



Evaluation of *In vitro* anticoagulant and antimicrobial activities of *Gymnema sylvestre*

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Abstract

The aim of this study was to investigate the *in vitro* anticoagulant and antimicrobial properties in *Gymnema sylvestre*. The ethanol extracts of the stem, leaves and root of *Gymnema sylvestre* were studied for the *in vitro* anti-coagulant activity by Plasma re-calcification method. Among the three plant extracts, leaf showed maximum anti-coagulant activity of (40:39mins) in 1000ppm and a minimum of (02:38mins) in 1500ppm of root extracts. The standard drug EDTA showed very good anti-coagulant activities of more than one hour. For the antibacterial activity, all the extracts are potent antimicrobials against all the microorganisms studied. Among the different plant extracts studied leaf extracts showed maximum antibacterial activity (17.3 mm) against *Proteus mirabilis*. In antifungal activity, *Candida albicans* (19.2mm) showed efficient antifungal activity for leaf extract.

Keywords: *Gymnema sylvestre*, Ethanol extract, *In vitro* anticoagulant, antimicrobial activity

Introduction

Currently, the cardiovascular disease is a leading cause of death all over the world. This disease is caused mainly due to the abnormal blood coagulation (clotting) in the arteries supplying blood to the heart. Blood clots that develop in the arteries can cause heart attack/stroke. The clots disconnect blood flow to the heart (Klausner, 1983). The undesired blood clot interferes in the free flow of blood leading to dysfunction/permanent damage to the heart. The usual coagulation process is essential to avoid unnecessary blood loss through the injured blood vessels; but undesired blood coagulation results in several life threatening diseases. Blood clots formation in the arteries supplying blood to the heart or brain is the common cause of heart attack and stroke (Dobesh *et al.*, 2004).

The existing anticoagulants agents consist of heparins, vitamin K-antagonists and their derivatives are principle drugs used in clinical practices. Present anticoagulants comprise the harmful life-threatening side effects. Limitations of existing anticoagulant and cost effectiveness have prompted scientist and biochemist to go for alternate novel agent from natural sources (Manicam *et al.*, 2010 and Kumar *et al.*, 2011). It is well documented that herbal medicines are used for health purposes although insufficient accurate information is available. Similarly, local population in many countries uses crude extracts for the treatment of number of ailments (Afonne *et al.*, 2000).

Antimicrobial resistance has become a global problem. Worldwide attention has been shifted towards finding new herbal chemicals for the development of new drugs. Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant forms. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery (Nisbet and Moore, 1997).

Gymnema sylvestre (Asclepiadaceae) is a slow growing, perennial, woody climber, distributed throughout the India, in dry forests upto 600 m height. It is mainly present in the tropical forest of Central and Southern India. *G. sylvestre* is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, family planning, snakebite, urinary complaints, stomach problems, piles, chronic cough, breathing troubles, colic pain, cardiopathy, constipation, dyspepsia and hemorrhoids, hepatosplenomegaly. In addition, it also possesses antimicrobial, antihypercholesterolemic, anti-inflammatory and sweet suppressing activities and it also acts as feeding deterrents to caterpillar (Kritikar and Basu, 1963 and Alam *et al.*, 1990).

Based on the medicinal properties of *G. sylvestre*, the present investigation was taken up with an objective to evaluate the *in vitro* anti coagulant activity and antimicrobial activity against various human pathogenic bacterial and fungal cultures.

Materials and Methods

Preparation of plant extracts

The leaves, stem and root of the plant were collected separately and washed with distilled water and then were sterilized with 0.2% HgCl₂ and subsequently washed with distilled water. Thereafter, 20g of plant samples were weighed separately and crushed using mortar and pestle. The crushed samples were kept in 500ml conical flask containing the ethanol (100 ml) whose extract is to be obtained. This was left undisturbed for 48hrs. After this, the extracts obtained were filtered and left in open air for evaporation of solvents.

