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PHARMACOGNOSTIC STUDIES, EVALUATION OF EX-VIVO THROMBOLYTIC AND INVITRO ANTIOXIDANT ACTIVITIES OF LEAVES OF GUAIACUM OFFICINALE

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ABSTRACT

The present study was an attempt to investigate macroscopic and microscopic characters, chemical nature, thrombolytic and antioxidant activity of Guaiacum officinale. Macroscopic, microscopic, qualitative phytochemical analysis, physiochemical analysis, extractive values in ethanol and water of the leaves were done. Macroscopic and microscopic study showed distinct morphological characteristics in the leaf. Physiochemical analysis of leaf powder revealed total ash 5%, acid-insoluble ash 2.7% alcohol soluble extractive 22.6%, water soluble extractive 31.4%. Pharmacognostic study of leaf is helpful in sample identification and to ensure quality and purity standards of Guaiacum officinale. We focused on the thrombolytic activity and anti-oxidant activity of Guaiacum officinale leaf extract using a simple in-Vitro clot lysis and reducing power assay methods respectively. The Results exhibited a maximum of 81.84% clot lysis at 600 µg/ml concentration in 72 hrs of incubation. The findings indicated that concentrations of leaf extract enhanced the percentage of clot lysis in dose dependent manner along with the incubation time factor. However, streptokinase SK a reference standard and water were used as a positive and negative control showed clot lysis maximum 91.77% and 4.22% in 72 hrs of incubation respectively. The antioxidant activity of the chloroform and hydroalcoholic extracts was found to be significant when compared with absorbance of standard ascorbic acid.

KEYWORDS: Guaiacum officinale, Pharmacognosy, Microscopy, Anti-oxidant, Thrombolytic.

INTRODUCTION

From times immemorial man is dependent on plants for his survival. The important necessities of life food, clothing, shelter and medicines are supplied to a great extent by plant kingdom. The relationship between man and plants has been close throughout the development of human culture. In traditional system of medicine, plants represent principle means of therapy for various types of ailments. In recent years, plants are being used extensively for treating different pathophysiological conditions. Pharmacological investigations of medicinal plants have provided important advances for the therapeutic approach to several pathological conditions.[1] The plant Guaiacum officinale has been choosen for present study which is widely used in traditional medicine and also literature revealed that much work was not reported on extracts of leaves of Guaiacum officinale. The objective of present study was to study the pharmacognostic parameters and evaluation of ex-vivo thrombolytic and in-vitro antioxidant of Guaiacum officinale.

Guaiacum officinale (Family: Zygophyllaceae) commonly known as lignum-vitae, Tree of Life. Guaiacum officinale grows to a height of 9-12 m. Stem is generally crooked, wood intensely hard, the branches knotty and bark deeply furrowed. The dense crown of close-growing foliage gives the tree a rounded, compact, net appearance. Each leaf is composed of 2 or 3 pairs of smooth, stalkless leaflets arranged on a slender mid-rib. The leaflets are 6-13 cm in length. There is much irregularity both in their size and shape: some are broadest above the middle (obovate), some almost blunt (obtuse). Beautiful blue flowers grow in great profusion and almost cover the tree and remain for a long time. As the older blooms fade from deep blue to paler shades, some becoming almost white, a striking variegation of colour is produced. The flowers grow in clusters at the ends of the branches. Each flower has 5 petals cupped in a small, finely hairy calyx, supported on a slender stalk. There are 10 stamens bearing golden yellow anthers. The fruit appears as small, round, compressed, yellow capsules, containing 5 cells; occasionally there are fewer. Each cell encloses a single seed.[2]

Guaiacum officinale (G.officinale) is native of South America and West Indies. This plant is introduced to Pakistan for ornamentation. The chemical studies on G. officinale were started in 1918. A number of compounds including lignans, quinones, sesquiterpenoids and various triterpenoid saponins have also been isolated from the same source. Phytochemical screening revealed the presence oflarreagenin, sitosterol and oleanolic acid.[3] The resin of G. officinale

is used in the initial stage of angina and arthritis in homeopathy. Viqar Uddin Ahmed and co-workers have isolated thirty saponins namely Guaianin A, A1, A2, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R and S and Guaiacin A, B, C, D, E and F. Beside them officigenin-24-O—oleanolic acid-3-O-β-D-glucopyranoside, Akebonic acid-3-α-L-arabinopyranoside and sitosterole-3-β-D-glucopyranoside were also isolated[4]. Antibacterial activities against Bacillus subtilis, Corynebacterium diptheriae, Enterobacter cloacae, Kelebsiella ozaenae, Proteus vulgaris, Salmonella typhi, S. pyogenes, S. lactis, S. cilreus, and shigella dysenteriae were reported.[5] The aqueous ethanolic extracts of G.officinale were reported with anti-inflammatory activity.[6] Different parts of the plant are used as diuretics, tranquilizing and abortifacient[7]. The leaf, seed and twig extracts of G. officinale were reported for anti HIV-1 properties in primary peripheral blood mononuclear cells (PBMCs) infected with the reference HIV-1 BaL strain.[8]

Though many activities were reported to this plant, till date no work was done to establish the pharmacognostic parameters, thrombolytic and anti-oxidant activity of this plant. So, the present study was designed to study the pharmacognostic parameters, phytochemical screening and evaluation of thrombolytic activity and antioxidant activity invitro.

