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## Antilithiatic Activity of Leaves, Bulb and Stem Of *Nymphaea Odorata* and *Dolichos Lablab* Beans.

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### ABSTRACT

The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing. *Nymphaea odorata*, fragrant water Lilly (Nymphaeaceae) traditionally claimed for Antiseptic, Astringent, Demulcent, kidney stone, Deobstruent, Discutient, Vulnerary, Roots are used for coughs, mouth sores, and for tuberculosis and *Dolichos Lablab* beans (Fabeceae) for stimulate gastric activities, as antidote against poisoning, to treat colic, cholera, diarrhea, rheumatism, antilithiatic and sunstroke. The juice from the fruit pods are used for inflamed ears and throats. The fruit is also astringent, digestive, and stomachic and used to expel worms. Owing to its traditional claim methanolic extract of leaves, bulb and stem of *Nymphaea odorata*, and *Dolichos Lablab* beans white and black variety were screened for antilithiatic activity by using *in-vitro* model. Phytochemical study showed the presence of various classes of constituents in both plants and pharmacological study reveals that the leaves & bulbs of the plant *Nymphaea odorata* are having good antilithiatic activity compared to stem and Black and white beans of *Dolichos Lablab*.

**Keywords:** *Nymphaea odorata*, *Dolichos Lablab*, antilithiatic.

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## INTRODUCTION

Urinary stones affect 10–12% of the population in industrialized countries [1, 2]. There are only a few geographical areas in which stone disease is rare, e.g., in Greenland and in the coastal areas of Japan [2]. The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing [3]. With a prevalence of > 10% and an expected recurrence rate of ~ 50%, stone disease has an important effect on the healthcare system [4]. Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened [1]. Features associated with recurrence include a young age of onset, positive family history, infection stones and underlying medical conditions [1]. Epidemiological studies revealed that nephrolithiasis is more common in men (12%) than in women (6%) and is more prevalent between the ages of 20 to 40 in both sexes [5]. The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices [6]. Increased rates of hypertension and obesity, which are linked to nephrolithiasis, also contribute to an increase in stone formation [7].

Management of stone disease depends on the size and location of the stones. Stones larger than 5mm or stones that fail to pass through should be treated by some interventional procedures such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL) [8]. Unfortunately, the propensity for stone recurrence is not altered by removal of stones with ESWL and stone recurrence is still about 50% [9]. In addition, ESWL might show some significant side effects such as renal damage, ESWL induced hypertension or renal impairment [10].

Although there are a few recent reports of beneficial effects of medical treatments in enhancing clearance of stones in the distal ureters [11], de facto there is still no satisfactory drug to use in clinical therapy, especially for the prevention or the recurrence of stones. In this regard, many plants have been traditionally used to treat kidney stones and have been shown to be effective [12].

The Ayurvedic concept appeared and developed between 2500 and 500 BC in India. The literal meaning of Ayurveda is “science of life,” because ancient Indian system of health care focused views of man and his illness. It is pointed out that the positive health means metabolically well-balanced human beings.

In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from the traditional healers [13]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants [22, 23]. Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. Research to find out scientific evidence for claims of plants used for Indian Ayurvedic system of medicine has been intensified. Detailed research on the chemistry and pharmacology of products of plant

origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases [14].

## MATERIALS AND METHODS

**Plant materials:** The plant *Nymphaea odorata* collected from the Ahmedabad, Gujarat and the seeds of *Dolichos Lablab* (White and black) were collected from the lake nearby Pune, Maharashtra. It was identified and authenticated by Head, Department of Pharmaceutical Sciences, Kashmir University, Kashmir and its voucher specimen no. is KUNO -01, KUDLW 02 and KUDLB- 03 respectively. The dried plant materials were powdered and stored in airtight containers prior to extraction. The Soxhlet extraction was performed using methanol as a solvent.

The aqueous extract of cystone was prepared by grinding a tablet to powder. This powder was mixed with 5 mL water and kept for 2-3 hrs and then centrifuged at 1000 rpm. The clear supernatant was used for study as a standard.

**Chemicals:** TRIS buffer, Hydrochloric acid (HCl), Sodium hypophosphate ( $\text{NaHPO}_4$ ), Calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ )

**Preparation of Buffer:** The buffer composition was; 0.1M TRIS buffer; Solution A was 0.4M TRIS [48.4g of Tris (trihydroxymethyl)amino methane per 1000 ml].; Solution B was 0.4M hydrochloric acid. [33.6 mL of concentrated acid per 1000 mL.; A working solution was made up of 25 mL solution A, 20.7 mL solution B made up to 100 mL, the pH was 7.4.

