Research Article

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EXPLORING THE HAEMOSTATIC POTENTIAL OF PANCHAVALKALA KASHAYA AND PANCHAVALKALA AQUEOUS EXTRACT: AN *IN VITRO* STUDY

Aiswarya Rose [1]*, Sreejith Satheesan [2]

[1] PG Scholar, Department of Shalya Tantra, Pankajakasthuri Ayurveda Medical College and PG Centre, Thiruvananthapuram, Kerala, India

[2] Associate Professor, Department of Shalya Tantra, Pankajakasthuri Ayurveda Medical College and PG Centre, Thiruvananthapuram, Kerala, India

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***Corresponding author**
E-mail: dr.aiswaryarose10@gmail.com

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ABSTRACT

Acharya Susrutha under the context of Rakthasthambhanopaya (methods of haemostasis) has explained about the different methods to attain Rakthasthambhana or haemostasis. which include Skandana (clotting of blood), Santhana (uniting), Pachana (ripening or digestive measures) and Dahana (thermal cauterisation). Acharya Susrutha while explaining about agantuja vrana chikitsa (treatment of wound) has explained that 'Pittavat sheeta kriya' should be done prior to santhana to mitigate the 'Kshatoshmana'. So, from this it is evident the primary method of management should be done with sheeta (cold) followed by Kashaya rasa dravya (astringent taste drugs) for Santhana. Panchavalkala is a group drug with sheeta veerya (cold potency) and Kashaya rasa (astringent taste) mentioned in Dalhana commentary for Skandana. This study investigates the haemostatic effect of Panchavalkala Kashaya and Panchavalkala aqueous extract by *in vitro* method. Preliminary phytochemical screening of both trial drugs was done. A total of 10 samples of healthy human platelet poor plasma were mixed in trial drugs, Prothrombin and Activated Partial Thromboplastin time were determined. Data was analysed using Kruskal Wallis test. Phytochemical analysis confirmed that the Panchavalkala kashaya contain alkaloids, tannins, glycosides and saponins. Panchavalkala aqueous extract sample consist of flavonoids, alkaloids, glycosides, tannins and saponins. LCMS Q-TOF analysis shows the presence of compounds Gallic acid, Epicatechin, Leucocynidin and Procyanidin B1. And the HPLC study confirmed that Gallic acid is an active phytoconstituent present in the Panchavalkala aqueous extract. In the present study the trial group Panchavalkala aqueous extract showed more desirable haemostatic effect than Panchavalkala Kashaya on comparison.

Keywords: Haemostatic, Panchavalkala Kashaya, Panchavalkala aqueous extract, Rakthasthambhana.

INTRODUCTION

Haemostasis is a process to prevent and stop bleeding, to keep blood within a damaged blood vessels and it is the first stage of wound healing[1]. Skandana and Santhana are the procedures through which haemostasis is achieved by inducing quick coagulation of blood using Sheeta veerya (cold potency) and Kashaya rasa dravya (Astringent taste) externally according to Acharya Dalhana. Panchavalakala is one among the group of drugs mentioned by Acharya Sushruta for skandana and santhana karma having kashaya rasa and sheeta veerya[2]. It is a formulation made up of the bark of five trees viz. Nyagrodha (*Ficus benghalensis* Linn), Udumbara (*Ficus glomerata* Roxb.), Asvattha (*Ficus religiosa* Linn.), Parisa (*Thespesia populnea* L.) and Plaksha (*Ficus lacor*). All five drugs have dominance of kashaya rasa and sheeta veerya which is useful in the management of rakta atisrava (haemorrhage). Panchavalkala aqueous extract obtained through standardized method has shown the presence of phytochemicals with haemostatic effect[3]. This signifies the importance of Panchavalkala kashaya as a prospective drug candidate in the management of superficial bleeding wounds and haemorrhage. Hence, the present *in vitro* study was carried out to evaluate the haemostatic effect of Panchavalkala kashaya and Panchavalkala aqueous extract as a stepping stone for future applications in surgical practice.

MATERIALS AND METHODS

Materials: 10 ml blood samples from 10 healthy volunteers, Coagulation Analyzer- KC1 Delta-Tcoag, Trisodium citrate, Prothrombin Reagent-Thromboplastin, Activated Partial Thromboplastin Test reagent - C.K. PREST[4], calcium chloride solution, Micropipettes, Panchavalkala kashaya, Panchavalkala aqueous extract.

