

# Evaluation of *Artemisia scoparia* for hemostasis promotion activity

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**Abstract:** Excessive hemorrhage through any reasons is a life threatening process. *Artemisia scoparia* of family Asteraceae has been used in local system of medicine to stop bleeding from wounds and in injuries, antiseptic, in healing urticarial and for removal of worms from the body. Aerial parts of *A. scoparia* was extracted with 95% methanol (ASM) and fractionated through liquid-liquid partition in ascending order of n-hexane (ASH), chloroform (ASC), ethyl acetate (ASE), and the remaining as the aqueous fraction (ASA). Phytochemical classes of the extract/fractions were determined by qualitative assays. Prothrombin time (PT) was estimated on the plasma of human blood by Owren method. Capillary tube method was applied to determine the hemostasis activity in Sprague-Dawley rat. Tannins, saponins, terpenoids, quinones, betacyanins and flavonoids were present whereas phlobatannins, anthraquinones and alkaloids were established absent in ASM, ASC, ASE and ASA. Prothrombin time was significantly decreased by mixing (10 µg) of ASM (16.67±1.15 sec), ASH (12.33±0.57 sec), ASC (15.33±0.57 sec) and ASA (9.0±1.0 sec) to that of vehicle (20.0±1.0 sec). Administration (200 mg/kg) of all the extract/fractions showed significantly less (26.00±11.79 sec - 41.00±7.21 sec) hemostasis time as compared to the (242.67±39.67 sec) control rats. The results suggested the therapeutics importance of *A. scoparia* use in bleeding pathologies.

**Keywords:** *Artemisia scoparia*, phytochemical, blood clotting, prothrombin time, hemostasis.

## INTRODUCTION

Blood loss from cuts, bruises and in clinical settings threaten the life safety of patients. The blood clotting is a very complex phenomenon and involves many factors resides in the plasma and tissues. Blood coagulation is the process of forming a clot to stop bleeding. The body relies on the interaction of three processes to stop bleeding. Primary hemostasis involves the vasoconstriction and platelet plug, while in secondary hemostasis clotting factors are engaged. These factors elicit each other in process called clotting cascade. Two separate pathways, the intrinsic and the extrinsic, take place in the clotting cascades that interacts each other. Activation of the extrinsic pathway occurs by external trauma that causes blood to get away from the vascular system. This pathway occurs more rapidly than the intrinsic pathway. Factor VII is involved in it and the intrinsic pathway involves factors XII, XI, IX and VIII. The intrinsic pathway's activation occurs by trauma inside the vascular system, and also activated by chemicals, platelets, exposed endothelium or collagen. This pathway is more vital but slower than the extrinsic pathway. Both pathways come to an end at the pathway of clot production in what is known as the common pathway. The common pathway entails factors I, II, V, and X (Chen *et al.*, 2012). On account of hemostasis importance it is necessary to evaluate new, easy and affordable agents for the therapeutic uses. In this context

medicinal plants provide alternative and complementary agents to achieve the required goal.

*Artemisia* common name wormwood is the part of most extensive dispersed genus of Asteraceae family. This genus is comprised of 300-400 kinds of herbs and shrubs which are used by medicinal industries on account of very valuable secondary metabolites and essential oils. *Artemisia scoparia* is dispersed in different regions of the world. It is found in China, India, Europe, Afghanistan, Iran, Russia, Thailand, Japan, Korea and Pakistan (Hayat *et al.*, 2009). At present, 38 *Artemisia* species have been recognized and confirmed in the arid as well as semiarid areas of Northern Punjab, Kashmir, KPK and Baluchistan province of Pakistan (Hayat *et al.*, 2009). It grows up in the summer season, in sandy soil of barren areas at an altitude of 450 to 4000m. It is a perennial and slightly aromatic herb. Locally it is called Jhahoo, Lasaj, Dona, Marua and Churi Saroj (Khan *et al.*, 2014).

In subcontinent (India-Pakistan) *A. scoparia* is traditionally used for the treatment of various disorders. It's extract was applied on wound to protect them from infectious agents (Schlimmer, 1970). It is considered to be very effective in burn conditions (Ahmad and Javed, 2007). This plant is affluent in volatile oils that display a broad spectrum of biological activity and has extensive application in medicine. *Artemisia scoparia* has been known for its antipyretic, anticholesterolemic, antiseptic, antibacterial, cholagogue, diuretic and vasodilator properties. It has also been used for the treatment of gall

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bladder inflammation, hepatitis and jaundice (Singh *et al.*, 2009). The plant is used to promote wound healing and removing parasitic worms from the body (Zargari, 1996). The aerial parts of the plant are used in conventional medicine as anti-phlogistic, antiobesity (Choi *et al.*, 2013), as anti-mold agent and for healing urticaria (Yahagi *et al.*, 2014). Singh *et al.* (2010) isolated chemical constituents of essential oils of *A. scoparia* (camphor, beta-caryophyllene, cineole). Isolation of many compounds have been done from this plant i.e.,  $\beta$ -sitosterol, isosambandin, 6-9-diethylesculetin, 8-methylesculetin, scopoletin, 9-ethoxycoumarin, chlorogenic acid ethyl ester, cappillarisin and magnolioside (Bachrouch *et al.*, 2015). The clot promoting activity has not been reported for this species. Therefore, we evaluated the methanol extract and its derived fractions for the presence of various phytochemical classes and blood clotting efficiency.