In vitro anti coagulant activity

This assay measures the prolongation of thrombin generation. When human plasma is incubated with a compound which inhibits blood coagulation, and

thrombin is added to initiate coagulation, the time taken for the clot formation will be prolonged as compared to the control (no inhibitor added) as stated by Dong *et al.*, (1998).

The blood samples were obtained from normal individuals by using sterile syringes. Its been withdrawn from vein of right arm of each individual and placed separately in containers containing tri-sodium citrate to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature.

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of CaCl₂ (25 mM) were added together in a clean fusion tube. They were then subjected to incubation at 37°C in water bath. EDTA & Sodium citrate were taken as reference standard. For control, extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time.

Antimicrobial activity

Microorganisms used:

The test organisms used in the present study included the bacteria; *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsilla pneumonia* and *E.coli*. And the fungi, *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Mucor* sp, *Candida albicans* were used.

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. About 50 µl of different plant extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours fungal pathogens. The diameter of the inhibition zone (mm) was measured and the

activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Results and Discussion

In vitro Anticoagulant activity

The ethanol extracts of the stem, leaves and root of *Gymnema sylvestre* were studied for the *in vitro* anti-coagulant activity by Plasma re-calcification method (BuLoeliger *et al.*, 1985 and Colman *et al.*, 1994). These extracts showed significant anti-coagulant activity based on the concentrations dependent manner (i.e.,) in ppm. Among the three plant extracts, leaf showed maximum anti-coagulant activity of (40:39mins) in 1000ppm and a minimum of (02:38mins) in 1500ppm of root extracts. The standard drug EDTA showed very good anti-coagulant activities of more than one hour (Table 1).

The **prothrombin time (PT)** is a measurement of blood coagulation, specifically the clotting time of plasma recalcified in the presence of excess tissue thromboplastin, which measures the interactions of fibrinogen, prothrombin, Factor V, Factor VII and Factor X (Anonymous, 2013).

Similar to that of our study, Manickam *et al.*, 2010 evaluated the aqueous leaf extract of *Melastoma malabathricum* Linn. possesses potent anticoagulant property. Lalitha *et al.*, 2015 reported that the aqueous extract exhibited significant anticoagulant activity compared to ethanol and methanol extract.

Antimicrobial activity

Antimicrobial activity is used to test whether the plant extract has capability to control the growth of the microorganism. The antimicrobial potential of both the experimental plants was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, viz., Ampicillin (1.0 mg/disc), Flucanazole (1.0 mg/disc).

Table 1: Effect of ethanol extracts of prothrombin time of normal human plasma

PLANT SPECIES	CONC(in ppm)	Prothrombin time (mins)
Stem	500	16:45
	1000	38:17
	1500	45.25
Leaf	500	24:00
	1000	40:39
	1500	25:15
Root	500	05:19
	1000	07:28
	1500	02:38
EDTA	500	01:26:05
	1000	01:21:22
	1500	01:15:27
Isosaline (control)	500	05:43
	1000	02:40:32
	1500	02:30:44

The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. Among the different plant extracts studied leaf extracts showed maximum antibacterial activity (17.3 mm)

against *Proteus mirabilis* and the minimum inhibition zone diameter was obtained in *E. coli* and in *S. typhi* with diameter 2.6 mm, 2.3, respectively. (Fig 1).

