Research Article

Phytochemical Screening of Some Important Medicinal Plants Used for Kidney Stone

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ABSTRACT

Phytochemicals are bioactive compounds obtained from the *Solanum xanthocarpum, Bryophyllum Pinnatum, Tridex procumbence* and *Phyllanthus emblica* plant are widely applied in the traditional herbal medicine. These four plants were collected from local nursery located in Katol taluka of Maharashtra, India. These plants are being used for the treatment of kidney stones disease in and around the region. The parts of plants are shade dried for seven to fifteen days. A fine powder has been prepared of dried leaves, stem and root. Phytochemical analysis is carried in aqueous and methanol extracts. It shows the presence of Alkaloids, Tannins, Saponins, Protein, Steroids, Quinones etc. in these extracts. Thin Layer Chromatography study constituted different colored phytochemical compounds with different Rf values. These four plants contain many active phytochemicals. It can be further investigated for the isolation and identification of active biochemical compound.

Keywords: biochemical compound, Medicinal plants, Kidney stone.

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INTRODUCTION

Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional herbal medicine. These herbal medicines are being used by the local people to cure the various diseases which include the major diseases such as kidney stone, Diabetes mellitus, Cancer, HIV etc. For thousands of years the nature is the best origin for the traditional agents[1]. About 20% of known plants have been used in pharmaceutical drug discovery and study their effectiveness of the bioactive compounds on the health care system such as harmful chronic diseases, cancer and kidney stone. Natural products have been used for the treatment of numerous human diseases for a long period of time. Recently, there has been a growing interest in natural occurring plant products as alternatives to synthetic medicines considered as unsafe to humans and environment[2-3].

Tridax procumbens belong to family Asteraceae. It is best known as a widespread weed and pest plant. It has been in use in India for wound healing and as an anticoagulant, antifungal, and insect repellent. It is used in Ayurvedic medicine for liver disorders and kidney stone disease[4].*Bryophyllum pinnatum* belongs to the family Crassulaceae and the common names include life plant, love plant, miracle leaf and Canterbury bells. It is a succulent plant, 50 – 200 cm tall and about 3.2 cm wide, and reproduces via seeds and also vegetatively from leaf bulbils[5-6]. *Solanum xanthocarpum* belongs to the family Solanaceae. It is an annual herbaceous plant. It is commonly called Kantkari. It is useful in treating worms, cough, hoarseness of voice, fever, painful urination, enlargement of the liver, muscular pain, and stone in the urinary bladder[7]. *Phyllanthus emblica* belong to family Phyllanthaceae. It is also known as emblic, emblic myrobalan, myrobalan, Indian gooseberry, Malacca tree, or amla, in Sanskrit amalaki. It is a deciduous tree plant. It has been used in Ayurveda and its major constituent is vitamin C which has effective free radical scavenging property[8].

MATERIALS AND METHODS

Tridex procumbence, Solanum xanthocarpum, Phyllanthus emblica and Bryophyllum pinnatum plant were collected from local nursery located in Katol tahsil of Maharashtra state, India. The whole plants were collected and washed carefully under running water and then with sterilized distilled water. Then the plants were dried under the shade for seven to fifteen days. The different part of the plant such as fresh leaves, stem and root were homogenized to a fine coarse powder using mortar and pestle separately and then stored in fine air tight container for further process.

Preparation of leaves, stem and root extracts

Preparation of leaves, stem and root powder was carried out of *T. procumbence, S. xanthocarpum, P. emblica* and *B.*

pinnatum. Fresh leaves, stem and root of plants were softly eroded in deionized water by which the dust particles were removed, shade dried for seven to fifteen days. Dried leaves, stem and root were ground using mortar and pestle. After the process of grinding, the leaves, stem and root powder were sieved to get very fine particles of uniform size.

Preparation of aqueous solvent: Three grams powder of *T. procumbence, S. xanthocarpum, P. emblica* and *B. pinnatum* of leaves, stem and root were taken and soaked in 15ml of aqueous solution (distilled water), allowed to stand overnight and filtered to obtain aqueous extract of parts of plants[9].

Preparation of methanol solvent: Three grams powder of *T. procumbence, S. xanthocarpum, P. emblica* and *B. pinnatum* of of leaves, stem and root was taken and soaked in 20ml of methanol, allowed to stand for overnight and filtered to obtain methanolic extract of parts of plants[9].

