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Evaluation of Thrombolytic Potential of *Elaeagnus rhamnoides (L.) A. Nelson*

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ABSTRACT

Plants find a special significance in the field of medicine due to their therapeutic value. Thrombolytic agents play a crucial role in the treatment of numerous human diseases including atherothrombotic diseases, pulmonary embolism, and myocardial infarction. The current study was performed to evaluate the thrombolytic potential of Elaeagnus rhamnoides (L.) A. Nelson (Sea buckthorn or SBT). The extract of leaves, stems, and berries of the investigated plant displayed 28%, 26%, and 44% blood clot lysis, respectively as compared to that of a standard thrombolytic agent namely streptokinase (59% lysis). The fruit extract of sea buckthorn was found to display higher thrombolytic potential as compared to that of its leaves and stem. It was concluded that the extract of leaves, stem, and berries of SBT may find applications in the future as a thrombolytic agent. The presence of important functional groups for instance, alcohol, aldehyde, alkyne, alkene, amines, and ester in different ariel parts of SBT were verified by FTIR spectroscopy.

Keywords: *E. rhamnoides (L.) A. Nelson* (sea buckthorn or SBT), FTIR analysis, thrombolytic agents,

INTRODUCTION

Plants-based research is a field of interest throughout the world due to superb medicinal [1, 2] and nutritional [3, 4] properties associated with phytoconstituents. *E. rhamnoides (L.) A. Nelson* (old name = *Hippophae*

rhamnoides) is commonly known as sea buckthorn (SBT) [5, 6] and is grown in many regions of the world including Pakistan [7], India, China, Russia, Germany, and Finland [8, 9]. It is a thorny nitrogen-fixing deciduous shrub which possesses great nutritional and therapeutic potential and health benefits [8, 10]. The plant can withstand high daytime temperatures even in summer [11] and can also survive even at a temperature as low as – 40°C [12]. It may rise in height from 0.5-6 m and even up to 18 m in central Asia [13]. Its berries are commonly called Siberian pineapple or sea berry [6] and vary in shape (globose or egg-shaped) as well as in color (orange to yellow) [14]. However, the mature berries are red/orange in color [13] (**Figure 1**). Its branches are thick and exceptionally prickly [12] which bear linear-shaped leaves having 7 mm width and 3- 8 cm length [15] (**Figure 1**).

Each berry contains a solitary seed [16] whose size is 2.8-4.2 mm [17]. A fantastic contrast between the color of the leaves and berries together, portrays the decorative worth of this plant [16]. In numerous Asian regions like Pakistan, China, and India, SBT is commonly known as a miracle plant. Its leaves have remained as a favorite food of the flying horses (Pegasus) as shown in Greek mythology. SBT has also been used in conventional and traditional medicinal systems for hundreds of years in Europe, Asia [18], India, and Tibetan [19]. Its berries, leaves, shrubs, and seeds all, have been utilized in medicines [20] , to treat digestive disorders, skin diseases [21-23], cancer [6], cough, digestive problems, and to improve blood circulation and relieve pain [24]. SBT oil is rich in lipophilic ingredients , unsaturated fatty acids, vitamins (A and E), and phytosterols. All these constituents have multifunctional benefits for human health especially the fatty acids which demonstrate an important role in the improvement of cardiovascular and cerebrovascular disorders. The oil also shows anti-depressive, anti-inflammatory, and anti-oxidant potential [25].

The oils and fatty acids contain majorly contain palmitic acid and palmitoleic acid [25, 26]. Due to the presence of its unique unsaturated fatty acids , palmitoleic acid and gamma-linolenic acid, SBT oil possesses skin repair, and regeneration potential [10]. Its oil is rich in valuable nutrients and shows an important role in the proper functioning of the human body [10]. It removes excess toxins from the body, facilitates oxygenation of the skin, improves blood circulation, and easily penetrates through the epidermis. Due to the conversion of gamma-linolenic acid to prostaglandins

inside the skin, SBT oil prevents the body from allergies/infections and also inhibits the aging process, and eliminates inflammation [27]. The oil is also helpful in the treatment/prevention of cancer. It relieves the hematological damage, improves the immune system, restores the liver and kidney functions, and also assists in returning the health of those people who have received chemotherapy [6]. Some studies also indicated the safety of its use as a food supplement [28]. The plant material is commonly screened for the compounds of interest. The presence of polyphenols in the leaves and tocotrienols, tocopherols, carotenoids, and fundamental poly unsaturated fatty acids in the berries have been reported by numerous investigators [29].