MATERIALS AND METHODS

Macroscopic Evaluation

Size: A graduated ruler in millimeters was used for the measurement of the length, width and thickness of crude materials.

Color: The untreated sample was examined under the diffused daylight. The color of the sample was compared with that of a reference sample.

Surface characteristics, texture and fracture

The untreated sample was examined for organoleptic characters. The material was touched to determine if it is soft or hard, it was made bend as ruptured to obtain information on brittleness and the appearance of the fracture plane - whether it was fibrous, smooth, rough, granular etc.

Odour

The sample was placed on the palm of the hand and slowly and repeatedly air was inhaled over the material. Further, the sample was rubbed between the thumb and index finger or

between the palms of the hands using gentle pressure. The strength of the odour (none, weak, distinct, strong) and then the odour sensation (aromatic, fruity, musty, rancid etc.) was determined. A direct comparison of the odour with a defined substance was done.

Microscopy of leaf

A thin section of plant tissue ranging from 5-12 µm was prepared and the section was fixed on the slide it was then subjected for staining with phloroglucinol and concentrated hydrochloric acid in 1:1 ratio.

Powder microscopy

Epidermal cells, Stomata, trichomes, palisade cells, phloem fibres, xylem vessels, vascular bundles, starch grains and calcium oxalate crystals are some of the important characters for leaf drugs, which are observed in powder microscopy. Powder microscopy was performed as per WHO guidelines where 1 or 2 drops of water, glycerol/ethanol or chloral hydrate was placed on a glass slide. The tip of a needle was moisten with water and was dipped into the powder. A small quantity of the material that adheres to the needle tip was transferred into the drop of fluid on the slide. It was stirred thoroughly, but carefully, and a cover-glass was applied. The cover-glass was pressed gently with the handle of the needle, and excess fluid was removed from the margin of the cover-glass with a strip of filter-paper.[9]

Physical Properties of powder

Various physical properties of powder like Bulk density, Porosity, Angle of repose and carr's index were determined as per the official procedures.[10]

Physiochemical analysis

Physiochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash, sulphated ash were determined according to WHO guidelines on quality control methods for medicinal plants.[11]

Phytochemical screening of Guaiacum officinale

Extraction Procedure (Using Various Solvents)

The fine powder was weighed about 50gms and macerated with 140ml of Hexane for about 3-4 days and subjected for vacuum filtration. Then the extract was further evaporated in a china dish to get more concentrated. To the residue we added chloroform and macerated for about 7 days and subjected for vacuum filtration. Then the chloroform extract was further

evaporated in a china dish to get more concentrated. To the residue, added ethanol: water (120:40ml) and macerated for about seven days and subjected for vacuum filtration. Then the Hydro alcoholic extract was evaporated in a china dish to a residue.

Preliminary phytochemical screening

Various qualitative tests were performed for the detection of phytochemical constituents present in the three extracts.[12]

Evaluation of thrombolytic activity

Thrombolytic activity of the hydroalcoholic extract (HA extract) of G.officinale was determined using the following procedure described by Fahad hussain et al.[13] Commercially available lyophilized Streptokinase vial (Zydus Cadila pharmaceutical Ltd.) of 15, 00,000 I.U. was procured and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for in vitro thrombolysis. 4ml of blood from goat was collected from the slaughter house and transferred to different pre weighed sterile alpine tubes (0.5ml/tube). The tubes were now incubated at 37^0 C for 45 minutes. After clot formation serum was aspirated out without disturbing the clot formed. Each tube having the clot was again weighed to estimate the clot weight.

Clot Weight= Weight of clot formed in the tube-weight of empty tube

To each alpine tube containing clot appropriate labeling was done and 200, 400 and 600 µl of hydroalcoholic extract of leaves of G. officinal was added. To positive control tube Streptokinase was added and distilled water was added to negative control alpine tube. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation the supernatant fluid released was removed slowly and the tubes were weighed again to observe the difference in weight after clot disruption.[14] Difference between weight taken before and after clot lysis was plot as ratio to obtain the percent of clot lysis and the results were tabulated.

