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In vitro Anti-coagulant Activity of *Dalbergia sissoo* Bark

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ABSTRACT

Dalbergia sissoo belonging to family Fabaceae is a widely growing plant and has been reported to possess multiple therapeutic properties like analgesic, anti-inflammatory, analgesic and antipyretic anti-ulcer, antioxidant, anthelmintic, antimicrobial, antidiabetic and is also used to treat syphilis, dysentery, nausea, eye and nose disorder, skin diseases and blood disorder. However, the plant yet has not been fully elucidated for its anti-coagulant property. Hence, in the present investigation an attempt has been made to explore the anti-coagulant potential of *Dalbergia sissoo* bark in comparison with a known standard drug as the positive control and normal saline as the negative control. The anti-coagulant activity of the aqueous and alcoholic extract of *Dalbergia sissoo* bark may be due to presence of phytochemicals such as flavonoids and phenolic compounds which reveals on preliminary phytochemical screening.

Keywords: *Dalbergia sissoo*, Quick's method, Prothrombin time, Phenolic compounds, Flavonoid.

INTRODUCTION

Herbal medicines are used to cure many life-threatening diseases and a way of most common forms of therapy widely available throughout the world. In developing countries herbal medicines are good way to cure many diseases [1]. About 80% of people in developing countries depend on traditional system of medicines for primary health care [2]. In recent years there has been a tremendous increase in the use of plant based products, resulting in massive growth of herbal products globally [3]. *Dalbergia sissoo* is also known as sisu, shisham, tahli, jag at different parts of world [4]. It is also known as Indian rosewood belonging to family Fabaceae (Leguminosae). The bark is 3-5 cm long, either curved or flat, and fibrous [5]. *Dalbergia* is scientifically reported for its wound healing, anti-inflammatory, analgesic, antipyretic and used against blood disorders [6]. Anti-coagulant includes antiplatelets that decrease the rate of blood clotting in the body or prevent clot formation that was already formed. These are specifically indicated for stroke and deep vein thrombosis [7]. Herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system since ancient time. Plant produced compounds are safer and effective over synthetically produced anti-coagulant agents [8]. There are a number of foods and supplements that are known to thin the blood as "Natural blood thinners" [9]. Plants may serve as the alternative source for the development of new anticoagulant agent due to their biological activity [10]. To the best of our knowledge, studies to evaluate the anti-coagulant activity of bark extract of *Dalbergia sissoo* have not been reported. We report herein the investigation of anti-coagulant activity of its aqueous and alcoholic extracts.

MATERIALS AND METHODS

Plant material

Dalbergia sissoo plant was collected from the natural population growing in the Vilad ghat, Ahmednagar, India during August 2016. The plant material was identified at the field using standard keys and descriptions.

Preparation of plant extract

The plant material was placed in plastic air tight bags and washed thoroughly with fresh and distilled water to remove the unwanted matter. These were dried in shade, powdered and extracted with 95 % ethanol by heating in Soxhlet apparatus and distilled water by maceration. The extracts thus obtained was concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (40- 50°C), concentrated to yield the reddish brown semi solid residue. The aqueous and alcoholic

extracts were subjected to preliminary phytochemical screening

Phytochemical investigation

The crude aqueous and alcoholic extracts of *Dalbergia sissoo* bark were subjected to preliminary phytochemical analysis in order to detect presence of various groups of phytoconstituents by carrying out the chemical analysis [5].

Determination of Prothrombin Time (PT) by Quick’s method [11]

Pipette out 0.1 ml of plasma in small test tube. Add 0.1 ml of brain Thromboplastin and mix. Wait for 2 min and add pre warmed calcium chloride solution at 37°C, mix and start the stop watch. Hold the tube in front a source of light and keep tilting the test tube gently. At first appearance of fibrin clot stop the watch immediately and record the time as control reading. Pipette 0.1 ml of plasma in small test tube; add 0.1 ml of different concentration of standard and test compound solution separately. Incubate for 5 min and repeat the procedure to record the elongation in mean prothrombin time.

RESULTS

In recent years, naturally occurring chemical substances derived from plants have attracted interest as possible treatments for coagulation disorders and as template molecules for the development of new drugs. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases [12].

No study to date has assessed the anticoagulant activity potential of *Dalbergia sissoo*. Therefore, with the aim of therapeutic purposes, the *in vitro* anticoagulant action of aqueous and alcoholic extracts of *Dalbergia sissoo* was investigated by the prothrombin time in the present study. *In vitro* anti-coagulant activity of aqueous and alcoholic extracts of *Dalbergia sissoo* were carried out by Quick’s method at concentration of 100, 200 and 500 mg per dl and results are shown in [Table 1 and 2]. The clot lysis of *Dalbergia sissoo* was found to be increased with increase in concentration of the sample. Results showed that the extracts exhibit moderate anti-coagulant activity.

Table 1: *In-vitro* anti-coagulant activity of alcoholic extract of *Dalbergia sissoo* bark

Sr. No.	Concentration (mg per dl)	Time (in seconds)			Mean
1.	100	46	48	47	47
2.	200	85	83	81	83
3.	500	180	187	182	183

Table 2: *In-vitro* anti-coagulant activity of aqueous extract of *Dalbergia sissoo* bark

Sr. No.	Concentration (mg per dl)	Time (in seconds)			Mean
1.	100	35	35	38	36
2.	200	75	72	74	73
3.	500	167	160	168	165

Table 3: *In-vitro* anti-coagulant activity of Ethamsylate

Sr. No.	Concentration (mg per dl)	Time (in seconds)			Mean
1.	100	112	110	113	111

DISCUSSION

Antithrombotic drugs are pivotal in the prevention and/or treatment of thrombotic disorders. Secondary metabolites from vegetal origin are a potential source of anticoagulant drugs [13]. Its popular use as well as some interesting studies in literature prompted us to evaluate the anticoagulant activity of *Dalbergia sissoo* and experimental studies validated it as anticoagulant agent.

The aqueous and alcoholic extracts of *Dalbergia sissoo* were subjected to preliminary phytochemical screening and revealed the presence of alkaloids, phenolic compounds/tannins and flavonoids. Phenolic compounds are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects. Extracts rich in phenolics are increasingly of interest to the pharmaceutical and food industry [14]. Literature reveals phenolic compounds/tannins and flavonoids are responsible for anti-coagulant property [15]. Hence, the presence of tannins and flavonoids may contribute to the said activity.

CONCLUSION

From the present study it is evident that aqueous and alcoholic extracts of *Dalbergia sissoo* possess anti-coagulant property. However, *in vivo* activity of *Dalbergia sissoo* is yet to be discovered and is in pipeline. By above result, it can be suggested that the application of *Dalbergia sissoo* may be accessible to the society for the treatment of cardiovascular diseases involving atherosclerosis, deep vein thrombosis, strokes, heart attacks, and hypertension which are the main causes of morbidity and mortality in world countries.

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