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Research Article

Phytochemical Screening and TLC Profiling of Various Extracts of *Reinwardtia indica*

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ABSTRACT

Reinwardtia indica, belongs to family Linaceae known as yellow flax or pyoli commonly found in the Himalaya. The plant has varied ethno medicinal importance such as aerial parts are used to prevent bleeding of cuts and as mouthwash; leaves are used in the treatment of paralysis and as natural antibiotic. Qualitative phytochemical screening of chloroform, acetone, ethanol, methanol and aqueous extracts was performed to explore scientific basis of ethno medicinal potential. It confirmed the presence of many phytochemicals like alkaloids, flavanoids, phenols, tannins, saponins, terpenoids, phlobatanins etc. in various extracts. Most of the phytochemicals were found in methanol and ethanol extracts. Thin Layer chromatography (TLC) of the acetone, methanol, chloroform and aqueous extract was performed for four important phytochemicals alkaloids, flavanoids, tannins and phenol. Flavanoids showed their presence in all extracts with one spot in each (Rf 0.8 for acetone, 0.918 for methanol, 0.816 for chloroform and 0.737 for aqueous extract). Alkaloids and tannins were found in acetone and methanol extract while phenol was present only in methanol extract (Rf 0.8). These findings provided the evidence that *Reinwardtia indica* is a potent source for some medicinally important phytochemicals and it justifies its use as a medicinal plant. This can be further investigated for the isolation and identification of active biochemical compound of medicinal utilities.

Keywords: *Reinwardtia*, ethno medicinal importance, phytochemical screening, TLC.

INTRODUCTION

Reinwardtia indica, commonly known as yellow flax or pyoli belongs to family Linaceae. Only two species of *Reinwardtia* are known that are native to Southern Asia namely *Reinwardtia indica* and *Reinwardtia sinensis*. In India the genus is represented by only one species *R. indica*, native of Himalayan foothills found in a wide altitudinal range of 500-2500 masl. The plant has high ethno medicinal and ornamental value, stem paste is applied on wounds, cuts, boils, and pimples[1]. The paste of root is used for headache and backache, paste of roots with the fruits of *Piper nigrum* is given to eat in measles and the juice of root is been used for the treatment of fever, scabies, wound and indigestion; the roots are used as abortifacient besides this the leaves are used in the treatment of paralysis[2]. The paste of aerial parts is applied on cuts to stop bleeding and used for mouth wash[3]. The extracts of whole plant was evaluated in mild to moderate depression cases, proving its anti-depressant activity thus beneficial in the management of mild to moderate depression[4]. It is also used in energy drinks and other formulations due to its high nutritive value. A yellow dye made from the flowers is used for dyeing clothes and making paints. The leaves can be taken as an ingredient in natural antibiotics, cosmetics, and medicine as it has shown potent antimicrobial activity towards wide range of microbes[2,4].

The presence of phytochemical compounds in the plants indicates its medicinal potential. The presence of tannins shows plant posses anti-parasitic, antiviral and antibacterial activities. Flavanoids are the phenolic compounds having antioxidant, anti-inflammatory, anti allergic and anticancer activities. Saponins acts as anti-feedants and used as adjuvant in vaccines. Presence of alkaloids shows antimicrobial, anticancer, antiarrhythmic and analgesic activity. Steroids acts as signalling molecules and are important against cardio tonic activity. Phenols are used as antiseptic and active ingredient in some oral analgesics such as carmex and chloraseptic spray. Knowledge of the chemical constituents of plants is desirable because such information may be of great value revealing new sources of economic compounds as tannins, oils, gums, precursor for the synthesis of new chemical substances which can be used in drug[5]. Discovery of the actual value of a traditional plant as well as discovery of a therapeutic agent solely depends upon the knowledge about the phytochemical composition of the plant.