## MATERIALS AND METHODS

### Preparation of extract

The plant of *Artemisia scoparia* Waldst. & Kit. was collected from the campus area of Quaid-i-Azam University Islamabad in April 2013. It was recognized by Prof Dr. Rizwana Aleem Qureshi, Taxonomist Department of Plant Sciences, Quaid-i-Azam University Islamabad. A voucher specimen (# 6335) was deposited at the herbarium Pakistan Museum of Natural History Islamabad Pakistan. The aerial parts of the plant was dried in shade at room temperature (25°C) for 14 days and grinded in a common electrical grinder. Extraction was carried out with 95% methanol. After filtration the filtrate was dried by rotary evaporator under the condition of reduced pressure and 40°C temperatures to get the methanol extract of *A. scoparia* (ASM). Fractions of ASM were obtained by suspending 10g in 250ml of distilled water in order of; *n*-hexane (ASH), chloroform (ASC), ethyl acetate (ASE) and the residual aqueous fraction (ASA). Each fraction was dried and stored at 4°C for use in phytochemical analysis and pharmacological evaluation.

### Phytochemical analysis

Different tests were accomplished to identify the phytochemical classes present in aerial parts of *A. scoparia*. Qualitative screening of terpenoids, alkaloids, saponins (Edeoga *et al.*, 2005) flavonoids, tannins (Sofowora, 1982), phlobtannins, triterpenoids, phenols, coumarins, quinones, anthraquinones, anthocyanins and betacyanins (Trease and Evans, 1989) was performed on the extract and various fractions.

### Blood coagulation activity

Coagulation activity of *A. scoparia* for various extract/fractions was estimated in terms of prothrombin time (PT). Blood sample of human was obtained by non-contact, clean venipuncture with 10ml disposable syringe

and collected in vials having EDTA. Blood collected was centrifuged at 13,000 rpm at 4°C for 10 min (Guder *et al.*, 1997). The clear platelet free plasma obtained was kept in a water bath at 37°C for 2 min. The prothrombin time was estimated by the Owren method (Tietz, 1995) with some modification. For this purpose we have used the extract fraction samples instead of thromboplastin reagent. An aliquot of 100 $\mu$ l (100 $\mu$ g/ml) suspended in 3% v/v Tween 85 from each sample was mixed with 1 ml of plasma and coagulation time was recorded in triplicate. Vehicle was used as control.

### Assessment of blood clot promoting activity

Sprague-Dawley male rats (36) weighing about 150-200 g were divided into six groups with 6 rats in each group. At the primate facility of Quaid-i-Azam University Islamabad, the rats were maintained in ordinary cages. Ethical Committee of Quaid-i-Azam University Islamabad approved the study protocol (Bch#264) for the animal care and experimentation.

Screening of crude extract/fractions of *A. scoparia* for blood clotting activity was carried out by capillary tube method as reported by Stiene *et al.* (2006). Group I served as control and received DMSO alone. Group II-VI received 200mg/kg bw of extract/fractions; ASM, ASH, ASC, ASE and ASA respectively. After one hour, spirit was used to clean the tail of each rat and pricked with sterile needle. Stop watch was started, as soon as first drop of blood was appeared. Then capillary tube was placed over the blood drop and filled with blood. After some time a small piece of capillary was busted and the same procedure was repeated, till fibrin thread was appeared at the broken end of the capillary tube. Time period between tail pricking and the first appearance of fibrin thread at the broken ends of capillary tube was calculated. This time was called the clotting time of blood.

