 ml	0.1 ml	0.2 ml	2.18
	300 µg/ml	0.2 ml	0.1 ml	0.2 ml	3.14
	400 µg/ml	0.2 ml	0.1 ml	0.2 ml	3.51
	500 µg/ml	0.2 ml	0.1 ml	0.2 ml	4.11
EDTA	100 µg/ml	0.2 ml	0.1 ml	0.2 ml	7.08

We have two concentration of the solution that is ethanol and aqueous concentration. We have observed the activity of different concentration of anticoagulant in both EELA and AELA after the experiment. We have noticed that the ethanol preparation has more anticoagulant activity than aqueous preparation in all concentration (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml).

IV. CONCLUSION:

The present study was aimed to evaluate the anticoagulant activity ofEELAandAELA fractionsofleavesand stemsandalsoactivityofthefractionarecompared.The EELAactivitywasmorepotentthanAELA.The detailed information in this review shows the potential therapeutic values and is arich source of biological active compounds. Considering the easy availability of Leucasasperain our country, it seems that still there is a scope for scientific studies to fully exploit itsmedicinalpropertiestosupporttraditionalclaimsas wellasexploringsomenewandpromisingleads.Itwillp rovide apathwayforfuturestudy.Thepharmacologicalstudie ssofarhave mostlybeenperformed for volatile principle of plant. In future study, the isolated principle of extractofLeucasasperaneedstobe evaluatedinscientificmanner.Itcouldbe concludedthattheLeucasasperais a rich source of compounds, interesting chemical structure and variousbiologicalactiveproducts.

V. ACKNOWLEDGEMENT:

We would like to thank the members of Vyrathi Charitable trust & the management and faculties of SSM College of Pharmacy.

DISCLOSURE OF CONFLICT OF INTEREST:

All authors have declared no conflict of interest.

STATEMENT OF ETHICAL APPROVAL:

The present research work does not contain any studies performed on animal's/humans subjects by any of the authors.

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