MATERIAL AND METHODS

Sample collection

The plant samples of *Reinwardtia indica* were collected from Chandrapuri, district- Rudraprayag (30˚27’0”N, 79˚4’0” E) in the month of November. Leaves were washed, air dried, homogenised to fine powder and then stored in air tight bottles.

Phytochemical screening

Extract preparation

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50gm of powdered plant sample was extracted with chloroform, acetone, methanol and water by subjecting it to maceration at room temperature for overnight and filtered with Whatman's filter paper. The extracts were subjected to chemical test as per the methods mentioned below for the detection of the various phytochemicals[6,7].

Detection of alkaloids Extracts were dissolved individually in dil. HCl, filtered and then subjected to following tests

Mayers test

To a few ml of filtrate, a drop or two of Mayer's reagent was added by the side of the test tube. A white or creamy ppt. indicates test as positive.

Wagners test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown ppt. indicates test as positive.

Detection of flavonoids

1 ml of extract was taken in a test tube and few drop of dilute NaOH solution added. An intense yellow colour was appeared in the test tube. It became colourless on addition of a few drop of dilute acid that indicated the presence of flavonoids.

4 ml of 1% NH_3 was added to 0.5 ml extract and then 1ml conc. H_2SO_4 was added. Appearance of yellow colour indicated positive result.

Detection of phenol

Ferric Chloride Test

1 ml extract was dissolved in 2ml of distilled water. To this few drops of 10% ferric chloride solution was added. A dark green colour indicates the presence of Phenolic compounds.

Detections of terpenoids

Salkowski's test

5 ml extract was dissolved in chloroform (2 ml) and then 3ml Concentrated sulphuric acid (1 ml) was added to the solution. Formation of reddish brown coloured interface showed the presence of terpenoids.

Detection of steroids

1 ml plant extracts was taken in a test tube and dissolved with 10 ml chloroform and then equal volume of concentrated sulphuric acid was added to the test tube by sides. The upper layer in the test tube turned into red and sulphuric acid layer showed yellow colour with green fluorescence. It showed the presence of steroids.

Detection of coumarins

Two ml of the extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Detection of resins

To 0.5 ml extract, 3-4 ml of CuSO4 solution was added separately and the tubes were shaken vigorously for 1-2 min. the resulting solution was allowed to separate. Formation of green colour precipitate indicated the presence of resins.

Detection of tannins

Ferric chloride test

1 ml solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples

Detection of xanthoproteins

1 ml each of the various extracts was treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

Detection of quinines

The extracts were treated separately with Alc. KOH solution. Appearance of colours ranging from red to blue indicates the presence of Quinones.

Detection of carboxylic acid

One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

Detection of Oxylates

4 ml extract was added to 2 ml acetic acid containing 1 drop $FeCl_3$. 2ml H_2SO_4 was added to it. Brown ring at interface indicated positive result.

Detection of carbohydrates

To the 2 ml extract, 5-8 drops of boiling Fehling's solution was added. A red-brick precipitate showed the presence of reducing sugar.

Detection of phlobatannins

2 ml of the extract was mixed with 2-3 ml of 10% HCl in tube and the content was boiled for 5-6 min. Formation of red colour precipitate indicated the presence of phlobatannins.

Detections of Cardiac glycosides

Keller Killiani test

1ml of the extracts were dissolved in 1ml of glacial acetic acid and cooled, after cooling, 2-3 drops of ferric chloride was added. To this solution 2ml of conc. H_2SO_4 was added carefully along the walls of the test tube. Appearance of reddish brown colour ring at the junction of two layers indicates the presence of glycosides.

Detection of proteins

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acids.

Detection of saponins

Froth Test

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponin.

