Comparative study of In-Vitro Antioxidant and Thrombolytic activities, In-Vitro Anti-Anemic activity of ethanolic fruit and leaf extracts of Phoenix sylvestris in anemic rats

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Abstract- In order to prevent problems from thrombosis in atherosclerotic arteries that affect the heart, brain, extremities, and stagnant blood in veins and heart chambers, it is crucial to treat patients with haemostatic problems effectively. Using chemical agents that are already on the market presents challenges because there is a possibility of numerous adverse effects. An investigation using medicinal plants was done in an effort to prevent that. This study was conducted to assess the in-vitro anticoagulant and thrombolytic thromboplastin time using human plasma activities by measuring clotting time, prothrombin time, and activated partial collected from healthy volunteers at various concentrations. Additionally, the in-vivo anti-anemic activity of *Phoenix sylvestris* fruit and leaf extracts was evaluated in phenyl hydrazine-induced anemic rats.

There were seven groups of rats, each containing six rats. While the other groups were given phenyl hydrazine 60 mg/kg b.w. for two days to induce anaemia Group I was given normal saline as a control. Group II is the positive control; it only received treatment with phenyl hydrazine, whereas Group III received standard treatment with a vitamin B12 complex. functioned as the norm. Groups VI and VII were treated with ethanolic leaf extract of *P. sylvestris* (250 and 500 mg kg-1 b.w.), whereas Groups IV and V were treated with ethanolic fruit extract at varying doses (250 and 500 mg/kg b.w.). All the treatments were given orally and continued up to 28 days. On 29th day, blood was withdrawn, through tail puncture and subjected to the estimation of RBC, Haemoglobin and percentage haematocrit using haematology analyser.

Both extracts showed a more notable effect on the prolongation of these coagulation parameters and significantly increased coagulation. The fruit extract produced preferential increase in bleeding and clotting time, nevertheless the effect was more perceptible compared to leaf extract. When compared to *P. sylvestris* leaf extract, the fruit extract of *P. sylvestris* significantly increased haemoglobin, red blood cells, and percentage haematocrit; the outcomes were comparable to those of standard vitamin B12 complex.

Thus, the present study provides the pharmacological basis for its medical use in cardiovascular and other thrombotic disorders due to its significant anticoagulant, thrombolytic and anti-anemic activities.

Keywords: *In-vitro* Anti coagul ant, Thrombolytic Activities, *In- vivo* anti-Anaemic Activity, *Phoneix Sylvestris.*

Introduction

Blood in health and disease is the subject of haematology. Anaemia, haemophilia, autoimmunity, throm-bosis, and blood coagulation are among the major blood disorders and diseases that occur in sick patients as a result of RBC destruction. These conditions can lead to mortality[1]. The WHO reports that 2 million people worldwide, ranging in age from children to new borns, suffer from anaemia, accounting for 30% of the total population[2]. Red blood cells (RBCs) are involved in both blood coagulation and thrombotic disorders[3]. Prolonged bleeding and thrombosis occur primarily in anaemic patients due to their action as pro-coagulant and pro-thrombotic blood components[4]. Diabetes, hypertension, coronary artery disease, and ischemic heart stroke are caused by changes in red blood cells both in-vitro and in-vivo in whole blood[5].

A variety of blood disorders include blood coagulation. It's persistent process linked to anaemia condition[6]. Thrombosis, haemor-rhage, and hypertension are among the C.V.S. diseases that result from clotting disorders. Factor deficiencies (II, V, VI, X) are found in the extrinsic pathways. Prothrombin time and clotting time are used to evaluate these deficiencies. When calcium ions are present, thromboplastin stimulates the coagulation system's extrinsic pathway. Deficiency of factors VII and X shows increased prothrombin time or clotting time. The four main anticoagulant medications are citrate, EDTA, heparin, and Warfarin[7].

Thrombosis is one of the blood disorders causing heavy blood clots due to which occurs alteration in flow of blood in arteries, veins and tissues leading to severe heart problems[8]. Numerous thrombosis types, such as arterial thrombosis, can result in an ischemic stroke and myocardial infarction. Deep vein thrombosis, which is linked to pulmonary embolism, chronic thrombus embolism, post-thrombotic syndrome, and pulmonary hypertension, is caused by venous thrombosis. Anticoagulant therapy which dissolves blood clots by activating plasminogen, which forms plasmin[10], is the standard treatment for thrombosis. Tissue plasminogen activators, streptokinase, and urokinase are additional medications[9].