Determining anti oxidant activity by reducing power assay method

The antioxidant activity of the chloroform and hydroalcoholic extracts was determined using Reducing power assay method.[15] Primary stock solution of extract such that each ml contains 1000 µg/ml was prepared. From primary stock solution 100,150,200,250µg/ml of secondary stock solutions were prepared. 1ml of extract from each secondary stock solution

was taken in a test tube and added 2.5ml of phosphate buffer and mixed well. Then added 2.5ml of Potassium Ferricyanide (1%) to the above test tubes and incubated at 50^0C for 20mins. Then added 2.5ml of 10% Trichloroacetic acid and centrifuged at 300rpm for 10mins. The upper layer of about 2.5ml was collected and mixed with freshly prepared 0.5ml Ferric chloride (0.1%). Absorbance was measured at 700nm (if absorbance is > 1, add 2.5ml of distilled water). Absorbance of blank solution is also observed and the results are tabulated. The absorbance was compared with standard ascorbic acid and blank.[16]

RESULTS

The pharmacognostic studies of the leaves of Guaiacum officinale revealed that the leaves are dorsiventral or bilateral, cordially ovate, dark green on upper surface and pale green on lower surface with accuminate apex, entire margin and petiolate as represented in figure-1. The size of leaf varies from 4-5cm length and 3-4cm width. The microscopy of transverse section of the leaf was shown in figure-2. Microscopic studies revealed the presence of Lamina is comprised of single layered epidermis followed by layers of thin-walled, tangentially elongated to oval palisade parenchymatous cells, followed by few layers of spongy parenchyma. Stomata are paracytic stomata. Midrib is comprised of vascular bundles phloem composed of sieve elements and phloem parenchyma, xylem composed of vessels, tracheids and fibre trachieds. Vascular bundles are surrounded by collenchyma patches above and beneath the vascular bundles. No covering and glandular trichomes were observed. The physico chemical properties of powdered leaf were analysed by determining the bulk density, porosity, carr's index and angle of repose. Powder characteristics revealed that powder has good flow property with a percentage porosity of 79.36 and the results were tabulated in Table-1. The result of ash value indicates the purity of a crude drug, whereas alcohol and water soluble extractive values indicate the amount of polar constituents present in the sample. The results of ash value and extractive value were given in Table-2. The results of preliminary phytochemical analysis were given in the table-3. Guaiacum officinale was found to have potent antioxidant activity, as evident from invitro antioxidant studies done by reducing power assay. The hydroalcoholic fraction showed a dose dependent increase in the antioxidant activity compared to the standard (Ascorbic acid) and the results in graphical representation were depicted in figure-4. The results of thrombolytic activity indicated maximum 81.87% clot lysis at 600 µl concentration in 72 hrs of incubation as mentioned and the results in graphical representation were depicted in table-5.

Figure 1: Leaves of Guaiacum officinale.

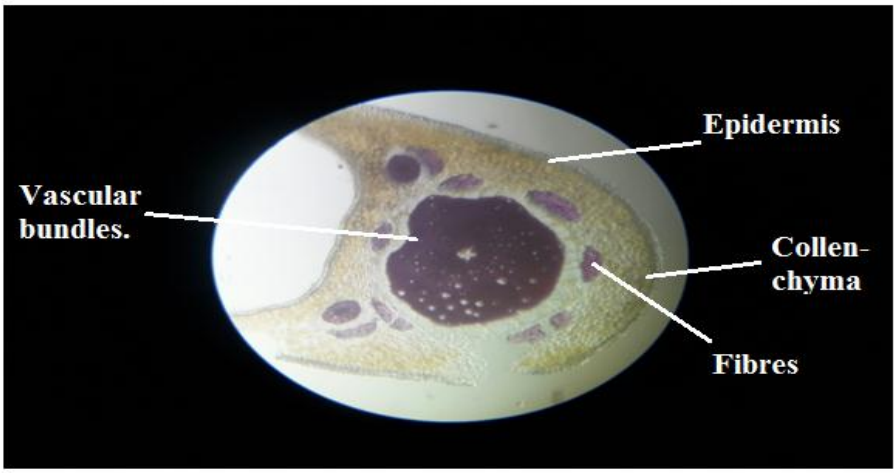

Figure 2: Transverse section of leaf of Guaiacum officinale

Table 1: Physical properties of powder.

S. No.	Flow properties	Result
1	Compressibility Index	15-25
2	Angle of repose	25-30
3	%Porosity	79.3
4	Flow property	Good

Table 2: Results of Physical evaluation.

