



Thin layer chromatographic profiling and phytochemical screening of six medicinal plants in Bangladesh

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Abstract

Medicinal plants contain different bioactive compounds which have great importance to the health of individuals and communities. These compounds produce definite physiological action on the human body. The present study was carried out to verify the thin layer chromatographic (TLC) profiling and phytochemical screening of water and ethanol extracts of six different plant parts in Bangladesh. These are Young Mango fruit, Radhuni leaves, Dill leaves, Thankuni leaves, Mature Chalta fruit and Olive fruit. Phytochemical screening was performed by various qualitative methods and TLC profiling was carried out using a various solvent system of varying polarity. Qualitative phytochemical determination by chemical test reflects the presence of alkaloid, steroid, saponin, tannin, leucoanthocyanin, coumarin, flavonoid, reducing sugar, glycoside and phenol in different plants. TLC profiling of these plants were carried out using different solvents of ethanol extracts and it showed different R_fvalue. The results obtained in the present investigation indicated that the six plants are rich source of various secondary metabolites. It is also observed that alkaloid and coumarin are common in most of the selected plants. This can be further investigated for the isolation and identification of the active biochemical compound of medicinal utilities in a cost-effective way.

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Introduction

Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plants materials trade within and across the country. In Bangladesh, there are no systematic cultivation process or conservation strategies for medicinal plants. The local people conserve traditional knowledge through their experience and practice, which is handed down orally without any documentation. This knowledge is now under threat to extinction. This is a very alarming situation with regard to the natural growth of medicinal plants in the wilderness in this country.

From the very beginning of the human civilization, people used different plant parts to treat various diseases. Till now plant extract are being widely used to prepare many drugs in pharmaceuticals. In village, there are many folk medicine practitioners known as Kobiraj, who are solely dependent on plant parts. They prepared the medicines from various plant parts by traditional methods. The therapeutic efficacy of the plants depends on some bioactive compounds or phytochemicals (Verpoorte, 1999). In our Indian sub-continent, around 95% of the prescribed medicines were based on the traditional treatment system of Unani, Homeopathy, Ayurveda (Satyawati and Gupta, 1987). These phytochemicals or secondary metabolites are being naturally synthesized in various parts of plants, such as leaf, bark, root, fruit, stem etc. These chemicals not used in the plant growth but these are playing important role in plant defense against herbivores and other interspecies defenses. There are possibly over 25000 secondary metabolites are in plants. These are usually classified based on the chemical synthesis pathway and are generally of 4 types. These are Alkaloids, Glycosides, Phenolic and Terpenoids. Most of them are being synthesized from some common amino acids such as tyrosine, ornithine, tryptophan, arginine and lysine. These are specific to an individual species (Pichersky and Gang, 2000).

These compounds are the basic sources of many pharmaceutical industries and are used for identifying the crude drug. Phytochemical screening is important for the identification of therapeutically and industrially important compounds such as alkaloid, steroid, etc. (Akindele and Adeyemi, 2007). Distributions of the active ingredients in the plants differ from parts to parts. Searching for phytochemical may be helpful for new drug development. Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic disease as well (Pandey *et al.*, 2013; Nandagoapalan *et al.*, 2016).

Thin layer chromatography (TLC) is a basic chromatographic technique that is used to separate mainly non-volatile compounds. TLC is generally done on the sheet of glass, aluminum foil, plastics which are generally coated with the thin layer of various absorbent materials such as silica gel, cellulosic materials and aluminum oxides (Factor, 1991). The solvent that is used for the separation is used as the mobile phase whereas the absorbent materials are used as the stationary phase. The both phase has the different polarity. Separation by the TLC is highly convenient as the components are separated on the plane. The separation occurs based on the polarity and migrating less than others (Stahl, 2013). The plant extract for the TLC studies can be made by various procedures; among those, the most convenient is to extract the plant material by alcohol and water mixture such as 80% ethanol (Khurram *et al.*, 2009). Separation of the active phytochemicals by TLC depends highly on the solvent used. The migration of the active compounds and the spot obtained highly dependent on the solvent system used. After the experiments, spots are obtained. Various chemical processes and light imprinting procedures are used to visualize the spots. These spots are quantified by the retention factor (R_f). Retention factors are general characteristics value and are changed depending on the polarity of the mobile and stationary phase (Stoddard *et al.*, 2007).

The solvent system used here is most convenient for the separation of the active components in these plants. TLC can be used to monitor the reaction progression, identification of the components of the mixture and the determination of the purity of the mixture. This analytical tool is used because of its simplicity, speed of separation, cost effectiveness and high sensitivity.