Phytochemical screening[9]

1. Test for tannins: 1ml of sample was added with 20µl of 0.1% ferric chloride and the appearance of brownish green or black colour indicates the presence of tannins.
2. Test for saponins : 1ml of sample was added with 2ml of distilled water, shaken vigorously and observed for foam appearance which indicates the presence of saponins.
3. Test for alkaloids: 1ml of sample was added to 20µl of Dragendroff's reagent (gram's iodine). Formation of orange colour indicates the presence of alkaloids.
4. Test for proteins : 1ml of sample was added to 100µl of Bradford reagent. Appearance of blue colour indicates the presence of protein.
5. Test for steroids: 1ml of sample was added to 200µl of 10% concentrated sulphuric acid and the appearance of green colour indicates the presence of steroids.
6. Test for Quinones: 1ml of sample was added to 200 µl of aqueous sodium hydroxide. Appearance of yellow colour in aqueous layer indicates the presence of quinones.
7. Test for Terpenoids: 1ml of sample was added to 400 µl of chloroform and 200 µl of concentrated sulphuric acid. The development of reddish brown colour indicates the presence of terpenoids.
8. Test for Cardio Glycosides: To 1ml of sample, 0.4ml of glacial acetic acid, 200µl of ferric chloride and 200 µl of concentrated sulphuric acid were added. The appearance of brown ring indicates the presence of cardio glycosides.

Thin-layer Chromatographic Studies (TLC)

Thin-layer chromatography was carried out on all the fractions using TLC pre-coated plates (silica gel 60F54) by using one way ascending technique. The plates were cut with scissors and marked with pencil about 1cm from the bottom of the plate. Each sample was faintly dissolved in methanol and capillary tubes were used to uniformly apply the dissolved samples on the plates and allowed to dry. The plates were developed in a chromatographic tank using the different solvent systems including; (1) chloroform: methanol (15:1), (2) chloroform: ethylacetate: methanol: water (15: 8: 4: 1). The plates were dried and visualized under normal day light, ultraviolet light (254nm and 366nm) and by spraying with 10% sulfuric acid followed by heating at 105°C for 5-10minutes in an oven[10].

The retention factor R^f for each active compound was calculated for each fraction using the following formula;

$$R^f = \frac{\text{Distance moved by the solute/ compound}}{\text{Distanced moved by the solvent (solvent front)}}$$

RESULTS AND DISCUSSION

The preliminary phytochemical screening had shown the presence of Alkaloid, Saponin, Tannin, Terpenoids, Protein, Flavonoids and cardioglycosides in leaves, stem and root extracts the summary of the results are presented in table 1-4. In this study four plant species belonging to different families were collected. Most of these plants were reported to treat a variety of diseases in traditional system of medicine. Alkaloids are the basic natural products which occur in plants. They generally found in the form of salt with organic acids. They are considered to be the most efficient therapeutic agent among plant substances. Purely synthesized alkaloid can be used as medicinal agents because of their analgesic and anti-bacterial properties[11]. Tannin used as acetylated mannose polymers hence it is used for medicinal purpose. Saponin used as a natural cleansers and cardiac glycosides are used for ulcer, kidney stone and diabetic treatment. Eating too much animal protein, such as red meat, poultry, eggs, and seafood, boosts the level of uric acid and could lead to kidney stones. A high-protein diet also reduces levels of urinary citrate, the chemical in urine that helps prevent stones from forming. Corticosteroid agents have antiinflammatory and antiedema effects as the presence of stones induces mucosal inflammatory reactions that cause edema, many studies used corticosteroids to treat or prevent these reactions to facilitate stones expulsion. Regardless of the underlying etiology, reactive oxygen species plays an important role in both acute kidney injury (AKI) and chronic kidney diseases (CKD). Depending on the oxidative state of the kidney, quinones can be nephrotoxoic or nephro-protective. Many factors play a role in the interaction between quinones and the kidney and the consequences of this are just beginning to be explored.Terpene combination (Rowatinex) is known to help with the expulsion of urinary stones. Rowatinex affects the expulsion of remnant stones after shock wave

lithotripsy (SWL). The preliminary phytochemical analysis of EEAG showed the presence of alkaloids, carbohydrates, cardiac glycoside, sterols & saponins, tannins, flavonoids are used against anti-urolithiasis activity.

Table 1: Phytochemical screening in aqueous and methanol extract of *Tridax procumbens*

Phytochemicals	Leaves		Stem		Root	
	Aqueous solution	Methanol	Aqueous solution	Methanol	Aqueous solution	Methanol
Tannin	-	-	-	-	-	+
Saponins	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Proteins	-	-	-	-	+	-
Steroid	-	-	-	-	-	-
Quinones	+	-	+	-	-	+
Terpenoids	+	-	+	+	+	+
Cardio Glycosides	+	+	+	+	+	+

Table 2: Phytochemical screening in aqueous and methanol extract of *Solanum xanthocarpum*

Phytochemicals	Leaves		Stem		Root	
	Aqueous Solution	Methanol	Aqueous Solution	Methanol	Aqueous solution	Methanol
Tannin	+	+	-	-	-	-
Saponins	+	+	+	+	+	-
Alkaloids	-	-	+	-	-	-
Proteins	-	-	-	-	+	+
Steroid	-	-	-	-	-	-
Quinones	+	-	-	+	+	+
Terpenoids	+	-	+	+	+	+
Cardio Glycosides	+	+	+	+	+	+