There had been a large number of investigations on the nutritional [30, 31] and therapeutic [32, 33] potential of medicinal plants. Numerous studies have reported the pharmaceutical potential of *E. rhamnoides (L.) A. Nelson* in Pakistan [34-37] and throughout the world [6,25, 38-42] but none of the current research studies have reported on its thrombolytic potential. Current studies have investigated the thrombolytic activity of leaves, stems, and berries of *E. rhamnoides (L.) A. Nelson*.

Figure 1. A stem of *Elaeagnus rhamnoides* bearing branches, leaves, and fruits

https://growing-wild.ca/2020/02/25/growing-propagating-and-eating-sea-buckthorn/

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Organic compounds (acetone, methanol, ethanol, and chloroform) of Unichem origin (India) were used for preparing solvent extracts of various parts of the plant. Standard streptokinase was obtained from PCSIR, Lahore (Pakistan). All glassware was washed with tap water then with detergent solution and dipped in 5% HNO_3 overnight and finally washed with excess quantity of deionized water. Whatman filter paper was used for the filtration of plant extracts. Water bath was used for evaporation of excess solvent in plant extracts. Micro centrifugal machine was used for evaluation of thrombolytic activity and sample was collected in micro centrifugal tubes.

2.2. Plant Material

The leaves, berries, and stems of *E. rhomboids (L.)* were collected from Skardu, Pakistan in September 2018. The plant is generally known as Sirmaa in Punjabi and its local common name is choq in Skardu region. The obtained parts of the plants were washed with clean water to dispose of the dust particles, dried under sunlight, ground into powder, and stored in a closed compartment at 25°C for the further use.

2.3. Preparation, Extraction, and Fractionation of Plant Material

The extraction was performed utilizing the evaporation technique. The powder segment (20 g) of SBT was soaked separately in 100 ml of methanol, ethanol, acetone, and chloroform for 10 days at room temperature with continuous blending. The obtained suspension was filtered by Whatman's filter paper and then evaporated in a water bath to obtain the plant extract in the dried form. The extracts of various parts of plant were weighed precisely; the obtained weights are shown in **Table 1**.

Table 1: Weight of SBT Stems, Leaves, and Berries (Mean of Triplicate Data)

Extracts	Stem(g)	Leaves(g)	Berries(g)
Chloroform	1.06 ± 0.02	1.15 ± 0.01	1.15 ± 0.02
Ethanol	1.2 ± 0.01	1.1 ± 0.02	1.26 ± 0.01
Methanol	1.08 ± 0.03	1.1 ± 0.03	1.66 ± 0.03
Acetone	-	1.14 ± 0.02	1.09 ± 0.02

Streptokinase (15, 00,000 IU) was utilized as a standard control; 500ul sterile refined water was added to 1 g streptokinase and mixed appropriately. From this suspension, 100µl (30,000 IU) was utilized for thrombolysis [43].

2.4. Thrombolytic Activity

For thrombolytic activity, the reported procedure [44] was used with slight modifications. Venous blood was collected in Gujrat (Pakistan) from 10 healthy male volunteers (graduate students) of age between 22-25 years, who had no history of oral contraceptives or anticoagulant therapy. 100 ul of venous blood was added to each of the five pre-weighed clean Eppendorf tubes which were then incubated at 37 °C for 45 minutes. After clot formation, fluid was totally discharged from each microcentrifuge tube. The clot weight was calculated by subtracting the weight of tubes containing clots from the weight of the tube alone. Then 0.1g of an extract was added to each of the microcentrifuge tubes containing pre-weighed clots followed by incubation at 37 °C for 90 minutes and removal of blood serum; clot weight was again calculated to observe the weight difference after clot lysis. The same experiment was repeated 3 times for each sample. During each experiment, streptokinase (100µl) and distilled water (100µl) were used as positive and negative controls, respectively in place of the extract [44].