Mango (*Mangifera indica*) belongs to genus *Mangifera* which has been using in Ayurvedic and indigenous medicine over 4000 years (Shah *et al.*, 2010). Radhuni (*Trachyspermum roxburghianum*) is a flowering plant of Apiaceae has reputed medicinal uses. These are being used as carminative, emmenagogues and cardioton. Dill (*Anethum graveolens*) is a family member of celery and it is an annual herb. It protects against different types of cancer (Callan *et al.*, 2007). Thankuni (*Centella asiatica*) is a perennial herb contains a high amount of carotenoid and terpenoid is used as a memory enhancer, anti-carcinogenic (Inamdar *et al.*, 1996). Chalta (*Delliana indica*) is an evergreen large shrub produces a very hard fruit has been used for the treatment of cancer and diarrhea (Sharma *et al.*, 2001). Jolpai (*Olea europaea*) is a deciduous tree having small fruits with a hard stony seed is being used to reduce cholesterol and blood sugar. It is also used as a laxative, mouth cleanser, vasodilator etc. In the Holy Quran *O. europaea* has been described as blessed tree and fruit (Pereira *et al.*, 2007). The ethno medicinal importance of the studied plants and their used parts are listed in Table 1.

The aims of the present study are to investigate the presence of qualitative phytochemicals and thin layer chromatography (TLC) of six commonly used medicinal plants in Bangladesh extracted with water and ethanol.

Materials and methods

Extraction procedure

Water extraction: The collected plant parts (fruits or leaves) were washed thoroughly with water. The materials were then dried under the sun. Then the materials were grinded in the blender.

The powder of the plant samples were shown in Fig. 1. The grinded materials were kept in an airtight plastic bag with labeling in to the cool chamber (15-20°C) until further use. Five grams of powder were mixed into 25 ml water and vortex properly. Then the mixtures were heated at 60°C for 45 minutes in a water bath. Then the mixture was filtered through Whatman filter paper for overnight. Then the filtrate was centrifuged at 2500 rpm for 20 min. Then the supernatant was collected and stored in sterile Falcon tube at 4°C for further screening (Nostro *et al.*, 2000).

Ethanol extraction: For the ethanol extraction procedure, 5 grams of each grinded sample was taken into conical flask, then 20 ml of 80% ethanol was added. Then the mixture was shaken in the rotatory shaker at 125rpm for 72 hours at room temperature. Then the mixture was filtered through Whatman filter paper and the filtrate was placed in the evaporator to evaporate the solvent at 55°C after complete evaporation and the concentrates were collected followed by 1% solution of the extract was made by using 0.9% NaCl solution and kept at 4°C for further analysis (Patil and Nasreen, 2016).

Qualitative phytochemical screening procedure

Test for Steroid (Sulphuric acid test): 10 ml of chloroform was added in 1 ml of extract and then 11ml of concentrated sulphuric acid was added by sides of the test tube. Turning the upper layer red and sulphuric acid layer yellow with green fluorescence indicates the presence of steroid (Gibbs, 1974).

Test for Saponin (Foam test): 3 ml distilled water was added in 1 ml of extract and then shaken for 5 min in a falcon tube and the presence of 1cm foam for 10 minute indicate the presence of saponin (Kumar *et al.*, 2009).

Test for Coumarin (NaOH test): 1 ml of extract was added with 1.5 ml of 10% NaOH. Chemical reaction will produce the yellow color and indicate the presence of coumarin (Rizk, 1982).

Test for reducing sugar (Fehling test): 1ml of extract was added in 2ml distilled water. Then heated the solution at 55-60°C for 15 min and then added 6-7 drops of Fehling's solution A and B was added respectively. Brick red precipitate indicates the presence of reducing sugar (Patil and Nasreen, 2016).
 Test for alkaloid (Wagner reagent test): In 1 ml of extract, 4-6 drops of Wagner's reagents [1.27gm of Iodine and 2gm of potassium iodide in 100ml of water] was added. The Radish brown precipitate indicates the presence of Alkaloid (Ugochukwu *et al.*, 2013).

Test for flavonoids (Alkaline reagent test): 3-4 drops of 20% NaOH solution was added in 2ml of extract. Intense yellow color formed and become colorless when 4-5 drops of diluted Hcl was added. This indicates the presence of Flavonoids (Ugochukwu *et al.*, 2013).

Test for tannin (Ferric chloride test): 0.5ml of extract was mixed with 1ml of distilled water, and then 4-5 drops of 1% ferric chloride was added. Blue color and the greenish black color indicate the presence of gallic tannin and catecholic tannin respectively (Patil and Nasreen, 2016).