Table 3: Phytochemical screening in aqueous and methanol extract of *Phyllanthus emblica*

Phytochemicals	Leaves		Stem		Root	
	Aqueous solution	Methanol	Aqueous solution	Methanol	Aqueous solution	Methanol
Tannin	+	+	+	+	+	+
Saponins	+	+	+	+	-	-
Alkaloids	+	+	+	-	-	-
Protein	+	+	+	+	+	+
Steroid	-	-	-	-	+	+
Quinones	+	+	-	+	-	-
Terpenoids	+	+	-	+	-	-
Cardio Glycosides	+	+	+	+	+	+

Table 4: Phytochemical screening in aqueous and methanol extract of *Bryophyllum pinnatum*

Phytochemical	Leaves		Stem		Root	
	Aqueous solution	Methanol	Aqueous solution	Methanol	Aqueous solution	Methanol
Tannin	-	-	-	-	-	+
Saponins	+	-	+	+	+	+
Alkaloids	+	+	-	-	-	-
Protein	-	-	+	+	-	-
Steroid	-	+	-	-	-	-
Quinones	+	+	-	+	+	+
Terpenoids	+	-	+	-	+	+
Cardio Glycosides	+	+	+	+	-	+

TLC Profiling

Rf values obtained from thin layer chromatographic analysis.

1. TLC study of the ethanolic extract of *T. Procumbence* are:

Solvent system Chloroform: Methanol (15:1) and Chloroform: ethylacetate: methanol: Water (15:8:4:1) used and 1 spot were visible and the Rf values as..

a. Leaves part were 0.79 and 0.76

b. Stem part were 0.87 and 0.78

c. Root part were 0.63 and 0.71

2. TLC study of the ethanolic extract of *S. Xanthocarpum* are:

Solvent system Chloroform: Methanol (15:1) and Chloroform: ethylacetate: methanol: Water (15:8:4:1) used and 1 spot were visible and the Rf values as..

a. Leaves part were 0.64 and 0.81

b. Stem part were 0.71 and 0.55

c. Root part were 0.75 and 0.73

3. TLC study of the ethanol extract of *P. Emblica* are:

Solvent system Chloroform: Methanol (15:1) and Chloroform:ethylacetate: methanol: Water (15:8:4:1) used and 1 spot were visible and the Rf values as..

a. Leaves part were 0.71 and 0.68

b. Stem part were 0.65 and 0.68

c. Root part were 0.82 and 0.64

4. TLC study of the ethanol extract of *B. Pinnatum* are:

Solvent system Chloroform: Methanol (15:1) and Chloroform: ethylacetate: methanol: Water (15:8:4:1) used and 1 spot were visible and the Rf values as..

a. Leaves part were 0.71 and 0.85

b. Stem part were 0.14 and 0.40

c. Root part were 0.66 and 0.85

Rf values of TLC solvent system for ethanol extract of studied plant parts.

Sr. No.	Plant species	Parts of Plants	Rf values
1.	*T. Procumbence*	Leaves	A. 0.79 & B. 0.76
		Stem	A. 0.87 & B. 0.78
		Root	A. 0.63 & B. 0.71
2.	*S. xanthocarpum*	Leaves	A. 0.64 & B. 0.81
		Stem	A. 0.71 & B. 0.55
		Root	A. 0.75 & B. 0.73
3.	P. Emblica	Leaves	A. 0.71 & B. 0.68
		Stem	A. 0.65 & B. 0.68
		Root	A. 0.82 & B. 0.64
4.	*B. Pinnatum*	Leaves	A. 0.14 & B. 0.40
		Stem	A. 0.66 & B. 0.85
		Root	A. 0.71 & B. 0.85

Solvent used:

A. Chloroform: Methanol (15:1) and

B. Chloroform: ethylacetate: methanol: Water (15:8:4:1)

CONCLUSIONS

Mostly plants play a major role in traditional medicinal system to combat several diseases. Generally, plants have many phytochemicals like Alkaloid, Tannin, Saponin, Protein, Steroids, Quinones, Terpenoids with specialized properties. The present study shows the presence of medicinally important bioactive compounds in four different types of selected medicinal plants which may be potential for novel drug discovery. TLC analysis of the phytochemicals showed the good sensitivity and separation. These findings may also lead to the further isolation, purification, characterization of the active compounds from the extract of the various plant parts using chromatographic techniques. The present study was carried out to gather the information about phytochemical screening process showed the presence of medicinally important constituents in the studied plant species. The present study will be useful for the researchers who are interested in phytochemicals related study. The further study is required to explore their potential therapeutic activities.

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