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THE HEMOSTATIC ACTIVITY OF GAMBHARI PHALA (GMELINA ARBOREA ROXB.) – INVITRO STUDY

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ABSTRACT

Medicinal plants with a variety of phytochemical ingredients remain a potential source for new drug discovery. The use of medicinal herbs in a wide range of diseases and symptoms, such as bleeding is prevalent in traditional and ethno medicine worldwide. There are only few drugs in *Ayurveda* having hemostatic activity. *Gambhari* is one among the potent and widely used drug in *Ayurveda* with promising hemostatic effect. It's one among the *Dashamoola*. *Gambhari phala* is mentioned as *Agrya* for *raktapitta* and *Raktasangrahi* by *Acharya Charaka*. The drug, Gambhari phala selected have *kashaya madhura rasa, sheeta veerya,* and *madhura vipaka* and pitta hara *karma* is assessed to have hemostatic activity in their respective aqueous and alcohol extracts. Aqueous and alcohol extracts of *Gambhari phala* prepared by Soxhlet extraction and invitro study for screening hemostatic activity was carried out by Lee white method. The aqueous and alcohol extracts of *Gambhari phala* were subjected to analytical evaluation. Invitro study revealed the presence of hemostatic activity. Presence of tannins, saponins, flavonoids, alkaloids, calcium ions are responsible for the hemostatic activity of aqueous and alcohol extracts of *Gambhari phala.*

KEYWORDS: *Gambhari phala*, Hemostatic activity, Aqueous and alcohol extracts, *Ayurveda*, In vitrostudy.

INTRODUCTION

The early bleeding control is essential since blood is valuable essence of life and uncontrollable hemorrhage could lead to life threatening conditions. Hemostatic agents can act through clotting factor activation, Vasoconstriction, platelet aggregation or anti-fibrinolytic activity. Unfortunately, none of the available hemostatic agents are perfect and each had their own drawbacks. For instance, biological agents like fibrin and thrombin are expensive and potential to transfer viral infections, as they are derived from human or bovine blood. Therefore, finding a new effective hemostatic agent with less adverse effect and disadvantages seems to be substantial. Since the use of herbal medicines is getting popular worldwide and with long history of ethno medicinal plants in controlling bleeding, *Gambhari phala* being an easily available drug and classically mentioned as *Agrya* for *Raktasangrahi* and *Raktapitta prashamana* by *Acharya Charaka* can be attributed for hemostatic activity.[1] The *kashaya, madhura rasa, sheeta veerya, madhura vipaka* and *Pittahara karma* of *Gambhari phala*[2] can help in producing hemostatic activity.

Gambhari phala also mentioned as an ingredient in various formulations like *Usheerasava, drakshadi kashaya, Sarivadi kashaya*[3] etc. indicated in *Raktapitta* (bleeding disorders) and also in several context of *Raktapitta chikitsa.* By considering all these the present research topic entitled "The hemostatic activity of *Gambhari phala (Gmelina arborea Roxb.)* - In vitro study" was carried out by Lee -white method.

METHODOLOGY

- Aqueous extract and Alcohol extract of *Gambhari phala* was prepared by Soxhlet extraction method.[4]
- The sensory and phytochemical evaluation of *Gambhari phala* was carried out.
- The In vitro study for hemostatic activity of aqueous and alcohol extracts of *Gambhari phala* was carried out using Lee- white method.[5]

Soxhlet extraction method- Preparation of aqueous and alcohol extracts of *Gambhari phala*

The aqueous and alcohol extract was carried out by Soxhlet extraction. The standard operating procedurewas followed.

Materials required

Coarsely powdered drug, solvent, Soxhlet apparatus, glass beaker

Procedure

- The solvent, 1000ml water or alcohol was taken in the round bottom flask.
- 100gms of dried, coarsely powdered drug kept on a piece of cotton in the extractor
- In order to avoid bumping of the solvent while boiling 3-4 pieces of small porcelain chips werekept inside the round bottom flask
- Soxhlet apparatus was set up.
- Care was taken for continuous flow of water in the condenser.
- Continuous extraction was carried out at 100°C till the complete extraction process.
- Complete extraction was confirmed when the extract in the syphon tube was colorless.
- The extracted liquid was transferred into a beaker, kept over water bath and evaporated to obtainextract.
- The weight of the residue was noted.

Lee-white methodPrinciple

The venous blood is collected in the tubes and kept on water bath at 37°c and time is noted from the timeof vein puncture till the blood clots.

Requirements

Eppendorf tubes, Blood sample, Syringes, Micropipette, Pipette tips, Stopwatch, Water bath.

Procedure

Five healthy individuals were selected for getting the blood samples.

- Eppendorf tubes were taken with the internal diameter 8mm and placed in 37°C water bath.
- A clean dry syringe was used to draw the venous blood.
- Stop watch was used to note the time immediately after drawing the blood.1ml of venous blood was put into Eppendorf tube, taken as control.

The Eppendorf tube was observed at 30 seconds of interval to note the clotting time by gentle tilting the tube.

- Time was noted when no blood flows even after the inverting the tube. This was noted as the clotting time.
- Then to other tube, 100 microliters of each Aqueous extract and Alcohol extract of both trial drugs and 1ml of blood was added.
- The tube was observed for clotting after 30 sec by gently tilting the tube.
- The sample procedure was repeated.
- Mean value of the five samples were calculated for comparison.