Thin layer chromatography profiling of various extracts

The TLC profiling was performed as per described by Biradar *et al*., 2013. Various extracts of young leaves of *Reinwarditia indica* were obtained by sequential Soxhlet extraction with different solvents of increasing polarity. 100gm of powdered sample of young leaves were sequentially extracted in a Soxhlet extractor using 800 ml of chloroform, acetone, methanol and water. The extraction was done until solvent in soxhlet became colourless. The extracts were then subjected to distillation

Table 1: Phytochemical screening of various extracts of *Rewardtia indica.*

Constituents	Test performed	Extracts				
		Chloroform extract	Acetone extract	Ethanol extract	Methanol extract	Aqueous extract
Alkaloids	Mayer's Test	-ve	-ve	+ve	+ve	-ve
	Wagner's Test	-ve	-ve	+ve	+ve	-ve
Flavanoids	Ammonia Reduction Test	-ve	+ve	+ve	+ve	-ve
Phenol	Ferric Chloride Test	-ve	+ve	+ve	+ve	-ve
Terpenoids	Salkowski's Test	-ve	-ve	+ve	+ve	+ve
Steroids	Salkowski's Test	-ve	-ve	-ve	+ve	-ve
Coumarins	NaOH Test	+ve	+ve	+ve	+ve	+ve
Resins	Copper Suphate Test	-ve	-ve	-ve	-ve	-ve
Tannins	Ferric Chloride Test	-ve	-ve	+ve	+ve	+ve
Xanthoproteins	Nitric acid+Ammonia Test	-ve	-ve	-ve	-ve	-ve
Quinones	Alc. KOH Test	-ve	-ve	-ve	-ve	-ve
Carboxylic acid	Effervescence Test	-ve	+ve	+ve	+ve	-ve
Oxylates	Acetic Acid Test	-ve	+ve	+ve	+ve	-ve
Carbohydrates	Fehling's Test	-ve	-ve	+ve	+ve	-ve
Phlobatannin	Hydrochloric Acid Test	-ve	-ve	-ve	-ve	-ve
Glycosides	Keller-Killiani Test	-ve	-ve	+ve	+ve	-ve
Proteins	Ninhydrin Test	-ve	+ve	+ve	+ve	-ve
Saponins	Froth Test	-ve	-ve	-ve	-ve	+ve

Table 2: Rf values for various phytochemicals in different extracts.

Phytochemical	Solvent system	Confirmatory test	Extract	Rf value
Alkaloids	EA : Chloroform: Water 5 : 3 : 1	Mayer's reagent spray	A	0.56
			M	0.25, 0.92
Flavonoids	N Butanol : EA : Water 5 : 10 : 15	3% boric acid + 10% oxalic acid spray	A	0.8
			C	0.816
			W	0.737
			M	0.918
Tannins	Chloroform : Water 6 : 4	$FeCl_3$ spray	M	0.85
			A	0.92
Phenols	Methanol: water 6 : 3	$FeCl_3$ spray	M	0.8

(EA: Ethyl acetate, A-acetone, M-Methanol, C-Chloroform, W-aqueous)

for preparation of crude extracts in respective solvents.

The TLC plates were prepared by using Silica gel 'G' as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 mins and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts of chloroform, acetone, methanol and water with respective solvent and then applied usually 1-10µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes.

Development of the chromatogram

After the application of the sample on the plate the plates were kept in TLC glass chamber (solvent saturated) than mobile phase was allowed to move through adsorbent phase up to 3/4th of the plate. TLC was performed for alkaloids, flavanoids, tannins and phenols, solvent system and confirmatory tests are shown in Table2.

RESULTS

Plants have been used since ages because of the antimicrobial and antioxidant properties due to various secondary metabolites that are synthesised in some or all its parts. Results of preliminary phytochemical screening are summarized in Table 1. This investigation reveals the presence of various important phytochemical in different extracts of this plant. These bioactive phytochemicals are the basis of therapeutic potential of medicinal plants and useful in the treatment of several diseases. The medicinal significance of the plant is because of the bioactive phytochemical compounds that generate characteristics physiological action on humans[8]. Different

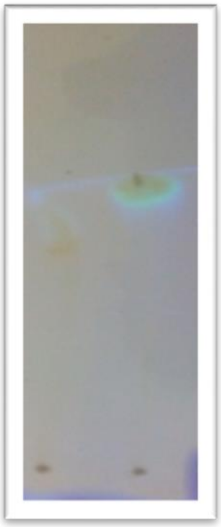
TLC profiling images of various extracts

A C M

Figure 1: alkaloids

A C

Figure 2: flavanoids



M W

Figure 3: flavanoids

C A M

Figure 4: phenols

(M- methanol; A-acetone; C-chloroform; W-water).