Setting: Department of Research and Development Pankajakasthuri Ayurveda Medical College and Post Graduate Centre, Biochemistry lab under Pankajakasthuri Herbal Research Foundation, Kattakada, Thiruvananthapuram, Kerala, India, final assays was carried out at NABL accredited lab at Neyyar Medicity.

Ethical Approval: The experimental protocol of the present study was reviewed thoroughly and approved by Institutional Ethical Committee of Pankajakasthuri Ayurveda Medical College and Post Graduate Centre, Thiruvananthapuram, Kerala, India, with reference number PKAMC/IEC/64/2020.

Figure 1: Nyagrodha

Figure 2: Udumbara

Figure 3: Aswatha

Figure 4: Plaksha

Figure 5: Parisha

Figure 6: Panchavalkala choorna

Figure 7: Panchavalkala choorna in 100 ml of water

Figure 8: Panchavalkala extract in centrifuge tube

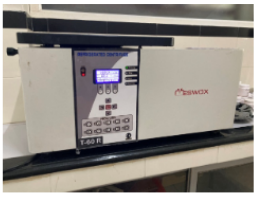


Figure 9: Refrigerated centrifuge

Figure 10: Supernatant obtained after Centrifugation

Figure 11: Supernatant obtained filtered using filter paper

Figure 12: Syringe filtering

Figure 13: Lyophilizer VirTis Genesis 25 L Pilot (Model SQ EL-85)

Figure 14: Coagulation Analyzer- KC1 Delta, Tcoag

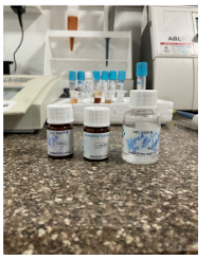


Figure 15: Reagents

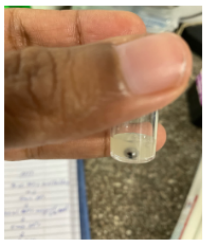


Figure 16: Immobile bead after coagulation

Method of Trial Drug Preparation: The fresh barks were collected with help of local herbalist. Drugs used in the preparation of trial drugs are; Nyagrodha (*Ficus benghalensis*), Moraceae family; Udumbara (*Ficus glomerata*), Moraceae family; Aswatha (*Ficus religiosa*), Moraceae family; Plaksha (*Ficus lacor*), Moraceae family; Parisha (*Thespesia populnea*), Malvaceae family. The barks of all the five plants were chopped into small pieces, shade dried at ambient temperature, and stored in airtight container. Panchavalkala kashaya was prepared by boiling coarse powder of drugs in 16 times water and reduced to 1/4th and strained using cloth[5]. The powders from each bark was taken in equal proportion for extract preparation. 5 g of drug was macerated with 100 ml of water in a closed flask for 24 hours, shaking frequently for 6 hours and was allowed to stand for 18 hours. The extract obtained was taken in a centrifuge tube and centrifuged at 13,000 rpm for 15 minutes in a refrigerated centrifuge machine, and the supernatant was filtered through syringe filter (pore size, 0.45 µm) after filtering with filter paper[6]. To prepare pre-determined concentration the Panchavalkala aqueous extract was lyophilised. The volume and concentration of control as well as trial drugs were determined by 9 pilot trials (with 3 samples each) before the commencement of study. Pilot trials were done with volumes 1ml, 400 µL, 200 µL, 50 µL, 40 µL,20 µL, 10 µL and with concentrations 5 mg/ml, 1.5 mg/ml and 750 µg/ml. 1mg/ml concentration and volume 10 µL, was fixed for the present *in vitro* study.

Method of *In vitro* Study: 10 ml of whole blood from 10 healthy volunteers of either sex above 18 years and not under any medication, was drawn into evacuated container tubes containing 0.129 M trisodium citrate (1vol/9vol blood). Further each sample was divided into 3 Groups for the experiment, mainly viz. Group D (control) and Group E (treated with Panchavalkala kashaya) and Group F (treated with Panchavalkala aqueous extract). The platelet poor plasma was separated by centrifugation at 3000 rpm for 20 minutes at 20 ^{0}C. The specimen of Group D (control) was incubated under the same condition with Distilled water, trial Group sample E was treated with Panchavalkala kashaya and Group F with Panchavalkala aqueous extract. Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and INR was measured using Beads method in Groups D, E and F[7].

