



PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND THIN LAYER CHROMATOGRAPHY OF *RHEUM EMODI*

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ABSTRACT

Preliminary phytochemical investigation of aqueous and methanolic rhizome extracts of *Rheum emodi* followed by their TLC profiling were carried out. Phytochemical analysis reveals the presence of diverse groups of phytoconstituents in two different extracts (aqueous and methanolic rhizome extracts). Chemical constituents also show different **Rf** values in two different solvent systems.

KEY WORDS: Photochemical screening, TLC, *Rheum emodi*

INTRODUCTION

Over recent decades, a substantial body of the evidence has demonstrated a wide range of pharmacological activities for a number of medicinal herbs. In addition, the investigation of the crude plant extracts through ethno- pharmacological evaluation of plants with folk medicinal value, showed that a number of plants exhibit medicinal properties which may include anti- oxidant, anti- inflammatory and anti- tumour activities, (Borgia *et al*; 1981, Heras *et al*; 1998). The presence of various compounds like flavonoids, polyphenolics, tannins and steroids have been implicated in a no. of medicinal properties of the plants (Mc Clure *et al*; 1975, Harborne *et al*; 1999, Hertog *et al*; 1998).

The present study is intended to study the preliminary phytochemical screening and TLC of the extracts of *Rheum emodi*. *Rheum emodi*, Pambchallan (Kashmiri) has been traditionally used to treat pathological ailments like fevers, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Peirce *et al*; 1999, Babu *et al*; 2003, Agarwal *et al*; 2000, Borgia *et al*; 1981). Some workers have worked anti - tumour activities of *Rheum emodi* (Huang *et al*; 2006), but very little is known about the mechanism involved.

MATERIALS AND METHODS

Plant material collection

Rheum emodi was collected from higher reaches of Kangdoori Gulmarg (J & K) in the month of May-June (2011) at an altitude of 3000m (a.s.l). The plant was identified at centre of plant taxonomy, Department of Botany, University of Kashmir using standard references. The rhizome part was cut and washed with water. The rhizome was then allowed to dry in shade at room temperature.

Plant extracts

Methanolic extract

Extraction of dried plant material was carried out using a method described by Harborne (1973). Dried plant material (rhizome) was grinded using electric grinder. 200 gm of the powdered rhizome was put into the thimble of the soxhlet, 250ml of 99% methanol (MERCK) was added in the flask of the soxhlet apparatus and extraction was started maintaining the temperature at about 55-60°C for about 48 hours. The filtrate was then taken and concentrated on a water bath and temperature was maintained at 55°C.

Aqueous extract

Similarly 200gm of dried and powdered rhizome of *Rheum emodi* was extracted with Distilled water for about 48 hours. The filtrate was then concentrated on a water bath and temperature was maintained at 60°C.

PROTOCOL FOR QUALITATIVE ANALYSIS

The following tests were carried out to detect the presence of active chemical constituents like alkaloids, tannins, glycosides, flavonoids, terpenes and saponins.

Alkaloids

To detect the presence of alkaloids, few drops of Mayer's reagent were added to the extract, cream coloured precipitate indicates the presence of alkaloids. (Siddiqui and Ali, 1997).

Tannins

1 ml of 5% FeCl₃ is added to the extract, presence of tanning is indicated by the formation of bluish black or greenish black precipitate. (Siddiqui and Ali, 1997).

Glycosides

To 2ml extract glacial acetic acid, few drops of 5% FeCl₃ and conc. H₂SO₄ were added reddish brown colour at the junction of two liquid layers and upper layer appears bluish green indicates the presence of glycosides. (Trease and Evans; 1989).

Flavonoids

Few drops of 10% concentrated sulphuric acid was added to the extract, followed by 1 ml of ammonia, formation of greenish yellow precipitate indicates the presence of flavonoids (Siddiqui and Ali; 1997).

Terpenes

To 2 ml of extract, 5 ml chloroform and 2ml conc. H₂SO₄ was added. Reddish brown colorations of interface indicates the presence of terpenes (Harborne; 1973).

Saponins

20ml Water is added to 150mg extract and shaken vigorously, layer of foam formation indicates the presence of Saponins (Siddiqui and Ali; 1997)

Carbohydrate

To 2.3ml extract, few drops of Molisch reagent was added, shaken well and conc. H₂SO₄ was added from sides of the test tube, violet ring formation at the junction of two liquids indicates the presence of carbohydrates (Krishnaveni *et al*; 1984).

RESULT

Table. 1 Results of the quantitative tests

S.No	Primary and Secondary metabolites	Aqueous extract	Methanolic extract
1.	Alkaloids	+	+
2.	Tannins	+	+
3.	Glycosides	---	++
4.	Flavonoids	++	++
5.	Terpenes	++	++
6.	Saponins	---	++
7.	Carbohydrates	+	---
	+ = Slightly present	++=Strongly present	--- = absent

T L C ANALYSIS OF RHIZOME EXTRACTS OF *Rheum emodi*

Both of the two extracts i.e. methanolic and aqueous were checked by thin layer chromatography. For methanolic extract solvent system were selected was chloroform : methanol (80 : 20) and for aqueous extract methanol : water : formic acid (18: 9 : 1).

Plant extract	Solvent system	R f values
Aqueous extract	Methanol :H2O :Formic acid (18:9:1)	0.38, 0.58
Methanolic extract	Chloroform: methanol (80:20)	0.65,0.712,0.75

After performing T L C of both the extracts, Rf.values were calculated for the spots which were seen under U V illuminator. Table 2



TLC of methanolic extract



TLC of aqueous extract

DISCUSSION

Qualitative photochemical screening is an essential step towards discovery of new drugs as it provides the information regarding the presence of a particular primary or secondary metabolite in the plant extract of clinical significance. The presence of any significant bioactive natural product indicates the necessity of separation of the compound from the mixture of compounds through suitable chromatographic techniques. In the present study, in aqueous extract flavonoids, terpenes were present strongly, but the presence of alkaloids, Tannins and carbohydrates is slightly positive. Similarly in methanolic extract presence of glycosides, Flavonoids, terpenes and saponins is strongly positive and the presence of alkaloids, tannins is slightly positive but the presence of carbohydrate is negative. These preliminary photochemical investigations focus on the importance of separation of the natural compounds from their mixtures as they may be used for various clinical practices. Thin layer chromatography was performed on two different extracts using two different solvent systems Methanol : Water : formic acid (18:9:1) for aqueous extract and chloroform : methanol (80:20) for methanolic extract. Rf values were calculated for all the fractions (table 2).

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