Anaemic is a blood disorder which can lead to many diseases. There are more than 400 types of anaemic conditions occurring by decrease number of circulating red blood cells are affecting tissue oxygenation[11]. It is diagnosed by decreased Hb less than 13g dl in males and in female's 12g dl[12]. For the synthesis of haemoglobin in our body the process of erythropoiesis is needed which contains several metabolites[13].

Various Treatment includes based on different types of aneamic condition are vitamin B12, immune suppressants, cortico steroids, erythropoietin injection, blood transfusion and osseous marrow transplan-tation[14]. Most of the pharmacological drugs which are used for the treatment of coagulation, thrombosis and aneamic conditions are not that much effective due to their unwanted side effects and risk factors. It was investigated on herbal medicines that their medicinal properties in plants helps to cure blood disorders.

Traditional plant of *P. sylvestris roxb*, together with 13 other species from the genus phoenix. belongs to family *Are-caceae*. It is widely distributed in India, it is unbranched, tall evergreen ornamental tree occurs at altitude of 1500m. Leaves are 3-4.5 m in length.

Fruits are 15-25mm long and 12mm broad. It possesses some of the medicinal properties which are heart related problems, diarrhea, asthma, lactation, tuber-culosis, cough, gonorrhoea, pains, toothache[15].

So far, much pharmacological work has not been carried out on *P. sylvestris* plant. The present study was carried out to evaluate

the *in-vitro* anticoagulant and thrombolytic, *in-vivo* anti-anaemic activities using rats were carried out and evaluated.

Material and Methods

Collection of Plant Material

The fruits and leaves of the *P. sylvestris* plant were collected in September from the Forest Research Institute in Dehradun, Uttarakhand, India. The authenticity of the plant was confirmed by the departmental authorities of FRI.

Preparation of Ethanolic Extract

The various leaf sections and immature fruits of *P. sylvestris* were gathered and cleaned with deionized water. Fruit and seeds were

separated, and leaves were chopped into tiny pieces. The dried extract materials were allowed to air dry for a week before being ground into a coarse powder using a mixer grinder. Two liters of ethanol were used to macerate 500 g of dried powder separately for four days. Filtered and collected, the fruit and leaf extract solvents were then further distilled and evaporated. The concentrated drug extract was finally produced, dried under a desiccator and stored in a china dish covered in aluminium foil.

Phytochemical Screening

The phytochemical screening of ethanolic fruit and leaf extract of *P. sylvestris* was carried out for the determination of phytochemical constituents like carbohydrate, vitamins, minerals, proteins, enzymes, sugars, iron, alkaloids, flavonoids, saponins, steroids, tannins and phenols[15,16] .

Acute Toxicity Studies

Oral doses of 50, 100, 200, 400, 800, and 1600 mg kg^{-1} of ethanolic *P. sylvestris* leaf and fruit extracts were given to groups of mice (n = 6); the mice were then monitored for signs of behavioural and neurological damage, and the % mortality was documented[24] hours later. The animals were given their doses. According to OECD guideline 42018, this plant species has not yet been the subject of any pharmacological research. Therefore, doses for acute toxicity tests were taken into consideration based on the other species in the same genus. At 1600 mg kg^{1}, the extracts were shown to be mortality-free.

In-Vitro Anticoagulant Activity

The *In-vitro* anticoagulant activity of ethanolic fruit and leaf extract of *P. sylestris* was determined by prothrombin time[17].

Blood Samples Collection

In order to obtain pure platelet plasma, five millilitres of blood were first drawn from the veins of each of the four healthy volunteers. The sterile syringes were used to remove the blood from each volunteer's right arm, and the samples were then placed individually in containers containing trisodium citrate to prevent the clotting process. Each person's obtained plasma sample was labelled and used right away to determine the prothrombin time test.

Blood Serum Samples Separations

The collected plasma samples were divided into 9 groups as given below in **Table-1**.