**Procedure:** The experiment consisted of the following tubes for both control and test, 25 mL each of 25 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 25 mM  $\text{NaHPO}_4$ . To the tubes of each set, the different extracts or an equal amount of vehicle was added. The tubes were incubated at 37 °C for 4 hrs. The precipitates of calcium phosphate were generated as follows. Calcium phosphate precipitate was generated by mixing 1 mL of solution from the tubes having calcium chloride dihydrate and disodium hydrogen phosphate monohydrate. Phosphorus was estimated using colorimetric analysis (Nepheloturbidometer). Appropriate standard curves were done with each set of experiments. The amounts of precipitates were determined in each set. The percent inhibition of the test was calculated in comparison with the control samples [15].

## RESULTS

### Pharmacognostical Studies:

#### *Nymphaea odorata*

This perennial plant is a floating aquatic that can grow in water up to 8' deep.

**Flower:** The flowers are fragrant with a yellow center and many spiral petals around 3-5" wide which tapers towards the ends. Long flowering stalks also develop directly from the rootstock; each stalk is un-branched and bears a single floating flower at its apex. The appearance of these stalks resembles the petioles of the leaves. Each flower is about 3-6" across; it has 4 greenish white sepals on the bottom that rest on the water surface, and about 20-30 white petals above. In the middle of the petals, there are many golden yellow stamens surrounding a single pistil. The petals are elliptic-lanceolate and they curve slightly upward. The outer stamens have winged filaments that are broader than the anthers, while the inner stamens have narrow filaments.

**Leaves:** The leaves are flat, spherical with a waxy water repellent upper side with green color and purplish color underneath around 8-14" that floats on the surface. The leaves develop directly from the rootstock on long petioles. They are 4-12" across, orbicular in shape, cleft toward the middle on one side, and smooth along the outer margins. The upper surface of the leaf is medium green, while the lower surface is often purplish; its texture is rather leathery and thick. Fine veins radiate outward from the center of the leaf. The air pores through which the leaf breathes are located on the upper surface, rather than the lower surface (unlike the leaves of most plants). The long petiole of each leaf is terete, stout, and purplish; it is often covered with algae.

**Stem:** The stem was thin light brown in color with striation on them. It was around 20-25 cm long.

**Bulb:** The bulb was green in color with round to oval shape. At the top there was hair like structures.

***Dolichos Lablab***

**Seed:** The seeds are kidney shaped with white hymen & of two color white & black.

**Table 1 : Qualitative Phytochemical Screening**

Sr. No.	Chemical Constituents	Type of Sample				
		<i>Nymphaea odorata</i>			<i>Dolichos Lablab</i>	
		Leaves	Bulb	Stem	White	Black
1	Alkaloids	+	+	+	-	-
2	Carbohydrates	-	-	+	+	++
3	Glycosides	-	-	+	+	++
4	Terpenoids	+	++	-	-	-
5	Saponins	++	+	+	+	+
6	Flavanoids	+	++	+	++	+
7	Polyphenols	++	+	-	+	+
8	Tannins	+	+	-	-	+
9	Pigments	++	+	-	+	+
10	Lipids	+	+	+	++	++