Test for glycoside (Glacial acetic acid test): 1ml of extract was mixed with 1ml of glacial acetic acid. Then 5-6 drops of 1% ferric chloride solution was added. Brown color ring produced at the top indicate glycoside's presence (Patil and Nasreen, 2016).

Test for phenol (Ferric chloride test): 1ml of ethanol was added in 1ml of extract. Then 6-7 drops of 1% ferric chloride solution was added in then solution. Formation of the green, blue, purple color indicates the presence of Phenol (Patil and Nasreen, 2016).

Thin layer chromatographic (TLC) analysis

Ethanol extract of each plant sample was subjected to TLC studies. For the TLC analysis, the dimensional ascending method was used (Gujjeti and Mamidala, 2013). 20×20 cm TLC plate coated with silica gel 60G F₂₅₄ (Merk, India), was cut with a scissor in 14×3 cm shape. The plate was then marked with the pencil softly 1.5 cm far from the both bottom and top.

Glass capillaries were used to spot the sample on the TLC plate on the pencil marked bottom line. Then it was placed in the fume hood to dry the plate and loaded the sample again until a dark spot is obtained. Then the solvent Hexane: Ethyl acetate: Acetic acid (4:4:2) about 20ml was taken in the chamber. The plate was placed in the chamber lining on the top. After the run, plates were dried in the fume hood and then used to detect the spots.

Detection of the spot

All the plates were dried and detected the spots with the help of UV light at 254nm and 366 nm (Biradar and Rachetti, 2013). The movement of the active compound was expressed by the retention factor (R_f).

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Results

Yield extract (%)

Yield of ethanol extract of different plants sample are shown in the Table 2. The amount of the extract found from *M. indica*, *T. roxburghianum*, *A. graveolens*, *C. asiatica*, *D. indica*, *O. europaea* are 44.26%, 20.17%, 23.2%, 7.41%, 17.24% and 21.9% respectively.

Phytochemical screening

The presence and absence of the phytochemical in the plants parts are listed in the Table 3. Our results revealed that Alkaloids are presents in water extract of *D. indica*, *O. europaea*, *T. roxburghianum*, *C. asiatica* and in the ethanol extract of *D. indica*, *O. europaea*, *T. roxburghianum*, *C. asiatica* and *A. graveolens*. Steroid is present in water extract of *O. europaea*, *A. graveolens* and *C. asiatica* and ethanol extract of *O. europaea* and *C. asiatica*. Tannin is present in the water extract of the *M. indica*, *T. roxburghianum*, *A. graveolens*, *C. asiatica* and the ethanol extract of the *M. indica* and *T. roxburghianum*. Saponin is present in the water extract of the *M. indica*, *T. roxburghianum*, *A. graveolens*, *C. asiatica* and the ethanol extract of the *C. asiatica*. Coumarin is present in the water extract of *M. indica*, *O. europaea*, *T. roxburghianum*,

C. asiatica and the ethanol extract of the *M. indica*, *O. europaea*, *T. roxburghianum*, *C. asiatica*. Flavonoids are present in the water extract of the *D. indica* and *O. europaea* and the ethanol extract of the *D. indica*, *O. europaea*, *T. roxburghianum* and *C. asiatica*. Reducing sugar is presents in the water extract of the *D. indica*, *O. europaea*, *T. roxburghianum*, *C. asiatica* and the ethanol extract of the *D. indica*, *T. roxburghianum*, *A. graveolens*

and *C. asiatica*. Glycoside is present in the water extract of the *M. indica*, *D. indica*, *O. europaea*, *T. roxburghianum*, *A. graveolens*, *C. asiatica* and the ethanol extract of the *M. indica*, *D. indica* and *O. europaea*. Phenols are present in the water extract of *M. indica*, *T. roxburghianum*, *A. graveolens*, *C. asiatica*, *D. indica* and in the ethanol extract of the *M. indica* and *T. roxburghianum*.

Table 1. Ethnobotanical importance of the six medicinal plants in Bangladesh.

Sample ID	Common name and scientific name	Family	Used part	Control diseases	Reference
1.	Mango (<i>M. indica</i>)	Anacardiaceae	Young fruit	Anti HIV, Anti tumor	Li <i>et al.</i> , 1998
2.	Radhuni (<i>T. roxburghianum</i>)	Apiaceae	Leaf	Bronchitis, Asthma, anti oxidant activity.	Peerakam <i>et al.</i> , 2014
3.	Dill (<i>A. graveolens</i>)	Apiaceae	Leaf	Liver Disease, anti oxidants	Oshaghiet <i>al.</i> , 2016
4.	Thankuni (<i>C. asiatica</i>)	Lamiaceae	Leaf	Neurodegenerative disease, Neuroprotectant.	Lokanathn <i>et al.</i> 2016
5.	Chalta (<i>D. indica</i>)	Dilleniaceae	Mature fruit	Anti Diabetic, Inflammatory bowel disease (IBD)	Kumar <i>et al.</i> , 2013
6.	Jolpai (<i>O. europaea</i>)	Oleaceae	Mature fruit	Cerebral ischemia, Neuroprotective	Qian <i>et al.</i> , 2016

TLC profiling

R_f values obtained from thin layer chromatographic analysis are listed in the Table 4.