RESULT

Results of *Gambhari phala* aqueous and alcohol extracts for hemostatic activity carried out by Lee-white method revealed as following,

Table no. 1: Showing characteristics (Sensory evaluation) of aqueous and alcohol extracts of *Gambhariphala.*

Observations	Aqueous extract of *Gambhari phala*	Methanol extract of *Gambhari phala*
Quantity of drug	20 g	40g
Extract obtained	2.83g	2.98g
Yeild	14.1%	7.45%
Colour	Black	Brown
Consistency	Semisolid	Powdery
Odour	Characteristic fruity odour	Characteristic fruity odour

Figure No. 1: Showing fresh fruits of Gambhari, Dry Fruits and Powder of Gambhari phala.

- Phytochemical screening of both the extracts of *Gambhari phala* reveals the presence of primary metabolites like carbohydrates, reducing sugar and quinones and secondary metabolites like alkaloids, flavonoids, saponins, tannins, glycosides, steroids, and resins. Inorganic constituents like Calcium, magnesium, sodium, potassium and iron.

In-vitro study: Hemostatic activity of aqueous and alcohol extracts of *Gambhari phala.*

Table No. 2: Screening of Hemostatic activity of Aqueous and Alcohol extracts of *Gambhari phala* by Lee and white method.

Sample No:	Only blood (Control group)	Blood with extract	
		Gambhari phala Aqueous	*Gambhari phala* Alcohol
1	4min 10 sec	4min 16 sec	5 min 10 sec
2	4min 15 sec	4min 22 sec	5 min 32 sec
3	4min 40 sec	4min 49 sec	6 min 05 sec
4	5 min	5 min 13 sec	6 min 15 sec
5	5 min 05sec	5min 15 sec	6min 1 sec

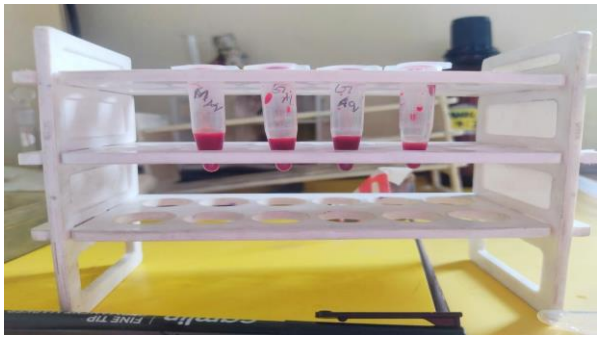

Figure no. 2: Showing blood samples with *Gambhari phala* extracts.

DISCUSSION

Gambhari,one among the *Dashamoola* and *Gambhari phala* ingredient of *madhura triphala*,easily and abundantly available drug.It is also used in various formulations and mentioned as one among *Agrya* for *Raktasangrahi* and *Raktapitta prashamana* is screened for its hemostatic activity -invitro using Lee-white method.

Lee-white method was followed because it's the ideal and suitable method for screening hemostatic activity.In Lee-white method with healthy volunteers showed a remarkable result with aqueous extract of *Gambhari phala* compared to its alcohol extract.

Mean value of blood clotting time of control group was 4.63 min. *Gambhari phala* Aqueous extract showed mean value of blood coagulation time with in 4.73 min, much similar to the control group (mean value 4.63 min) and Alcohol with mean value of 5.87 min .Presence of alkaloids, tannins, saponins, glycosides, flavonoids, phenolic compounds, quinones and triterpenoids in might be contributing for its hemostatic action.[6] Presence of Calcium in the extracts, one among the clotting factors (Clotting factor IV) and it helps by activating several coagulation factors and platelets. Calcium also plays an important role in conversion of prothrombin to thrombin in presence of prothrombinase and in conversion of solublefibrinogen to insoluble fibrin.[7]

Madhura rasa does *Raktavardhana, balya, Sandhanakara karma. Madhura rasa has prithvi and jala mahabhuta sanghatana, due to this they possess snigdha, sheetha, guru, sthira and sanghatakara guna* which could alter the *dravatva guna* of *rakta dhatu*.[8] It could also help in promoting tissue granulation and helps in arresting bleeding. *Kashaya rasa* does *sthambana, sandhaniya, raktapitta prashamana and asra vishodhana karma*. *Kashaya rasa* has *vayu* and *prithvi mahabhuta sanghatana*, due to this they possess *sthira, guru, ruksha, sheeta and sanghatakara guna* by which they perform *sthambana* and *shoshana karma*.[9] It could be related with vasoconstriction action. *Sandhana karma* of *Madhura* and *Kashaya rasa* helps in tissue binding action *(Sandhana-Shareere ante samhatikara bhava -by Indu)* and promote coagulation of blood. *Sheetha veerya* does *sthambana karma (Stambanam stambayathi yat gati mandam chalam druvam-Ch. Su 22/11-12)* [10]which could act on aggravated *rakta dhatu*, and does vasoconstriction.

The relatively prolonged clotting time with Alcohol extract could be because of the presence of alcohol, which is a known blood thinner and could probably delay the fibrin clot formation. The other reason might be, the phytoconstituents responsible for hemostatic activity like tannins etc. might be more solublein aqueous media than in alcohol, causing better hemostatic action of the drugs in their aqueous media.

By considering the above-mentioned rasa panchaka and phytoconstituents, the aqueous and alcohol extracts of *Gambhari phala* exhibited Hemostatic activity.

CONCLUSION

Finding a new effective hemostatic agent with less adverse effect and disadvantages could be a great boonto the science. The Hemostatic activity carried out for *Gambhari phala* has given significant pharmacological activity, which can be further screened for its efficacy in animal as well as clinical models also in various dosage forms.

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