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In-Vitro Anti-Urolithiasis Activity, Preliminary Phytochemical Investigation of *Bougainvillea glabra* Choisy Stems

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ABSTRACT:

Medicinal plants are sources of bioactive phytochemicals that, can be used to treat numerous of medical conditions due to their strong pharmacological action on the human body. *Bougainvillea glabra* is one of the medicinal plants with the many different of medicinal uses. Qualitative analysis is performed to find out bioactive phytochemicals present in the plant extract. *Bougainvillea glabra* secondary metabolites include Alkaloids, flavonoids, glycosides, phenols, and tannins. Strong anti urolithiasis activity is confirmed. It is confirmed by using calcium oxalate dissolution method which show 82% dissolution of calcium oxalate crystals. The extract shows significant activities.

Keywords: Ethanolic extract, Qualitative, calcium oxalate, urolithiasis.

INTRODUCTION

Plant-based medications have been utilized worldwide in traditional medical systems to treat a variety of illnesses. In areas where access to medications is likely to be limited, 80% of the global population still relies on medicinal plants for their urgent medical needs. Additionally, the use of plants in ethnomedicine is progressing globally. We are blessed by nature with an abundance of botanical diversity, with many different types of plants growing throughout the nation.[1] *Bougainvillea glabra* is one of the medicinal plants with the many different of medicinal uses.

The current research looks at the "phytochemical and pharmacological investigation of bioactive constituents of "*Bougainvillea glabra* (paper flower) stem." *Bougainvillea glabra* or paper flower of the genus *Bougainvillea* belongs to the *Nyctaginaceae* family.[2] *Bougainvillea glabra* is an evergreen climbing plant with thorny stems, it bears clusters of tiny white flowers surrounded by colorful bracts in a range of colors from pink and purple to red. [3]

Various phytochemicals are responsible for number of medicinal properties and pharmacological activities such as *Bougainvillea glabra* has been shown to have antioxidant, antiviral, anti-diabetic, antipyretic, anti-cancer, anti-bacterial, anti-arthritic, anti-fungal, anti-parasitic, and fertility properties. [4]

Qualitative analysis is performed to find out phytochemicals present in the plant extract. *Bougainvillea glabra* secondary metabolites include Alkaloids, flavonoids, Carbohydrates, glycosides and phenols and tannins.

Nephrolithiasis is the term used to describe the formation of renal stones inside the kidney. Urolithiasis is the condition that results when those stones travel from the renal pelvis into the bladder, urethra, and the ureters. Many patients with urolithiasis can be treated with analgesics and antiemetic medications as a component of their prenatal care; however, stones that cause infection, renal failure, and obstruction require increasingly intensive therapies.[5] Anti-urolithiasis medications or therapies help prevent or treat urolithiasis, also known as kidney stones.

Materials and Method:

Collection of plant

The Stem of *Bougainvillea glabra* were collected from local region of Malshiras, Solapur (District), Maharashtra, India during

September 2024. The stems of *Bougainvillea glabra* were shade dried at room temperature for 3 weeks. The dried parts were later coarsely powdered with help of electric grinder after passed through sieve no 20 to obtain coarse powder. The plant powder was stored at room temperature to protect it from moisture.

Preparation of extract

Coarsely powdered stems of *Bougainvillea glabra* were extracted with Soxhlet extraction method by using 95% ethanol as solvent. For this 35 gm of powder was extracted using 100 ml of above-mentioned solvent. [6]

Phytochemical evaluation of Extract

Extract was evaluated for detection of phytochemicals present in the extract. Phytochemical evaluation is carried out by performing different chemical tests such as test for Alkaloids, Flavonoids, Glycosides, carbohydrates, tannins and phenolics.[7]

Invitro Anti urolithiasis Activity

Principle

The specific principles may differ based on the method of action of the chemical under investigation. Stone formation inhibition: Some drugs exert anti urolithiasis action by preventing the production of urinary stones. They may, for example, disrupt the crystallization process by preventing crystal nucleation or growth. When stone forming elements are reduced, the solubility product of crystallizing salts, such as calcium phosphate and calcium oxalate, as decreased as well, which may have anti urolithiasis activity. [8]

Preparation of Calcium Oxalate Stones

1.34 g of sodium oxalate was dissolved in 100 ml of 2 N sulphuric acid and 1.47 g of calcium chloride dihydrate was dissolved in 100 ml distilled water. Both were mixed equally in a beaker to precipitate calcium oxalate. The precipitate free from traces of sulphuric acid by ammonia solution. Washed the precipitates with distilled water and dried at 60 ºC for 4 h.

Preparation of Semi-permeable Membrane

Semi-permeable membrane of eggs is prepared by following procedure,

The apex of the eggs was punctured with a glass rod. Empty eggs shell washed thoroughly with distilled water and placed in a beaker filled with 2 M hydrochloric acid for an overnight, which results in complete decalcification. It was then cleaned with distilled water, immersed in ammonia solution for a while to neutralize acid residues in a wet condition, and rinsed with distilled water.