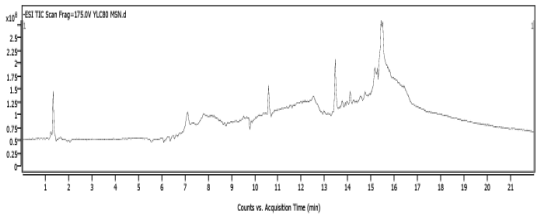
RESULT

Panchavalkala aqueous extract and Panchavalkala kashaya analysis: Phytochemical analysis of Panchavalkala aqueous extract revealed that the extract is a rich source of bioactive components like flavonoids, alkaloids, tannins, glycosides and saponins. Panchavalkala kashaya revealed that the extract is a rich source of bioactive components like flavonoids, alkaloids, tannins, glycosides and saponins (Table 1).

Table 1: Phytochemical screening of Panchavalkala aqueous extract

Test	Aqueous extract	Kashaya
Flavonoids	+	-
Steroids	-	-
Alkaloids	+	+
Tannins	+	+
Glycosides	+	+
Coumarins	-	-
Saponin	+	+

LCM- Q-TOF of Panchavalkala Kashaya

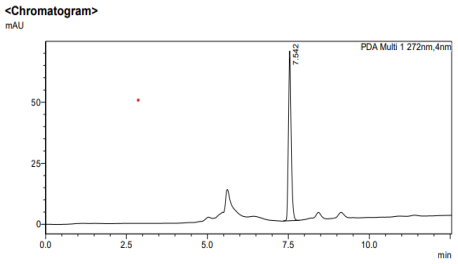


Graph 1: LC-QTOF-MS/MS chromatogram of the Panchavalkala kashaya.

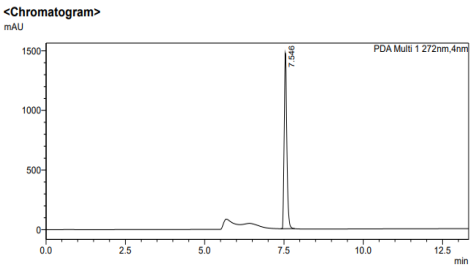
Panchavalkala kashaya showed the presence of Gallic acid.

HPLC of Gallic Acid

The HPLC analysis resulted in a peak of Gallic acid standard at the retention time of 7.546 min shown in (Graph 2) and authentic sharp peaks of Panchavalkala aqueous extract at the retention time of 7.542 min respectively shown in (Graph 3) The HPLC studies confirmed that Gallic acid is an active phytoconstituent present in the Panchavalkala aqueous extract. The HPLC result confirms the presence of gallic acid via LCMS. The above mentioned HPLC method is a simple, rapid and precise method for the estimation of gallic acid in Panchavalkala aqueous extract. The HPLC estimation resulted the Panchavalkala aqueous extract contain 66.1 ppm of Gallic acid. Literature shows that the presence of gallic acid in Panchavalkala aqueous extract.



Graph 2: HPLC chromatogram of standard Gallic Acid



Graph 3: HPLC chromatogram of Panchavalkala aqueous extract

***In Vitro* Study**: The mean APTT of Group D is 35.12±3.30 (sec), Group E is 48.34 ± 8.72 (sec) and Group F is 32.88±3.24 (sec). The P-value showed that there was significant difference between Groups D and E & Group E and F (Table.1). The and Group F (Panchavalkala aqueous extract), Group F was effective in reducing Activated Partial Thromboplastin Time.

Table 2: Kruskal Wallis Test – Effect of intervention on APTT

Pairwise Comparisons Kruskal Wallis test	Test Statistic	Std. Error	Std. Test Statistic	P-Value	Adj. Sig.[a]
Group D -Group F	5.800	3.935	1.474	0.141	0.422
Group D- Group E	-11.200	3.935	-2.846	0.004	0.013
Group F- Group E	17.000	3.935	4.320	0.000	0.000

The mean PT of Group D is 13.91± 0.79 (sec), Group E is 14.42±1.59 (sec) and Group F is 12.78±0.60 (sec). The P-value showed that there is significant difference between groups D and F & Group E and F (table. 2). The Group F reported the lowest mean PT which denotes that while pairwise comparing the trial Group F (Panchavalkala aqueous extract) was effective reducing Prothrombin Time compared to trial Group E (Panchavalkala Kashaya).