2.5. Statistical Analysis

Statistical analysis was performed by applying one-way ANOVA which showed significant results ($p < 0.05$) column-wise. The standard deviation was also calculated using MS Excel 2016.

2.6. FT-IR Spectroscopic Analysis

Fourier Transform Infrared Spectrophotometry (FTIR) is an important tool for identifying the types of chemical bonds/functional groups present in phytochemicals. Dried powder of leaves, stems, and berries of sea buckthorn was subjected to FTIR analysis in a range of 400–4000 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1. Thrombolytic Activities

The thrombolytic potential of leaves, stems, and berries of *E. rhamnoides (L.) A. Nelson* extracts were investigated. The obtained results (in terms of % clot lysis) are displayed in **Tables 2-5**. Streptokinase and distilled water

were used as standard controls. Streptokinase (positive control) has shown 59% thrombolytic action whereas the distilled water (negative control) displayed only 4% (negligible) thrombolytic action on human blood samples (**Table 6**).

Table 2a: Thrombolytic Activity of SBT Plant (Mean of triplicate data)

Extracts	Stem (g)	Leaves (g)	Berries (g)
Chloroform	19 ± 0.18	24 ± 0.21	47 ± 0.42
Ethanol	33 ± 0.31	26 ± 0.29	43 ± 0.39
Methanol	26 ± 0.23	38 ± 0.34	44 ± 0.52
Acetone	-	24 ± 0.22	46 ± 0.62
Oil	-	-	42 ± 0.39

Table 2b: Statistical Analysis by using One-way ANOVA

Source of Variation	SS	df	MS	F	p	F crit
Rows	961.07	4	240.27	2.63836	0.11	3.84
Columns	2266.13	2	1133.07	12.44217	0.004	4.459
Error	728.533	8	91.067			
Total	3955.73	14				

Table 2a gives a comparison between the thrombolytic activity of organic extracts of stems, leaves, and berries. Statistical analysis was performed by using one-way ANOVA which showed significant results (P<0.05) column-wise; the statistical results are represented in **Table 2b**. The stem extracts in chloroform, ethanol, and berries have displayed the thrombolytic activity of 19%, 33%, and 26%, respectively (**Table 3**). The oil of SBT berries showed 42% thrombolytic action (**Table 2**). The stem didn't produce any extract with acetone (**Table 3**); it means that no ingredient of the stem is soluble in acetone. On the other hand, chloroform, ethanol, methanol, and acetone extracts of leaves have shown 24%, 26%, 38%, and 24% activities, respectively (**Table 4**).

Table 3: Thrombolytic Activity of SBT Stem Extracts

Extracts	Wt of empty tubes (A) g	Wt of tubes with clots (B) g	Wt of clot (C=B-A) g	Wt of tube with clot after analysis (A) g	Wt of lysis (E=B-D)g	%
Chloroform	0.80	1.11	0.31	1.05	0.06	19
Ethanol	0.83	1.22	0.39	1.09	0.13	33
Methanol	0.80	1.18	0.38	1.08	0.1	26

Table 4: Thrombolytic Activity of SBT Leaves Extracts

Extracts	Wt of empty tubes (A)g	Wt of tubes with clots (B)g	Wt of clot (C=B-A) g	Wt of tube with clot after anlysis (C)g	Wt of lysis (E=B-D) g	%
Chloroform	0.82	1.07	0.25	1.01	0.06	24
Ethanol	0.80	1.10	0.30	1.02	0.08	26
Methanol	0.80	1.30	0.5	1.11	0.19	38
Acetone	0.78	1.19	0.41	1.09	0.10	24

Table 5: Thrombolytic Activity of SBT Berries Extract

Extracts	Wt of empty tubes (A)g	Wt of tubes with clots (B)g	Wt of clot (C=B-A) g	Wt of tube with clot after analysis (D)g	Wt of lysis (E=B-D)g	%
Chloroform	0.79	1.15	0.36	0.98	0.17	47
Ethanol	0.82	1.12	0.30	0.99	0.13	43
Methanol	0.81	1.17	0.36	1.01	0.16	44
Acetone	0.79	1.29	0.50	1.06	0.23	46
Oil	0.80	1.29	0.49	1.08	0.31	42