Fig 1: The antibacterial activity of *Gymnema sylvestre*

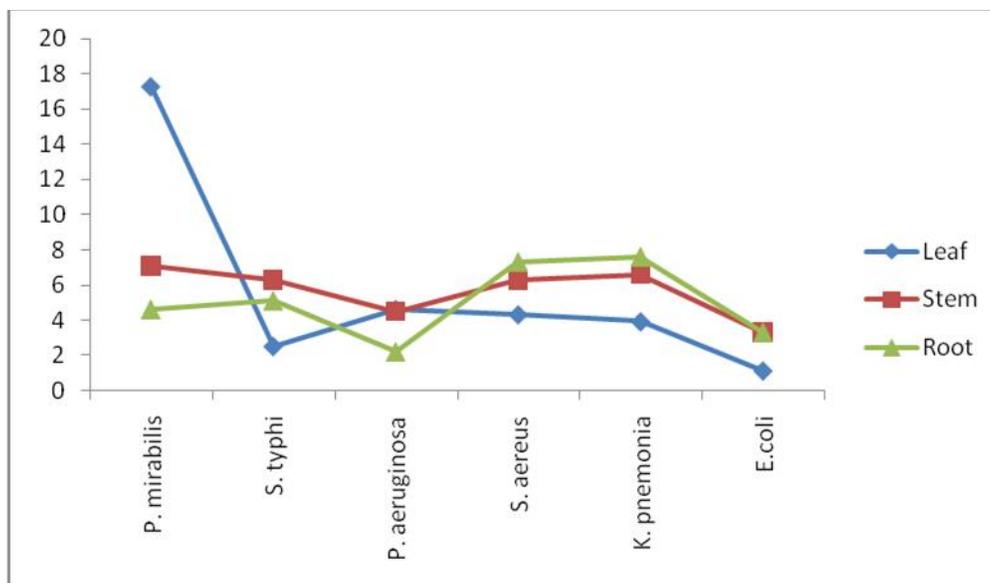
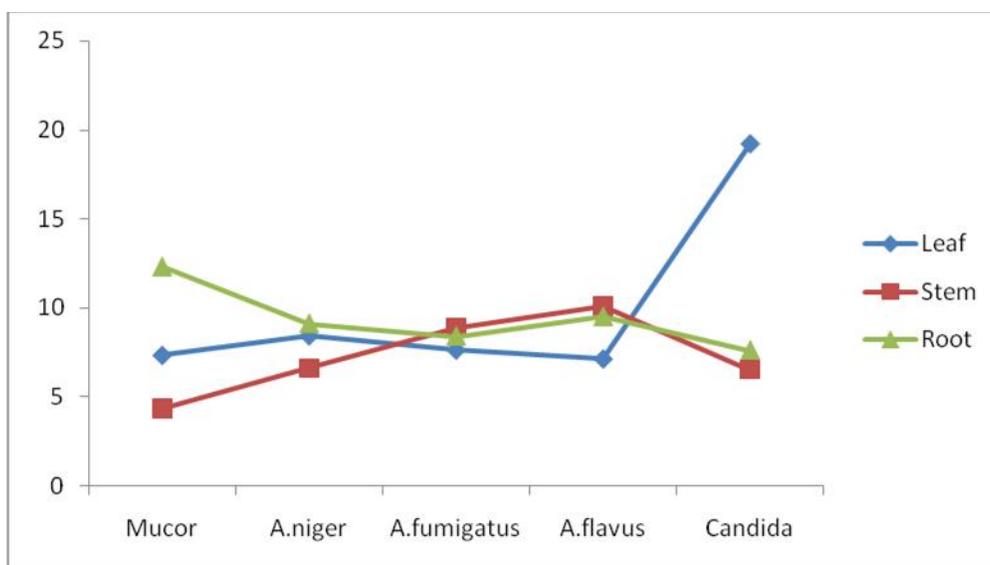


Fig 2: The antifungal activity of *Gymnema sylvestre*



For the antifungal activity, *Candida albicans* (19.2mm) showed efficient antifungal activity for ethanol leaf extract. The stem extract showed lowest inhibition zone with diameter against *Mucor* sp. (Fig 2). Similar results showing that the alcoholic extract having the best antimicrobial activity is also reported by Preethi *et al.*, 2010 in *Leucas aspera*, *Holarrhena antidysenterica*.

In conclusion, of the present investigation *Gymnema sylvestre* contain potential anticoagulant and antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases.

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