"+", "++", "+++" Present & "-" Absent

**Table 2: Nature of Extract of *Nymphaea odorata***

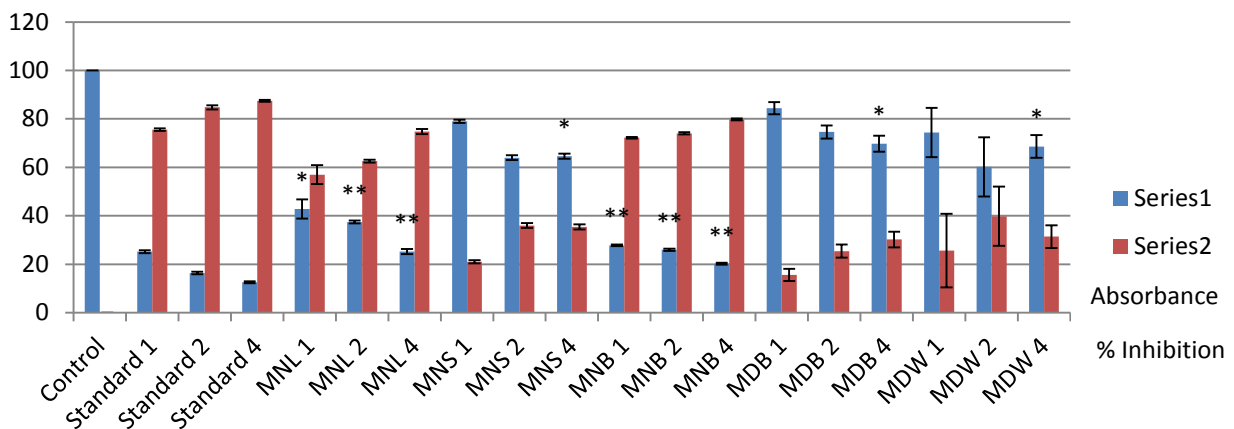
Sr No	Evaluation Parameters	<i>Nymphaea odorata</i>			<i>Dolichos Lablab</i> seeds	
		Leaves	Stem	Bulb	White	Black
1	Colour	Dark green	Brown	Dark Brown	Yellow	Dark Yellow
2	Odour	Characteristic & nauseous	Characteristic & nauseous	Characteristic & nauseous	Characteristic & nauseous	Characteristic & nauseous
3	Appearance	Sticky mass	Sticky mass	Sticky mass	Sticky mass	Sticky mass

**Pharmacological Studies:**

**Antilithiatic Activity**

The results of different groups studied using a methanolic extract of leaves, stem & bulb *Nymphaea odorata* & White & Black seeds of *Dolichos Lablab* at the concentration of 1 mg/ml, 2 mg/ml & 4 mg/ml are shown in Fig.1. In this study, the extracts from leaves & bulb of the *Nymphaea odorata* showed significant decrease in the concentration of stones compared to control and rest of the extract showed non-significant result.

**Fig 1: Antilithiatic Activity of different parts of *Nymphaea odorata* & seeds of *Dolichos Lablab***



\*\*=p<0.1, \*\*\*= p < 0.01

MNL - Methanolic extract of powdered leaves of *Nymphaea odorata*

MNS - Methanolic extract of powdered stem of *Nymphaea odorata*

MNB - Methanolic extract of powdered bulb of *Nymphaea odorata*

MDW - Methanolic extract of *Dolichos Lablab* seeds (White)

MDB - Methanolic extract of *Dolichos Lablab* seeds (Black)

Standard – Aqueous extract of CYSTONE (Marketed formulation)

(1= 1mg/ml, 2= 2mg/ml, 3= 3mg/ml)

## DISCUSSION

The plant *Nymphaea odorata* shows the presence of various phytochemicals like alkaloids, terpenoids, tannins, lipids, etc. while that of *Dolichos Lablab* shows the presence of carbohydrate, glycosides, saponins. Our study clearly shows the utility of leaves & bulbs of the plant *Nymphaea odorata* in the treatment of renal & urinary calculi. It can be inferred that leaves & bulbs of the *Nymphaea odorata* are more active as a prophylactic than its stem & both the varieties (White & Black) of seed of *Dolichos Lablab*. The exact mechanism of action of the plant is not known but the active components seem to be non-protein, non-tannin molecule that may act through inhibition of calcium & phosphate accumulation.

MNL at high dose (2 mg/ml & 4 mg/ml) showed significant activity ( $p < 0.01$ ) and that for low dose (1 mg/ml) showed less significant activity ( $p < 0.1$ ). MNB at all dose (1 mg/ml, 2 mg/ml & 4 mg/ml) showed significant activity ( $p < 0.01$ ). MNS at highest dose (4 mg/ml) showed significant activity ( $p < 0.01$ ) and that for low doses (1 mg/ml & 2 mg/ml) showed non-significant activity ( $p > 0.05$ ). MDB & MDW at highest dose (4 mg/ml) showed less significant activity ( $p < 0.1$ ) and that for low doses (1 mg/ml & 2 mg/ml) showed non-significant activity ( $p > 0.05$ ).

## SUMMARY AND CONCLUSION

The pharmacological evaluation of the all extract showed the extract of leaves & bulbs of the plant *Nymphaea odorata* showed that maximum antilithiatic activity than its stem & both the varieties (White & Black) of seed of *Dolichos Lablab*. Thus we can conclude that leaves & bulbs of the plant *Nymphaea odorata* are having good antilithiatic activity.

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