Table 6: Thrombolytic Activity of Standards

Standards	Wt of empty tubes (A)g	Wt of tubes with clots (B)g	Wt of clot (C=B-A)g	Wt of tube with clot after analysis (C)g	Wt of lysis (E=B-D) g	%
Streptokinase	0.78	1.30	0.52	0.99	0.31	59
Distilled water	0.80	1.30	0.50	0.85	0.02	4

The extracts of berries have shown greater thrombolytic activity as compared to leaves and stems; their thrombolytic actions in chloroform, ethanol, methanol, and acetone extracts were observed to be 47%, 43%, 44%, and 46% (**Table 5**), respectively which is near to the action of streptokinase (standard thrombolytic agent). The highest thrombolytic action (47%) was exhibited by the chloroform extract of SBT berries. All the above measurements were made as compared to streptokinase (thrombolytic agent, positive control) and water (non-thrombolytic agent, negative control) which have displayed 59% (highest) and 4% (negligible) of thrombolytic action on a human blood sample (**Table 6**).

The thrombolytic results (43-47% lysis) of the investigated SBT berries was very close to those (45.47%-50.86%) of four Bangladeshi medicinal plants namely *Mesua nagassarium*, *Hydnocarpus kurzii*, *Justica gendarussa*, and *Sansevieria trifasciata* by using the same standard (streptokinase, 61%) as reported earlier [45]. The plant extracts of *Drynaria quercifolia*, *Clerodendrum viscosum*, and *Averrhoa bilimbi* from Bangladesh displayed the 28.64%-34.38% *in vitro* thrombolytic activity with reference to streptokinase (41.05%) [46]. In another study, the root extracts of *Pistia stratiotes*, *Smilax zeylanica*, *Pandanus foetidus*, and *Pandanus foetidus* have displayed 35.85%, 43.35%,41.49%, and 47.54% of clot lysis as compared to streptokinase (70.24%) [47]. The percentage clot lyses of *Spilanthes paniculata* Wall., *Eclipta alba* (L) Hassk., *Emilia sonchifolia* (L.) DC., and *Wedelia chinensis* Osbeck. Merr. were found to be 42.77%, 15.19%, 28.71%, and 24.48%, respectively with reference of water (2.96%) and Streptokinase (71.43%) [13].

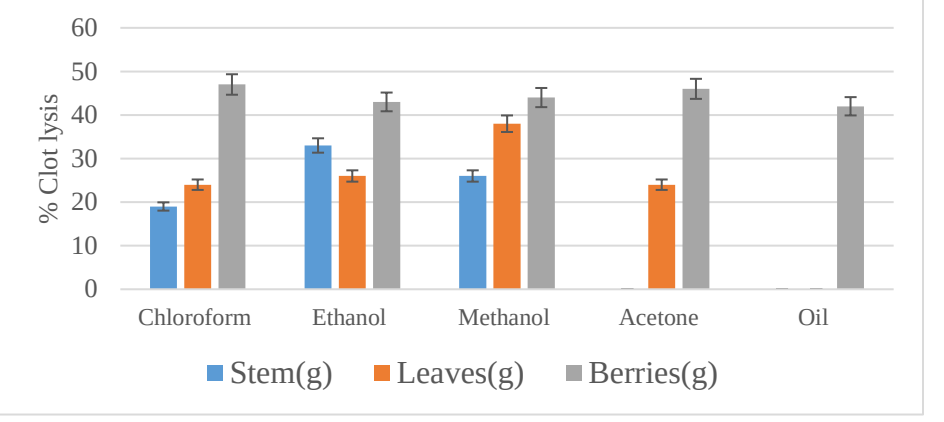

Figure 1. Graphical representation of SBT thrombolytic action

Figure 1 describes a graphical comparison between thrombolytic activities of stem, leaves, and berries extracts. The percentage of clot lyses is shown on Y-axis while the investigated SBT extracts of leaves, stem, and berries are placed on X-axis in the representation (**Figure 4**).