TLC study of the ethanol extract of *M. indica* Solvent system Hexane: Ethyl acetate: Acetic Acid (4:4:2) was used and 2 spots were visible and the R_f values were 0.23 and 0.75 respectively.

Table 2. The percentage yield of ethanol extract of different plant parts.

Sample ID	Plants	Color of extract	Yield of the extract (in gram)	Percentage yield (%w/w)
1.	<i>M. indica</i>	Walnut Brown	2.21	44.26%
2.	<i>T. roxburghianum</i>	Greenish Black	1.00	20.17%
3.	<i>A. graveolens</i>	Light Brown	1.16	23.2%
4.	<i>C. asiatica</i>	Greenish Black	0.370	7.41%
5.	<i>D. indica</i>	Peanut Brown	0.862	17.24%
6.	<i>O. europaea</i>	Tortilla Brown	1.09	21.9%

TLC studies of the ethanol extract of *T. roxburghianum* solvent system Hexane: Ethyl acetate: Acetic Acid (4:4:2) was used and 4 spots were visible and the R_f values were 0.50, 0.62, 0.87, 0.95 respectively.

TLC studies of the ethanol extract of *A. graveolens* solvent system Hexane: Ethyl acetate: Acetic Acid (4:4:2) was used and 2 spots were visible and the R_f values were 0.54 and 0.66 respectively.

TLC studies of the ethanol extract of *C. asiatica* solvent system Hexane: Ethyl acetate: Acetic Acid (4:4:2) was used and 3 spots were visible and the R_f values were 0.64, 0.74 and 0.86, respectively.

TLC studies of the ethanol extract of *D. indica* solvent system Hexane: Ethyl acetate: Acetic Acid (4:4:2) was used and 2 spots were visible and the R_f values were 0.54 and 0.68 respectively.

TLC studies of the ethanol extract of *O. europaea* (4:4:2) was used and 2 spots were visible and the R_f solvent system Hexane: Ethyl acetate: Acetic acid values were 0.55 and 0.78 respectively.

Table 3. Screening results of secondary metabolites on different plants.

Test	<i>M. indica</i>		<i>T. roxburghianum</i>		<i>A. graveolens</i>		<i>C. asiatica</i>		<i>D. indica</i>		<i>O. europaea</i>	
	W	E	W	W	E	E	W	E	W	E	W	E
Alkaloid	-	-	+	+	-	+	+	+	+	+	+	+
Steroid	-	-	-	-	+	-	+	+	-	-	+	+
Tannin	+	+	+	+	+	-	+	-	-	-	-	-
Saponin	+	-	+	-	+	-	+	+	-	+	-	-
Coumarin	+	+	+	+	-	+	+	+	-	+	+	-
Flavonoid	-	-	-	+	-	-	-	+	+	+	+	+
Reducing sugar	-	-	+	+	-	+	+	+	+	+	+	-
Glycoside	+	+	+	-	+	-	-	-	+	+	-	+
Phenol	+	+	+	+	+	-	+	-	+	-	-	-

Here “+” indicates presence and “-” indicates absent. W= Water extract, E= Ethanol extract.

Discussion

Phytochemical studies:

The presences of phytochemicals are involved in the various disease protections and curing. Functions of alkaloid are mostly related to the protection. It is widely used as an analgesic, inhibitor of acetylcholinesterase (Hesse, 2002).

It is also used as a muscle relaxant, cough release. Steroid functions as the signaling molecule. It is associated with the decreasing membrane fluidity. The steroid is also used as the immune suppressor. The steroid is being widely used in the pharmaceutical industry as it is highly associated with the sex hormone (Santhi *et al.*, 2011).

Table 4. R_f values of TLC solvent system for ethanol extract of various plant parts.

Sample ID	Plant species	No. of spots detected	R_f Value
1.	<i>M. indica</i>	2	0.23
			0.75
2.	<i>T. roxburghianum</i>	4	0.50
			0.62
			0.87
			0.95
3.	<i>A. graveolens</i>	2	0.54
			0.66
4.	<i>C. asiatica</i>	3	0.64
			0.74
			0.86
5.	<i>D. indica</i>		0.54
			0.68
6.	<i>O. europaea</i>	2	0.55
			0.78

Solvent used: Hexane: Ethyl acetate: Acetic acid (4:4:2).