M

Figure 5: tannins

A

Figure 6: tannins

phytochemicals show their presence in different solvents. Solvents of changing polarity were used to get maximum information about phytochemicals. Many of the phytochemicals such as alkaloids, phenols, flavanoids, and tannins were found in methanol and ethanol extract while some were also present in acetone extract. Chloroform gave positive result only for coumarins. Saponins were absent in any other extracts except water. Tannins may possess the potential values as cytotoxic agents[9]. Terpenoids are credited for analgesic and anti-inflammatory activities while flavonoids have been reported to be responsible for many useful characteristic properties including anti inflammatory, estrogenic, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumor activity[10]. Presence of glycosides enhances cardiac output and lowers heart diseases like congestive heart failure and cardiac arrhythmia. Saponins have been considered as bioactive antibacterial agent. Findings of phytochemical investigation form the basis of the use of the plant as traditional medicines in treatment of wounds, pimples, depression etc. Similar finding were observed by Rossi *et al*., (1965); and Archana *et al*., (2012) in different plants

Thin layer chromatography

The results of TLC profiling are summarized in Table 2. Chloroform extract showed the presence of flavonoid (Rf 0.86); in acetone extract alkaloid (Rf 0.56), flavonoid (Rf0.8) and tannin (Rf 0.92) were present; Methanol extract showed the presence of alkaloid (Rf value 0.25, 0.92), flavonoid (Rf 0.918), tannins (Rf 0.85) and phenol (Rf 0.8); while in aquous extract only flavonoid (Rf 0.737)was present. Acetone and methanol extracts showed the

presence of alkaloids confirmed by appearance of creamy precipitate on the plate on spraying mayer's reagent. A flavanoid compound was found in all four extracts (methanol, chloroform, acetone, aqueous extract) as it gave a green fluorescence when viewed under UV transilluminator. A phenolic compound in methanol extract while a tannin in methanol as well as acetone extract was present, it was confirmed by the grey colour and brownish grey colour of the spot after $FeCl_3$ spray respectively. The Rf values of each phytoconstituents present in various extracts in different mobile phases is given in the following table.

Thin layer chromatography is usually done for a better identification of the bioactive compounds. In the present study the TLC profiling of all the plant extracts again revealed the presence of different metabolites such as alkaloids, flavonoids, phenols and tannins. It was observed that among the four solvents (chloroform, acetone, methanol and water), methanol was found effective in extracting maximum number of secondary metabolites. Different Rf values of the compounds provides an idea about their polarity that may also help in selecting a particular solvent system for further isolation of any compound from the plant extracts using chromatographic and spectroscopic techniques[11]. Compound showing high Rf value in less polar solvent system have low polarity while those with a low Rf value have high polarity[12].

CONCLUSION

Seeing the results of phytochemical analysis it can be concluded that *Reinwardtia indica* produces many secondary metabolites of medicinal value. TLC profiling further confirmed the presence of alkaloids, flavonoids, tannins and phenols. Methanol was found the best suited solvent for extraction purpose. The plant showed potential for development of drugs against many diseases. Thus, the plant can be used as a source to produce phytochemicals using advance techniques of extraction, screening, identification and isolation. Present study confirms the presence of many important phytochemicals in the unexplored plant *Reinwardtia indica* from Uttarakhand Himalayan Region.

The presence of some medicinally important phytochemicals such as alkaloids, flavanoids and terpenoids were further strengthened by thin layer chromatography and comparing the RF of corresponding spot with that of standards.

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