3.2. FTIR Spectroscopy

The dried powder of leaves, stems, and berries were subjected to FTIR analysis to verify the presence of various functional groups. The vibrational bands were observed in the region between 400–4000 cm^{-1}. The resultant spectra are displayed in **Figures 2-4**; the obtained data is summarized in **Table 7**. FTIR spectroscopy has confirmed the presence of alcohol, aldehyde, alkyne, alkene, amines, and ester.

Table 7: FTIR data (cm^{-1}) of Various Parts of Sea Buckthorn

	υ =C-H	υ $CH_{2(asym)}$	υ C=O	υ =CH	υ C-$O_{(sym)}$	YC-$O_{(asym)}$
Leaves	2999,72	2914,88	1737,55	1464,76	1167,69	1098
Stem	3004,55	2920,66	1743,33	1463,71	1158,04	1096
Berries	2923,6	2854,2	1743,33	1457,2	1141,01	1097

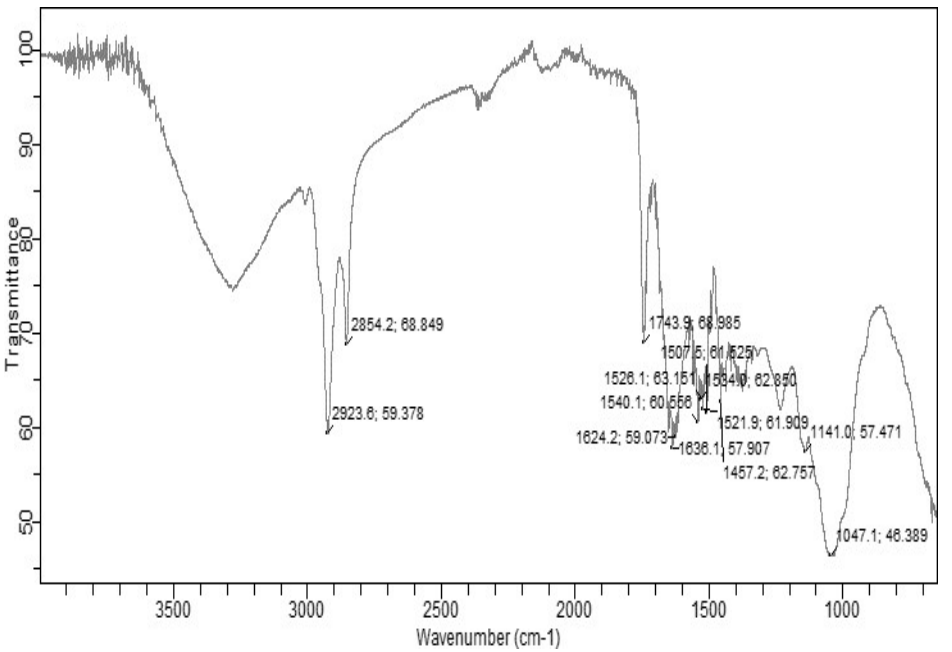

Figure 2. FTIR spectrum of sea buckthorn berries powder

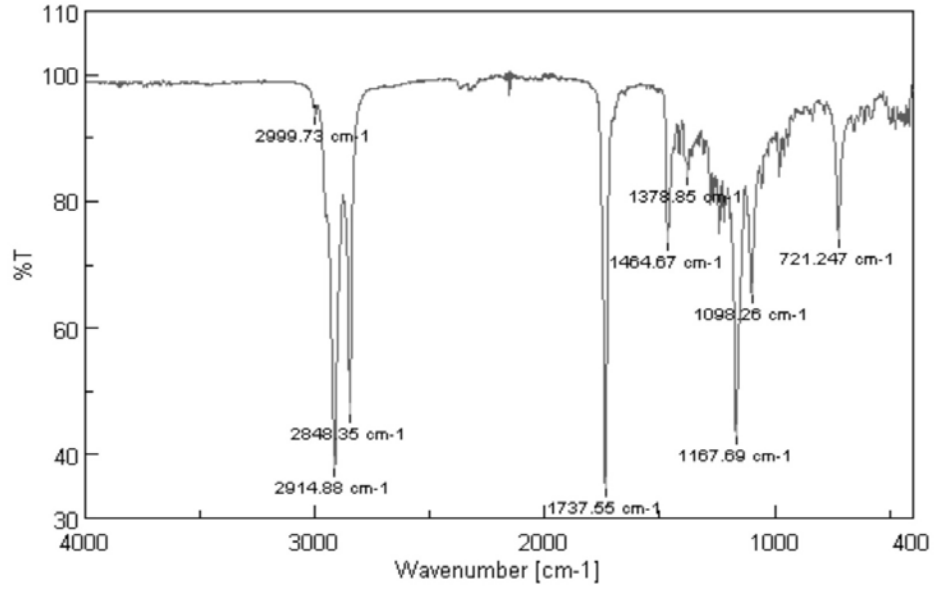

Figure 3: FTIR Spectrum of Sea Buckthorn Leaves Powder

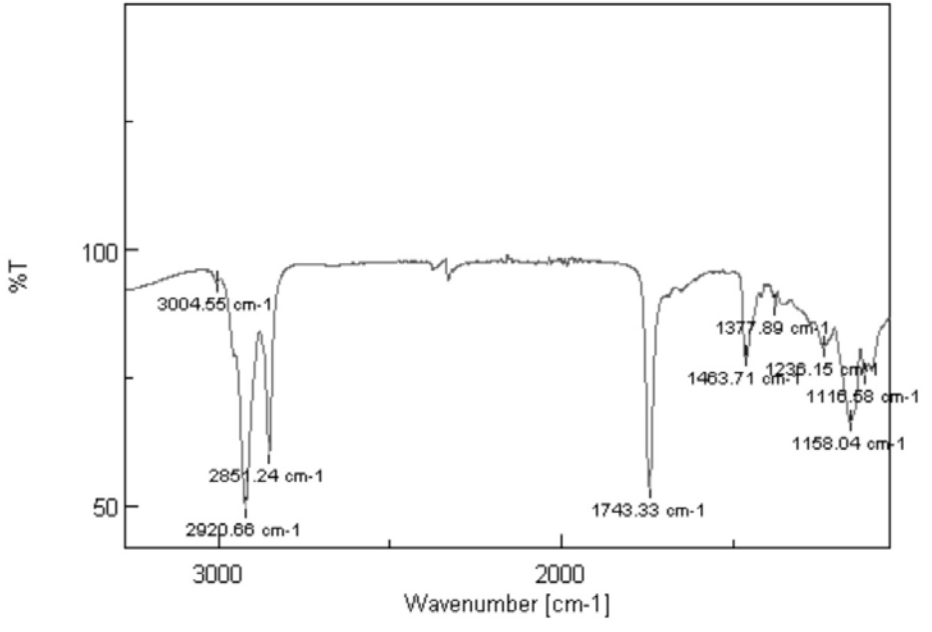

Figure 4. FTIR Spectrum of Sea Buckthorn Stem Powder

4. CONCLUSION

The extract of leaves, stem, and berries of SBT showed 28%, 26%, and 44% blood clot lysis, respectively with reference of as compared to standard streptokinase (59%). So, this plant can be used as a potential thrombolytic agent in future medication. The fruit of SBT was found to display higher thrombolytic potential as compared to that of its leaves and stem. From the FTIR analysis, the presence of important functional groups of alcohol, aldehyde, alkyne, alkene, amines, and ester in the aerial parts of SBT was verified. However, further studies are needed to isolate and screen its bioactive compounds. Future research is also required to increase the thrombolytic activity of SBT by mixing it with other effective thrombolytic plant extracts.

Conflict of Interest Statement

There is no conflict of interest among author of this article.

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List of Abbreviations

Sea buckthorn = SBT; FTIR = Fourier transform infrared spectroscopy; mm = Millimeter; cm = Centimeter; µl= Microliter; IU = International Units

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