Finally, the clotting time was recorded with a stop watch by titling the test tube for every 30 seconds. This time was noted as prothrombin time.

Table-1 Blood Serum Samples

Group	Type	Composition
I	Negative Control	0.2 mL plasma + 0.1 mL 0.9% saline + 0.3 mL $CaCl_2$
II	Test (Fruit Extract)	0.2 mL plasma + 0.05 g/mL fruit extract + 0.3 mL $CaCl_2$
III	Test (Fruit Extract)	0.2 mL plasma + 0.1 g/mL fruit extract + 0.3 mL $CaCl_2$
IV	Test (Fruit Extract)	0.2 mL plasma + 0.3 g/mL fruit extract + 0.3 mL $CaCl_2$
V	Test (Fruit Extract)	0.2 mL plasma + 0.5 g/mL fruit extract + 0.3 mL $CaCl_2$
VI	Test (Leaf Extract)	0.2 mL plasma + 0.05 g/mL leaf extract + 0.05 mL $CaCl_2$
VII	Test (Leaf Extract)	0.2 mL plasma + 0.1 g/mL leaf extract + 0.3 mL $CaCl_2$
VIII	Test (Leaf Extract)	0.2 mL plasma + 0.3 g/mL leaf extract + 0.3 mL $CaCl_2$
IX	Test (Leaf Extract)	0.2 mL plasma + 0.5 g/mL leaf extract + 0.5 mL $CaCl_2$

Table-2 Treatment details of different controls and test groups

Group	Type	Treatment Details
I	Normal Control	0.1% Carboxy Methyl Cellulose (CMC)
II	Anaemic Control	Phenylhydrazine – 60 mg/kg/day for 2 days
III	Reference Control	Vitamin B12 syrup for 28 days
IV	Test Group (Fruit Extract – Low Dose)	*P. sylvestris* fruit extract – 250 mg/kg for 28 days
V	Test Group (Fruit Extract – High Dose)	*P. sylvestris* fruit extract – 500 mg/kg for 28 days
VI	Test Group (Leaf Extract – Low Dose)	*P. sylvestris* leaf extract – 250 mg/kg for 28 days
VII	Test Group (Leaf Extract – High Dose)	*P. sylvestris* leaf extract – 500 mg/kg for 28 days

In-vitro Thrombolytic Activity

The *In-vitro* thrombolytic activity of ethanolic fruit and leaf extracts of *P.sylvestris* were assessed by thrombolytic test. Aliquots (5 mL) of venous blood sample was withdrawn from healthy volunteers which are dist-ributed in eight different preweighed sterile micro centrifuge tube (1 mL/ tube) and incubated at 37^{O}C for 45 minutes. After the clot formation occurs the serum is completely removed without clot disruption with the help of micropipette for determining clot weight of each individual volunteer. The tube clot was weighed once more **(tube clot weight = tube weight with clot – tube alone weight)**.

Following the addition of a pre-weighed clot to each sterile micro centrifuge tube, 100µL of an aqueous solution containing various partitions of fruit and leaf crude extracts at varying concentrations (0.05, 0.1, 0.3, and 0.5 g mL-1) was added to the pre-weighed clot. 100µL of distilled water was used as the negative control, and 100µL of streptokinase was added as the standard control. The clots were then incubated at 37^{O}C for 90 minutes to observe clot lysis. Following the release of the incubation fluid, the tubes are weighed once more to measure any weight differences following clot disruption. Finally, the difference obtained in

weight taken before and after clot lysis was determined as percentage clot lysis [18].

$$\% \text{ Clot lysis} = \frac{\text{weight of released clot} \times 100}{\text{clot weight}}$$

In-Vivo Anti-Anaemic Activity

Experimental Animals

Wistar male albino rats of either sex (150-180gms) were used for study. Animals were housed in colony cages at ambient temperature of 25±2^{0}C, 12 h light/dark cycle and 50±5% relative humidity with free access to food and water ad *libitum*. Prior to experimentation, the animals were given at least a week to become used to the laboratory setting. Throughout the experiment, participants went without food but not water for the entire night. Every experiment was run from 9:00 to 16:00 hours during the light period. Every group had five animals in it. Based on the approval of the Institutional Ethics Committee (IEC) and the guidelines provided by REG. No. 1269/a/10/ CPC SEA, animal experiments were conducted.