## STATISTICAL ANALYSIS

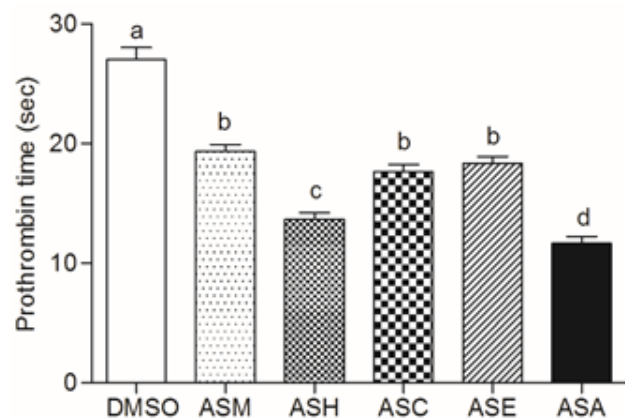
The experimental results were defined as mean  $\pm$  standard deviation i.e. mean  $\pm$  SD. For *in vivo* studies, the consequences of different treatment given to animals were evaluated by one way analysis of variance by computer software Statistix 8.1. Level of significance among various treatments was determined and a P-value  $\leq$ 0.01 and  $\leq$ 0.05 was believed to be statistically significant in every case.

## RESULTS

### Phytochemical analysis of *A. scoparia*

The analysis of phytochemical constituents of plant helps to predict the functioning potential of respective species. In phytochemical analysis the methanol extract and its derived four fractions of aerial parts of *A. scoparia* were examined. The results obtained in this study confirmed

the presence of flavonoids, tannins, terpenoids, coumarins, saponins, betacyanin in the extract and its derived four fractions. However, the presence of alkaloids, anthraquinones and phlobatannins was not exhibited in the extract/fractions.



a-d (means with different letters) indicate significance at  $P < 0.05$ .

**Fig. 1:** Coagulation time of human normal blood plasma (prothrombin time sec). Mean  $\pm$  03. DMSO; dimethylsulfoxide. ASM; *Artemisia scoparia* crude methanol extract. ASH; *Artemisia scoparia* n-hexane fraction. ASC; *Artemisia scoparia* chloroform fraction. ASEE; *Artemisia scoparia* ethyl acetate fraction. ASA; *Artemisia scoparia* soluble aqueous fraction.

#### Blood coagulation activity

The coagulation time after mixing the extract/fractions with plasma is depicted in fig. 1. The coagulation time obtained by mixing all the extract/fractions was significantly ( $P < 0.05$ ) decreased with respect to the control coagulation time ( $27.0 \pm 1.00$  sec). Minimum time to coagulation of plasma was taken by ASA ( $9.0 \pm 0.57$  sec) followed by ASH ( $12.33 \pm 0.57$  sec), ASC ( $15.33 \pm 0.57$  sec), ASM ( $16.67 \pm 1.15$  sec) and ASE ( $18.33 \pm 0.57$  sec). The coagulation time of ASM, ASC and ASE was non significantly ( $P > 0.05$ ) different among each other whereas ASH and ASA took significantly ( $P < 0.05$ ) less time to plasma coagulation to that of ASM, ASC and ASE.

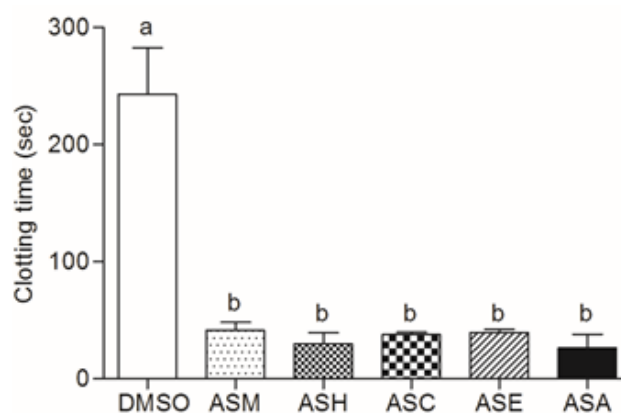
#### Clot promoting activity

The effect of different fractions of ASM on the blood coagulation time was investigated in rats by administering 200 mg/kg bw of the extract/fractions. Blood coagulation time was determined by capillary tube method. It was determined that blood coagulation time was significantly decreased in all the extract/fractions. The blood coagulation time was decreased from  $242.67 \pm 39.67$  sec in DMSO (control) rats to minimum of  $26.00 \pm 11.79$  sec for ASA. The blood coagulation time of other extractions was;  $29.66 \pm 9.60$  sec (ASH),  $37.66 \pm 2.08$  sec (ASC),  $39.33 \pm 3.05$  sec (ASE) and  $41.00 \pm 7.21$  sec (ASM).