Calcium Oxalate dissolution method

Weigh 10 mg of calcium oxalate and 10 ml of extract, standard and packed together in the semi-permeable membrane by suturing model, these semi-permeable membranes allow to suspended in a conical flask filled with 100 ml of 0.1 M Tris HCl buffer. First group served as a negative control, containing only 10 mg of calcium oxalate. Second group contain 10 mg of calcium oxalate crystals and 10 ml of standard drug which act as positive control, and lasty third group prepared by adding 10 mg of calcium oxalate crystals and 10 ml of plant extract. The conical flask containing all groups was placed in an incubator, preheated to 37 °C for 2 hours, for about 7 to 8 hours. The content of the semi-permeable membrane from each group was squeezed out into the conical flask, and add 2 ml of 1 N sulphuric titrated with 0.9494 N $KMnO_4$ till light pink color end point was produced. 1 ml of 0.9494 N $KMnO_4$ equivalent to 0.1819 mg of calcium. The amount of dissolved calcium oxalate by extracts is determined by subtracting the amount of residual undissolved calcium oxalate from the total amount. The Percentage of dissolution was calculated. [9]

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Result and discussion

Phytochemical evaluation of Extract

Table no.1: Result of Qualitative phytochemical test for 95% ethanol stem extract of *Bougainvillea glabra*

Sr. no	Chemical constituent	95%Ethanolic extract
1	Alkaloids	+ +
2	Flavonoids	+ +
4	Tannins &phenol	++
5	Glycoside	+
6	Carbohydrates	+ +

(+ + strongly present, + present, - absent)

Different qualitative phytochemical tests are performed to detect the presence of the metabolites, for example, alkaloids, glycosides, flavonoids, carbohydrate, Tannin and Phenolics in stem concentrate of *Bougainvillea glabra* utilizing the 95% ethanol solvent. Outcome is Alkaloids and flavonoids majorly present in extract. Test for tannins and Phenolics also shows positive results.

Invitro Anti urolithiasis activity

Calcium oxalate dissolution method:

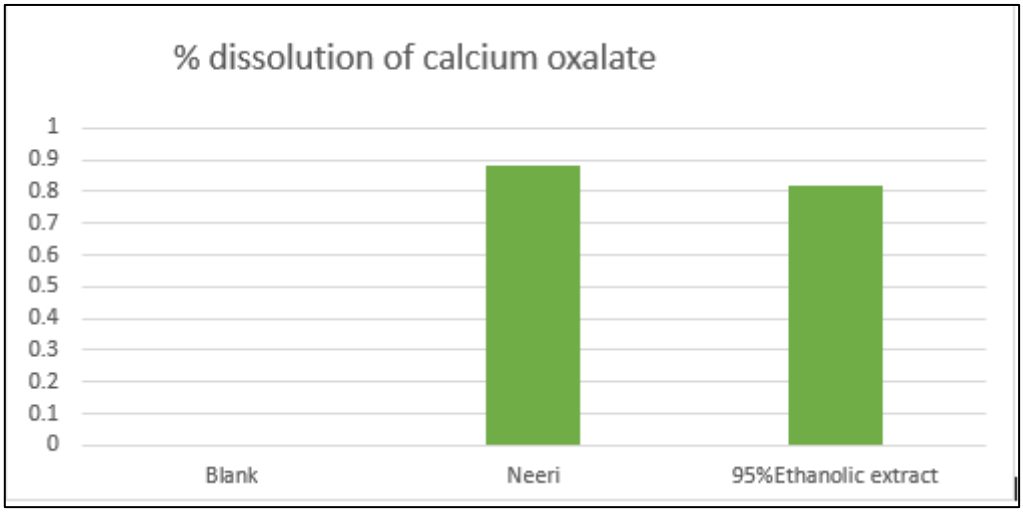
Fig no 1: Decalcified of egg shell In 10% acetic acid

Fig no 2: egg membrane along with the content suspended into 0.1 M tris buffer

The percentage of dissolution of ethanolic extract was calculated from dissolved and undissolved calcium oxalate crystals.

Table no 2: Result of *Invitro* anti urolithiasis activity of Stem of *Bougainvillea glabra*

Drug	Undissolved calcium oxalate	% dissolution of calcium oxalate
Blank	0	0%
Neeri (std)	1.2	88%
95% ethanolic extract	1.8	82%



Graph no 1: Result of *Invitro* anti urolithiasis activity of stems extract

According to the ongoing *invitro* study, the 95% ethanolic extract shows good enough result with reference to standard drug Neeri with a maximum dissolution is 88% and the 95% ethanolic extract maximum dissolution is 82%, the study reveals that the dissolved calcium oxalate of extract is low (1.8 mg) when compared to the standard (1.2 mg). Standard medicine (Neeri) medication has powerful anti-urolithiasis activity. This study conclude that plant extract had strong anti-urolithiasis activity as near standard (Neeri) medicine.

Conclusion :

Phytochemical screenings of the stem's extracts were investigated according to the standard procedures. The crude Stem extract of *Bougainvillea glabra* were investigated to preliminary phytochemical screening which showed the presence of various Phyto-constituents such as alkaloids, flavonoids, glycosides and tannins and phenolics. The *invitro* anti-urolithiasis activity of Stem extract of *Bougainvillea glabra* is also confirmed with the help of Calcium Oxalate Dissolution Method. From this method it is concluded that it shows 82% dissolution of calcium oxalate in 95% ethanolic extract of *Bougainvillea glabra* of stems, which is significant.

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