Evaluation of Anti-Anaemic Activity

In-vivo anti-anaemic activity in rats was evaluated by induction of anaemic. Used phenyl hydrazine (60 mg kg^{-1}) by intraperitoneal administration for 2 days for all groups of animals except normal control group. Animals were divided into 7 groups of 5 each.

Finally, on 29th day treated anaemic rats with different plants extracts of *P. sylvestris* activity was evaluated by withdrawn of blood sample through tail puncture. Blood is taken in blood collecting tubes containing EDTA for prevention of blood clotting. Further, blood was subjected to centrifugation process for plasma separation. Finally blood samples were used for the purpose of estimation of blood parameters like red blood cells (RBC), haemoglobin (Hb) and percentage Haematocrit (% Hct) by using Haematology analyser apparatus[19].

Statistical analysis

All the values are expressed as the mean ± SEM and were analyzed *via* one-way analysis of variance (ANOVA) followed by Dunnett's test, using SPSS 18.0 software. Statistical significance was set at $P < 0.05$.

Results and Discussion

Preliminary Phytochemical of plant extracts revealed the presence of phytochemical constituents like carbohydrates, sugars, alkaloids, flavonoids, saponins, phenols and tannins.

The effects of various ethanolic fruit and leaf extracts of *P. sylvestris* show that dose escalation increases prothrombin time at different concentrations. The ethanolic fruit extract exhibits greater anticoagulant activity than the leaf extract at 500 µg/mL (50:35±0.13), while the leaf extract at 500 ug/ml (41:14±0.02) yields similar results. (**Table-3**).

Table-3 *In-vitro* Anticoagulant Activity of Ethanolic Fruit and Leaf Extracts of *P. Sylvestris*

Drug Treatment	Concentration (µg/mL)	Time of Coagulation (Mins)
Normal control	0.9%NaCl	2:13±0.002
Fruit extract	50	7:15±0.04
	100	13:52±0.08
	300	24:14±0.15
	500	50:35±0.13*
Leaf extract	50	6:12±0.04
	100	10:14±0.06

	300	22:23±0.17
	500	41:14±0.02*

All the values are expressed as Mean ± SEM, n= 5 ,* p<0.001 when compared with Normal Control.

Table-4 In-*vitro* Thrombolytic Activity of Ethanolic Fruit and Leaf Extract of *P. Sylvestris*

Drug Treatment	**Concentration (µg/mL)**	**Weight Of Clot Before Lysis**	**Weight Of Clot After Lysis**	**Clot Difference**	**%Clot Lysis**
Normal Control (Distilled Water)	100	0.121 ±0.0012	0.0063± 0.0012	0.115 ±0.0017	5.2
Standard Control (Streptokinase)	100	0.118 ±0.0010	0.016± 0.00089	0.102 ±0.00034	86.2
Fruit Extract	50	1.25 ± 0.014	0.60 ± 0.015	0.7± 0.14	80.66
	100	1.12± 0.015	0.49 ± 0.012	0.6± 0.12	81.65
	300	1.22± 0.013	0.53 ± 0.0089	0.62 ± 0.015	83.88
	500	1.18 ± 0.012	0.48 ±0.015	0.57± 0.012	84.22 *
Leaf Extract	50	1.2 ± 0.044	0.36 ± 0.17	0.67± 0.014	71.4
	100	1.46± 0.17	0.66± 0.014	0.81± 0.0189	73.43
	300	1.35± 0.0067	0.61± 0.021	0.90± 0.012	75.66
	500	0.95± 0.017	0.53 ± 0.017	0.42 ± 0.014	79.5 *

All the values are expressed as Mean ± SEM, n=5,* P<0.001 when compared with standard values

Anaemic rats induced with phenyl hydrazine were used to test *Phoenix sylvestris* ethanolic fruit and leaf extracts for their anti-anemic properties. The table-3 displays the results. In comparison to the leaf extract (500 mg/kg), which had RBC (5.69), Hb (10.8), and %HCT (38.6), the fruit extract of *P. sylvestris* (500 mg/kg) significantly increased the haemato-logical parameters of red blood cells (7.17), hemoglobin (14.7), and percentage heamatocrit (48.53).By comparing fruit and leaf extracts with anaemic control group , fruit extract shows maximum activity with standard control group of vitamin B_{12} syrup. The results mentioned in **Table-5**.