Table 3: Kruskal Wallis Test – Effect of intervention on Prothrombin Time

Pairwise Comparisons Kruskal Wallis Test	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Group D- Group E	-0.800	3.930	-0.204	0.839	1.000
Group D- Group F	10.700	3.930	2.722	0.006	0.019
Group E- Group F	11.500	3.930	2.926	0.003	0.010

The mean INR of Group D is 2.31± 3.9 (sec), Group E is 1.09±0.12 (sec) and Group F is 0.94±0.05 (sec). The P value showed that there is significant difference between Group D and Group E and also in Group E and Group F (table.3). Group F reported the lowest mean INR which denotes that while pairwise comparing Group F was effective reducing INR compared to Group E.

Table 4: Kruskal Wallis Test – Effect of intervention on INR

Pairwise Comparisons Kruskal Wallis Test	Test Statistic	Std. Error	Std. Test Statistic	P-value	Adj. Sig.[a]
Group D- Group E	-11.200	3.935	-2.846	0.004	0.013
Group D- Group F	5.800	3.935	1.474	0.141	0.422
Group F - Group E	17.000	3.935	4.320	0.000	0.000

As noted in the results of the *in vitro* study, the trial group with Panchavalkala kashaya prolonged APTT and PT values but in contrast, Panchavalkala aqueous extract demonstrated a pronounced decreasing effect on APTT and PT compared to the control group.

DISCUSSION

The findings of *in vitro* study reveal the haemostatic effect of Panchavalkala aqueous extract. Panchavalkala aqueous extract is found to enhance the intrinsic and extrinsic pathway of coagulation cascade thus acting as Haemostatic agent. Panchavalkala Kashaya prolongs APTT beyond clinical limits whereas PT is within normal limits. Since Panchavalkala Kashaya prolongs APTT, it can be inferred that the trial drug is interfering antagonistically on the initiation of intrinsic pathway of coagulation cascade. The observed effects of the plant extract investigated in this study can be attributed to the presence of specific phytochemicals identified in the qualitative screening. The literature highlights that certain phytochemical, such as tannins, phenols, flavonoids, and serine proteases, have been linked to a reduction in prothrombin time (PT) and activated partial thromboplastin time (APTT) in treated plasma. Moreover, saponins have been associated with the shortening of plasma coagulation time, particularly affecting APTT. For instance, saponins isolated from the *Paris forgesii* plant were found to be linked with a reduction in prothrombin time in normal plasma[8]. The presence of saponins and phenols in plant extracts has been associated with their ability to reduce PT in normal plasma. Saponins, on the other hand, have been associated with reduced blood clotting time when applied topically. Studies involving *Chromolaena ordata* indicated that flavonoids and tannins possessed blood clotting properties, leading to accelerated plasma coagulation[9]. Gallic acid and vanillin acid, both phenols isolated from *Sedum aizoon* (L.) leaves extract, were associated with a reduction in plasma Prothrombin Time and APTT[10]. Phenols and flavonoids have also been linked to reduced coagulation time affecting the extrinsic pathway. Serine glycoprotease isolated from *Cucumis savitus L*. fruit extract also significantly reduced APTT in treated plasma[11]. Tannins have been shown to reduce APTT as demonstrated by activities of *Mirabilis jalapa* and *Paris polyphylla V.* extracts in normal rabbit plasma. Strong complexes formed when tannins are mixed with proteins are believed to contribute to the phytochemicals ability to shorten coagulation time[12]. In addition, chalcone (X), a flavonoid from *Oxytropis falcate* extract, reduced plasma re-calcification time significantly[13]. The various phytochemicals found in Panchavalkala extracts, such as saponins, flavonoids, tannins, and phenols and a Gallic acid have proven effect on coagulation assays, facilitating Haemostasis and reducing Prothrombin Time and Activated Partial Thromboplastin time.

The possible Haemostatic mechanism of trial drugs from APTT and PT assays is that APTT is related to the intrinsic coagulation pathway and PT mainly reflects the extrinsic coagulation pathway. In present study Panchavalkala aqueous extract inhibited the time taken by PT and APTT, indicating that its Haemostatic activity was related to the extrinsic and intrinsic coagulation pathway. So, Panchavalkala aqueous extract demonstrated enhanced activity in lowering the PT and APTT of Platelet poor plasma from Healthy human blood.

CONCLUSION

Panchavalkala aqueous extract showed significantly high haemostatic effect in the *in vitro* study when compared to that of Panchavalkala kashaya, establishing its effect on both extrinsic and intrinsic pathways of human blood coagulation. Thus, the study proved that Panchavalkala aqueous extract has significant effect in human blood coagulation *in vitro*. Panchavalkala kashaya was observed to have significant effect in decreasing the APTT only, thus showing its effect in intrinsic pathway of human blood coagulation.

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