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A Study on the Anti Urolithiatic Potential and Optimizing the solubility of Gallstones in selected plants

[1]Vidhyashri.A, [2]Dr.D.Chandra Prabha

Post Graduate, Department of Biochemistry, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamil Nadu, India

ABSTRACT

Nature bestowed our country with an enormous wealth of medicinal plants. Medicinal plants have been known for its rich source of therapeutic agents for the prevention of various diseases. They are used from centuries due to its safety, efficacy, cultural acceptability and less side effects as compared to synthetic drugs. Alarming rise in the cholithiasis- deposition of oxalate stone in the gall bladder cases due to life style modified diets are prevailing in the current era. The anti-urolithiatic activity and the phytochemical analysis were assessed in *Zingiber officinale* (ginger), *Macrotyloma uniflorum* (horsegram), *Phyllanthus emblica* (amla) and *Musa sp* (banana stem) with four different solvents namely distilled water, ethanol, chloroform, hexane to study the anti-urolithiatic activity. We observed that *Zingiber officinale* (ginger), *Phyllanthus emblica*(amla), *Macrotyloma uniflorum* (Horsegram) and *Musasp* (banana stem) exhibits anti urolithiatic activity.

Keywords: Medicinal plants, Gall stones, Anti-urolithiatic activity, Phytochemical analysis,

INTRODUCTION

Over the centuries, plants and herbs are used as an important source of medicines. The vast knowledge of the medicinal values of plants is the result of long evolution through trials and error. The use of traditional medicines depend on local availability of natural resources and their indigenous knowledge. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits. The unique biological activity of the plants can be identified by their phytochemicals properties. Most parts of the plants used for the analysis of the phytochemical properties were leaves, roots, stem barks, and fruits (Misganaw Gedlu Agidew 2022).

Cholelithiasis is a gallbladder inflammatory disease, resulting in desquamation of cells and the production of abnormal substances. About 10 - 20% of adult population suffers from gall stones and its predominance in India is 3 - 6 % .Prevalence of Cholelithiasis is high in adults as compared to children(Salman Ahmed, 2020).

Anticholelithiatic plants are utilized since ancient time for treating, dissolution or elimination of gallstones and to avoid their recurrence. The use of anticholelithiatic plants in the form of decoction, infusion, juice, powder are less expensive than current medication and procedures . Extraction and isolation along with clinical trials may develop proactive anticholelithiatic compounds. This could be helpful in creating mass

awareness about conservation of such plants to promote ethno-medico botany knowledge within the region, besides contributing to the preservation of such medicinally important species before they are extinct (Salman Ahmed, 2020).

Ginger (*Zingiber officinale*) has been used to treat GI tract ailments. Ginger has the potential to metabolize cholesterol (which is part of gallstones) into bile acids, thus reducing the formation of gallstones (Suzy Cohen, 2020). Horse gram (*Macrotyloma uniflorum*) seed is reported to possess antioxidant, hepatoprotective and anti-hyperlipidemic activity along with anti-hypercholesterolemic effect. Banana Stem (*Musa sp*) provides plenty of fiber, unsaturated fat and saturated fat supports gallbladder health (Meg campbell 2010). Phytocomponents residing in these plants contribute potentially toward reduction of cholesterol secretion subsequently super saturation of bile with cholesterol by controlling hepatic lipid regulatory enzymes responsible for cholesterol biosynthesis (Papiya Bigoniya *et.al* ; 2014).

The present study aims in assessing anti urolithiatic potential and optimizing the solubility of gallstones in ginger, horse gram, banana stem and amla.

METHODOLOGY

The materials and methods used for the present study are discussed below;

EXTRACTION

12.5gm of ginger rhizome, horse gram seeds, banana stem and amla were separately weighed, blended with 50ml of water and left in dark for overnight extraction and filtered using whatman no.1 filter paper. The same procedure is repeated with ethanol, chloroform, and hexane. The filtrate was stored in refrigerator.

PHYTOCHEMICAL ANALYSIS

Phytochemicals screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents.

Test for Alkaloids:

Add few drops of diluted Hydrochloric acid and filter the extract. And then filtrate the tested for Wagner's reagent (red brown precipitate).

Test for Flavonoids:

Alkaloids test: Add two to three drops of sodium hydroxide to 2ml of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating the presence of flavonoids.

Test for Terpenoids:

A 3ml of extract was added with 3ml of chloroform and sulfuric acid to form a reddish brown layer indicates the presence of terpenoids.

Test for Saponins:

Foam test: 1ml of extract was shaken with 2ml of distilled water. If foam produced persists for 10 minutes it confirms the presence of saponins.

Test for steroids:

2ml of chloroform and concentrated sulfuric acid were added with the 5ml of plant crude extract. In the lower chloroform layer red color indicates the presence of steroids.

Test for Phenols:

A small amount of extract was taken with 1ml of water in a test tube and 1 to 2 drops of ferric chloride was added. A blue, green, red or purple color indicates the positive test.

Test for Proteins:

Ninhydrin test: To 1ml of extract added few drops of freshly prepared 0.2% Ninhydrin reagent and heated. A blue colour develops indicates the presence of protein.

Test for Tannins:

Ferric chloride test: To 2 to 3ml of extract was taken and added 5% of ferric chloride, the appearance of brownish green colour indicated the presence of tannins.

Test for Quinones:

One drop of concentrated sulphuric acid was added to the 1ml of extract. The appearance red colour indicates the presence of quinones.

Test for carbohydrates:

1ml of extract was added in the test tube and 1ml of Benedict's reagent was added and heated for about 10 minutes in a boiling water bath. The blue color turns into reddish brown color indicates the presence of carbohydrates.