## DISCUSSION

Therapeutic potential of the extract/fraction emanates the presence of various metabolites. The results obtained in

this experiment suggested a crucial role of all the extract/fractions in hemostasis by reducing the time of plasma coagulation (PT) as well as blood clotting. In this study presence of flavonoids, tannins, terpenoids, coumarins and saponins while absence of alkaloids, anthraquinones and phlobatannins in all the extract/fractions have been determined. The results obtained in this study were similar to earlier reports where the presence of flavonoids, tannins, terpenoids, coumarins, saponins has been established in *Artemisia parviflora* (Ahuja et al., 2011). However, contradictory results for the absence of alkaloids were recorded in our study to that of Ahuja et al. (2011). Similarly presence of flavonoids, tannins, terpenoids and saponins was confirmed in the ethanol extract of *Artemisia nilagirica* (Devmurari et al., 2010). Presence of terpenoids, flavonoids and tannins was also confirmed in *Artemisia absinthium* (Amat et al., 2010). Similar reports to our results Ahameethunisa and Hopper (2010) studied the existence of flavonoids, quinones and terpenoids in *A. nilagirica*. However, contrary to our results they reported the presence of alkaloids. The results of Ashok and Upadhyaya (2012) are similar to our studies where presence of alkaloids was not established in *A. absinthium* and *A. annua*.



a-b (means with different letters) indicate significance at  $P < 0.01$ .

**Fig. 2:** Blood clotting time with capillary tube method in Sprague-Dawley rats. Mean  $\pm$  06 rats. DMSO; dimethylsulfoxide. ASM; *Artemisia scoparia* crude methanol extract. ASH; *Artemisia scoparia* n-hexane fraction. ASC; *Artemisia scoparia* chloroform fraction. ASEE; *Artemisia scoparia* ethyl acetate fraction. ASA; *Artemisia scoparia* soluble aqueous fraction.

The presence of blood coagulation activity in all the fractions obtained from the methanol extract of *A. scoparia* indicated that solvent partitioning has not succeeded in separating the blood coagulation factor into a single solvent fraction. The occurrence of various metabolites may be implicated for the various therapeutic effects of *A. scoparia* aerial parts. In this context the tannins and saponins especially tannins might play a prominent role in hemostasis by arresting bleeding from

**Table 1:** Phytochemical analysis of *A. scoparia*

Phytochemical	ASM	ASH	ASC	ASE	ASA
Terpenoids	+	++	++	++	++
Coumarins	+	-	+	++	++
Flavonoids	++	-	+	++	+
Tannins	+	+	+	++	++
Phlobtannins	-	-	-	-	-
Anthraquinone	-	-	-	-	-
Alkaloids	-	-	-	-	-
Saponins	+	+	++	+	++
Betacyanin	++	+	+	++	++
Quinones	+	-	+	++	++

++, abundant; +, present; -, absent. ASM; *Artemisia scoparia* crude methanol extract. ASH; *Artemisia scoparia* n-hexane fraction. ASC; *Artemisia scoparia* chloroform fraction. ASE; *Artemisia scoparia* ethyl acetate fraction. ASA; *Artemisia scoparia* soluble aqueous fraction.

injured vessels through coagulation of proteins to form vascular plug. Similar hemostatic results have been reported during *in vivo* conditions in rat for the *Aspilia africana* extract in which it was suggested that tannins might play crucial role in hemostasis (Okoli *et al.*, 2007). They also determined less coagulation time for the whole blood of rat with the same extracts. During hemostasis spontaneous arresting of blood occur which involves vascular spasm of the ruptured vessels, platelet aggregation and blood coagulation. These results imply that various extract and fractions might enhance the vasoconstriction and reduce the bleeding from injuries and or wounds.

The phenomenon of blood clotting is not restricted to the platelet aggregation and vasoconstriction mechanism and involve cascade of reactions. This process starts with activation of prothrombin with its subsequent conversion to thrombin which in turn converts fibrinogen to insoluble fibrin. The extract and fractions of *A. scoparia* when mixed with the plasma of human blood reduces the time of coagulation and eliciting the possibility that extract/fraction interferes with the blood coagulation process. Similarly decrease in prothrombin time was recorded by Dasgupta *et al.* (2014) by various extracts of *Tagetes erecta* leaves. They reported minimum time of 13.14±0.06 sec with ethanol/water extract. These results suggested that extract and fractions of *A. scoparia* may compel the hemostasis effect by coagulation pathway with the consequent reduction in clotting time and vasoconstriction which are necessary to deplete the loss of blood from injuries.

The results of present study indicated that the plant may have clinical implications as a coagulant for the treatment of various pathological states, including neoplastic disorders, infectious diseases, premature aging, atherosclerosis and diabetes and also during injuries. The lower time lapse for blood coagulation by *A. scoparia* in rat may involve the effect on plasma fibrinogen activity

and/or fibrinogen antigen. The *A. scoparia* administration to rats might increase the efficiency of erythrocytes leading to enhanced protein network formation during bleeding.

## CONCLUSION

The results of this study suggested the therapeutic use of *A. scoparia* in the management of hemorrhage. It is a novel effective hemostatic agent and can be used as complementary and alternative medicine to reduce the morbidity and mortality in clinical settings.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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