Table-5 Effect of Ethanolic Fruit and Leaf Extracts of *P. Sylvestris* in Phenyl hydrazine Induced Anaemic in Rats.

S. No.	Drug Treatment	RBC	HB(G/DL)	HCT%
1	Normal Control	8.81±0.65	13.72±0.65	48.75±0.03
2	Anemic Control (Phenyl hydrazine-60mg/kg)	2.51 ±0.018	9.76 ±0.18	21.4±0.21
3	Reference Standard (Vitamin B_{12} syrup)	8.43±0.42	13.18±0.73	46.25±0.54
4	Test Group (Fruit Extract-250mg/kg)	5.86 ±0.18	13.6 ±0.13	36.8±0.43
5	Test Group (Fruit Extract-500mg/kg)	7.17 ±0.027	14.7 ±0.13	48.53±0.21
6	Test Group (Leaf Extract-250mg/kg)	4.03± 0.0144	9.3± 0.077	31.61±0.18
7	Test Group (Leaf Extract-500mg/kg)	5.69 ±0.0044	10.8±0.15	38.6±0.41

All the values are expressed as Mean± SEM, n=5,*p<0.001 when compared with standard values

The present study performed to evaluate *In-vitro* anticoagulant, throm-bolytic and *in-vivo* anti-anaemic activity in anaemic rats using *P. sylvestris* of ethanolic fruit and leaf extracts. During study research plant extracts activity assessed by phytochemical screening tests and acute toxicity studies. The phytochemical tests show presence of carbohydrates, alkaloids, tannins, phenols, sugars, flavonoids, saponins[15,16]. In acute toxicity studies reveal plant extracts does not have adverse effects and risk factors showing presence of low toxicity profile in animals by administration of ethanolic plant extracts of *P. sylvestris* at various doses. By comparative study it has been clearly shown that ethanolic fruit extract shows maximum effect than leaf extract. It possesses anticoagulant, thrombolytic and anti-anaemic properties. The coagulation occurs due to heavy bleeding and clots[6.] The condition of coagulation is treated by using *P. sylvestris* plant of leaf and fruit ethanolic extracts exhibits reduce blood clotting on human blood sample. *In-vitro* study based on prolongation of dose at 500µg/mL fruit extract shows maximum anticoagulant activity compared to the leaf extract. *In-vitro* thrombolytic activity revealed that ethanolic extracts of *P. sylvestris* show maximum blood clot-lysis. condition of thrombosis cause heavy blood clots leading to severe CVS diseases and blood disorders[8]. By using medicinal plant of *P.sylvestris,* we can cure thrombosis by decreasing blood clots and increasing clot lysis. Based on increasing dose concentration at 500 µg ml^{-1} shows maximum *in-vitro* lysis of clot in fruit extract. Mild effect in leaf extract was noted. By comparing fruit extract shows significant activity as compared to leaf extract. *In-vivo* anti-anaemic activity using phenyl-hydrazine induced anaemic rats were investigated by administration of Phenyl-hydrazine (i.p) for 2 days treatment at dose of 60mg/kg. There is decreased haematological parameters, leads to destruction of red blood cells, haemoglobin and percentage haematocrit. The decreased parameters attain in normal level[20.] When treated with plant extracts of *P.sylvestris, it* shows increase of RBC, Hb and % haematocrit. From results it is shown that ethanolic fruit extract of *P. sylvestris* have significant effect at 500 mg kg^{-1} dose based on dose prolongation. Ethanolic leaf extract shows minimum activity at dose of 500 mg kg^{-1}. On comparison fruit extract shows better activity than leaf extract.

Conclusion

The findings of the present study suggest that the ethanolic extracts of *Phoenix sylvestris* fruits and leaves possess anticoagulant, thrombolytic, and anti-anemic properties, indicating their potential in the treatment of various blood-related disorders.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The data are contained within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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