ANTI-UROLITHIATIC ACTIVITY- SOLUBILITY TEST

5ml of extract were taken in the boiling test tube and the gallstones (obtained from the local hospital) were added in five different concentrations (10, 20, 30, 40, 50µg). For each 1 hour the samples are observed for varying degrees of gall stone solubility.

RESULTS AND DISCUSSON

PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical surveys and the knowledge of the chemical constituents of plants are desirable to understand herbal drugs and their preparations. Therefore, the phytochemical investigation of *Zingiber officinale* (Ginger rhizhome), *Macrotyloma uniflorum* (Horse gram seed), *Phyllanthus emblica* (Amla), *Musa sp* (Banana stem) in the present study was assessed so as to reveals the presence of various

potential phytochemical constituents which may be useful for pharmaceutical industries and could be used as an effective nutraceutical. The results of phytochemical screening such as flavonoids, terpenoids, saponins, steroids, alkaloids, phenols, proteins, tannins, quinones, carbohydrates, in the selected plants extracted with various solvents like aqueous, ethanol, chloroform, hexane are showed in table-1.

Table-1: Phytochemical analysis

	Zingiber officinale extracts				*Macrotyloma uniflorum* extracts				*Phyllanthus emblica* extracts				*Musa sp* extracts			
	Aq	Et	Ch	He	Aq	Et	Ch	He	Aq	Et	Ch	He	A	Et	C	H
Flavanoids	+++	++	+	+	+++	+	+++	-	--	-	-	-	+++	+++	-	-
Terpenoids	+	+++	++	+	+++	+++	+	-	-	+	-	-	-	+++	-	-
Saponins	++	-	+++	+++	++	-	++	+++	+	+	++	++	++	-	+++	++
Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+++	-	+	-	-	-	-	-	+++	+	++	-	-	-	-	-
Phenols	-	-	-	-	-	-	-	-	+++	-	-	-	+++	+++	-	-
Proteins	-	+++	-	-	-	++	-	-	-	++	-	-	-	+++	-	-
Tannins	-	-	-	-	-	+	++	++	+	++	+	-	+	++	-	-
Quinones	+++	+++	++	+	+++	+++	+++	+	+	++	-	-	+++	+++	-	-
Reducing sugars	+	+++	+	-	+++	-	-	-	-	-	-	-	+++	+++	-	-

+++-Increased ++ moderately + traces - absence

In the present study, we observed promising results for the phytoconstituents such as flavonoids, terpenoids, saponins, quinones, carbohydrates in all the extracts. Flavonoids possess a number of medicinal benefits, including antiurolithiatic, anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects. These biological activities depend upon the type of flavonoid, its possible mode of action, and its bioavailability (Asad Ullah *et al* 2020). Terpenoids with the potent radical scavenging property exhibit , antimalarial activity, anti inflammatory effect, anticancer property, and antiulcer activities (Stephen Adeniyi *et al*, 2022). Saponins are reported to exhibit cholesterol-lowering action in animals (Aziza Ashour *et al* 2019). The alkaloids protect against chronic diseases. Phytoconstituents protect against hypercholesterolemia and possess antibiotic properties.

(Dandge *et al.,* 2016). Flavonoids and tannins are the group of phenolic compounds that act as primary antioxidants and possess antimicrobial, anti-inflammatory, anti-allergic, anticancer, antineoplastic activity, and for the treatment of intestinal disorders (Rievere, C *et al.,* 2009).

ANTI-UROLITHIATIC ACTIVITY- SOLUBILITY TEST

The various test extracts were treated with 10µg gall stones and observed the degree of solubility. Among all the extracts ethanolic extracts had maximum solubilizing capability. (Table 2)

	Zingiber officinale extracts				*Macrotyloma uniflorum* extracts				*Phyllanthus emblica* extracts				*Musa sp* extracts			
	Aq	Et	Ch	He	Aq	Et	Ch	He	Aq	Et	Ch	He	A	Et	C	H
Time in mins	5.37	3	5.52	4.24	6.07	4.01	6.58	6.24	6.3	4.21	6.27	6.29	4.32	2.11	4.14	4.24

Table-2: Solubility of gall stones

From the results it was inferred that the selected concentration of 10µg gall stones had maximum clearance within short period of time in all test samples. Anti-urolithiatic activity significantly reduces the size of stones and enhancing the excretion of calcium, phosphate, oxalate while maintaining the level of magnesium, which is reported to be one of the stone inhibiting factors. Vyas*et al*(2011) reported that the reduction of stone-forming constituents reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of the extract Basavaraj *etal*(2021) studies on the isolated quercetin and betulin from *A. lanata* have shown mild diuretic effect as well as antiurolithiatic effect by significantly reducing the size of calculi in the kidneys and enhancing the excretion of calcium, phosphate, oxalate while maintaining the level of magnesium, which is reported to be one of the calculi inhibiting factors.

SUMMARY AND CONCLUSION

From the present study aqueous and ethanolic extract of *Zingiber officinale*, *Macrotyloma uniflorum, Phyllanthus emblica*, and *Musa sp* were enriched with flavanoids, terpenoids, saponins, quinones and carbohydrates. Presence of these phytochemical compounds accounts for the potent medicinal benefits. The ethanolic extract of, *Zingiber officinale*, *Macrotyloma uniflorum*, *Phyllanthus emblica*and *Musa sp* have high potency of anti-urolithiatic activity. However, further studies are required to elucidate the mechanism of action in and drug production.

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