The growth of many bacteria, fungi, yeast and viruses are inhibited by tannin (Chung *et al.*, 1998). Tannin has been reported to have anticarcinogenic and antimutagenic properties. Saponin has also industrial application in foaming industry and can also be used as the surface active agents. Saponin is also being used hugely in the detergent industry (Shi *et al.*, 2004).

Coumarin is used in the dietary supplement. It has a function in Alzheimer's diseases and treatment of liver cancer. Coumarin also functions as the metal chelator, scavenger of free radical and also as the antioxidant (Tseng, 1991). Flavonoids serve as the largest nutrient family.

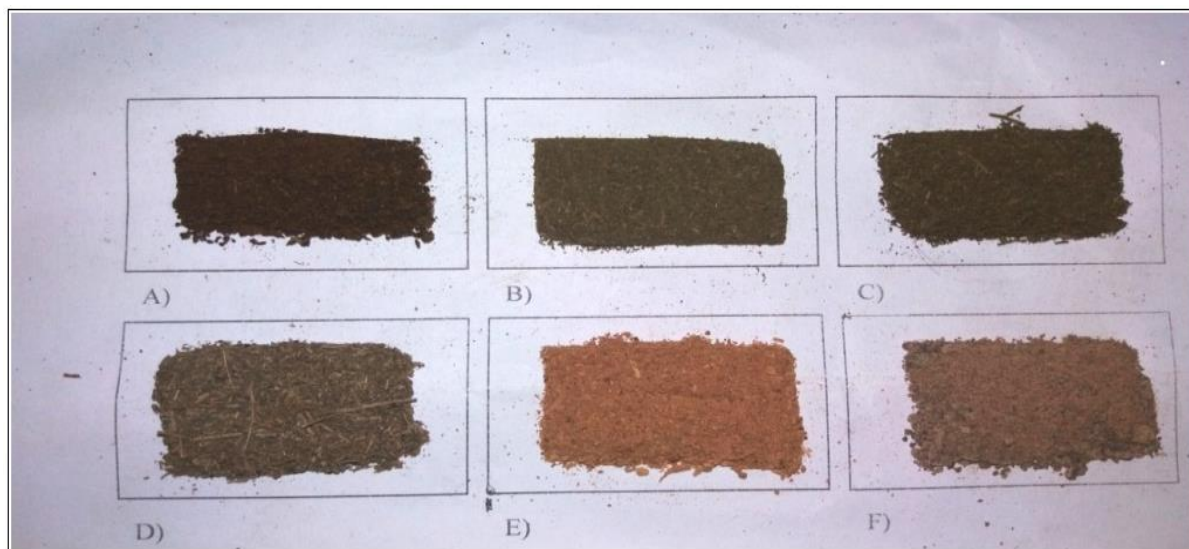


Fig. 1. Powder of each samples are shown. A) *M. indica*, B) *T. roxburghianum*, C) *A. graveolens*, D) *C. asiatica*, E) *D. indica*, F) *O. europaea*.

It contributes to the antioxidant and anti-inflammatory health benefits. It also supports the nervous system. Flavonoid may be used as the non-immunogenic drug also (Lee *et al.*, 2007). Reducing sugar helps in weight loss. It helps in improved mental health, clearer skin, and better digestion. It also serves in healthier brain function. Reducing sugars are being used for the reduction of the coronary heart disease, hypertension and obesity. Glycoside helps to supply the heart with the freshly oxygenated blood. Glycosides are being used as the active ingredient for many drugs associated with various heart diseases (Kren and Martínková, 2001). It helps to increase the ability to dilate the coronary arteries. It functions as antioxidants; it helps in healthy aging and cancer prevention. Phenol provides the protection against the ultraviolet light and pathogenic attack (Beckman, 2000).

TLC analysis

TLC profiling of all six plants extracted by ethanol gives an impressive result that directing towards the presence of number of phytochemicals.

Various phytochemicals in the plant extract has given different R_f values.

These R_f values obtained from the phytochemicals provide the important information about their polarity and important clues for the separation of these phytochemical in the separation process. Different R_f values of the compound also reflect an idea about their polarity by the use of the various solvent systems for TLC studies could be important for the selection of the appropriate solvent system.

This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

Conclusion

The present study shows the presence of medicinally important bioactive compounds in six different types of medicinal plants in Bangladesh 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 and spectroscopic techniques.

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