Type of parameter	Weight obtained	Weight of Sample taken	Results
Total ash value	0.15	3	5%
Acid insoluble ash value	0.08	3	2.8%
Alcohol Soluble Extractive	1.13	5	22.6%
Water Soluble Extractive	1.57	5	31.4%

Table 3: Results of Phytochemical Screening.

Plant constituents	Hexane fraction	Chloroform fraction	Hydroalcoholic fraction
Carbohydrate	Absent	Absent	Present
Alkaloids	Absent	Present	Present
Saponins	Absent	Absent	Present
Phytosterols	Present	Absent	Absent
Phenolic compounds	Absent	Absent	Present
Tannins	Absent	Absent	Present
Flavanoids	Absent	Absent	Present
Proteins	Absent	Absent	Absent
Terpenoids	Absent	Present	Absent
Cardiac glycosides	Absent	Present	Present
Fixed oils & Fats	Absent	Absent	Absent

Table 4: Results of Anti-oxidant Activity.

S. No.	Concentration(µg/ml)	Standard	CGO	GOE
1	100	0.2002	0.1101	0.1198
2	150	0.3865	0.2124	0.2813
3	200	0.5975	0.2882	0.4842
4	250	0.8107	0.3123	0.5931

CGO: Chloroforrm fraction, GOE: alcoholic fraction

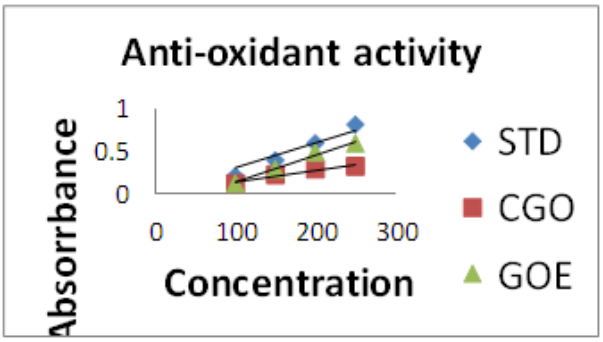

Figure 3: Comparing the Anti-oxidant activity of two extracts.

Table 5: Results of Thrombolytic Activity.

S. No.	Extracts used	Percentage of Clot lysis
1	GOE(200µl)	34.8±0.315**
2	GOE(400µl)	74.20±0.580***
3	GOE(600µl)	81.84±0.605***
4	STK(100µl)	91.77±0.304***
5	DW	4.20±0.790***

Values are presented as Mean ±S.E.M (Standard error of mean) n=3

****P<0.05, *****P<0.001**

DISCUSSION

Medicinal plants are comprised of various phytoconstituents that are responsible for the pharmacological activities. Preliminary phytochemical screening was done for hexane, chloroform and hydroalcoholic extracts of Guaiacum officinale using various qualitative tests. The preliminary phytochemical analysis reveals that the hydroalcoholic extract of Guaiacum officinale showed positive results towards carbohydrates, alkaloids, tannins, phenolic compounds, flavonoids, saponins, terpenoids and glycosides; chloroform fraction showed positive results towards alkaloids, phytosterols, terpenoids and cardiac glycosides; hexane fraction gave positive results for phytosterols. Antioxidants can donate an electron to free radicals, which leads to the neutralization of the radical there by reducing them into more stable and unreactive species. This reducing power was measured by direct electron donation in the reduction. The product was visualized by forming the intense Prussian blue color complex and then measured at wavelength of 700nm. A higher absorbance value indicates a stronger reducing power of the samples. HA extract showed concentration-dependent reducing power. However, its reducing power was weaker than that of Ascorbic acid, which exhibited strongest reducing power. In this study, investigation of thrombolytic activity of leaf extract was carried out using a simple and rapid in-vitro clot lysis model. The results indicated maximum 81.87% clot lysis at 600 µl concentration in 72 hrs of incubation as mentioned. The results indicated clearly that concentrations of leaf extract enhanced the percentage of clot lysis. However, streptokinase STK a reference standard and water were used as a positive and negative control that showed clot lysis of maximum 91.77% and 4.27% respectively.

CONCLUSION

The pharmacognostic studies of leaves of G officinale revealed in detail about the morphological and microscopical characteristics of the leaf. Till date no specific data was reported regarding the pharmacognostic studies of Guaiacum officinale, therefore our work is useful for the standardisation of the herb Guaiacum officinale. As the major constituents reported for the plant are saponins and phytophenols, we focused to evaluate the thrombolytic activity and antioxidant potential of the plant. From the results, it was evident that the leaf extract is a good thrombolytic and antioxidant. Therefore, further studies are required to evaluate the specific phytoconstituents in G officinale responsible for therapeutic activities in order to develop “new lead molecule” as novel therapeutic agent.

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