WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 1673-1684. Research Article ISSN 2277– 7105

PHYTOCHEMICAL ANALYSIS OF *PIMPINELLA TIRUPATIENSIS* BAL.&SUBR. - AN ENDEMIC PLANT TO EASTERN GHATS OF TIRUMALA HILLS, ANDHRA PRADESH

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Article Received on
19 March 2018,
Revised on 09 April 2018,
Accepted on 30 April 2018
DOI: 10.20959/wjpr20189-12207

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ABSTRACT

Phytochemicals are naturally present in the plants. They show biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes with antimicrobial activity by inhibition or by killing mechanisms. The use of plants and plant products as medicines could be tracked as far back as the beginning of human civilization. The present study was aimed to investigate the Phytochemical screening of *Pimpinella tirupatiensis* Bal. & Subr. leaves, stem, and root tuber. The alkaloids, flavonoids, glycosides, tannins, saponins, sterols, anthocyanidins, phenols and lignins were observed in high, moderate and low concentrations, which are used for many ailments.

KEYWORDS: *Pimpinella tirupatiensis*, Phytochemical analysis, endangered, endemic medicinal plant.

INTRODUCTION

Phytochemicals are the chemicals naturally occur in plants, these phytochemicals highly popular due to their medicinal uses. Phytochemicals play a major role against various diseases. During the past 20 years, at least one novel compound from higher plants has been marketed every 2.5 years (Deans and Sovoboda, 1990). Furthermore, increasing reliance of the medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutic from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Numerous studies have identified

compounds within herbal plants that are effective antibiotics (Basile *et al.*, 2000). The products from the plants are healthier, safer and more reliable than synthetic products (Benli *et al.*, 2008).

Taxonomic classification of *Pimpinella tirupatiensis*

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Apiales
Family : Apiaceae
Genus : *Pimpinella*
Species : *Tirupatiensis*
Species Authority : N.P.Balakr. & Subram.

Other linguistic names of *Pimpinella tirupatiensis*

Common name in English : Forest coriander
Telugu names : Adavi Dhaniyalu, Adavi Kothamiri, Komali Gadda, Konda Dhaniyalu, Konda Kothamiri.

The species of *Pimpinella tirupatiensis* Bal. & Subr. (Family Apiaceae; local name, kondakothimera) is a endangered and endemic medicinal plant and restricted to the Seshachalam hills of the Eastern Ghats, India. (Balakrishnan & Subramanyam 1960). It is an erect herb with perennial tuberous root stock, Stem is simple, branched, veins prominent, margins cartilaginously crenate - serrate. Leaves are simple, obtuse or acute, deeply cordate, petiolate. Flowers are white, compound umbles, Fruits ovoid, papillose-scrabrous. The tuber consists of a tap root arising from a stout root stock which persists throughout the life of the plant. The boiled tubers of *P. tirupatiensis* can be used as food. This plant is prescribed for venereal diseases and peptic ulcers (Nagaraju and Rao,1989).The local Adivasi tribal (Erukalas, Nakkalas, Sugali, Yanadis) community uses the tuberous roots of *P. tirupatiensis* to cure severe ulcers of stomach, throat and genital organs and also as aphrodisiac and abortifacient agents. Fruits are used to cure asthma and are considered as an effective remedy for 'flatulent colic'(Thammanna and Narayana Rao, 1990). Dried roots of *P. tirupatiensis* are administered along with few other ingredients to cure colic and rheumatic ailments in cattle (Sudarsanam *et al.*, 1995). This plant root extract is also used to treat skin diseases and is used as an antimicrobial agent (Jeevan Ram *et al.*, 2004). The whole plant of *P.*

tirupatiensisis used to treat cough, stomach, liver problems, asthma, ulcer and toothache (Madhava Chetty *et al*., 2008).

The screening of leaves, stem, and root tuber of *P. tirupatiensisis* for their biologically active components shall lead to their potential applications for the treatment of various ailments. With this intention the present work has been carried out.

Fig:1 (a) Pimpinella tirupatiensis Habitat (b) P.tirupatiensis tubers

MATERIALS AND METHODS

Collection of Plant Material

The plant material *Pimpinella tirupatiensis* was collected from Japaliteertham, Tirumala hills, Chittoor district, Andhra Pradesh. The plant material was washed thoroughly 2-3 times with running tap water and sterile distilled water. Then the plant parts were shade dried and coarsely powdered separately and stored in well closed bottles for further analysis in laboratory.

Authentication of Plant Material

The plant was authenticated by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and voucher specimen was (Pt 2207) preserved in the herbarium, Plant taxonomy lab, Division of Botany, Department of Sericulture, Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupati, Chittoor District for future reference.

Preparation of plant extracts

The method of Odebiyi and Sofowora (1978) was followed to prepare the extracts. Finely powdered plant samples (5.0 g of each) were soaked in 50 ml each of Aqueous, Chloroform, Petroleum Ether, and Benzene separately in 100ml sterile conical flasks. The flasks were covered with sterile cotton plugs followed by wrapping with aluminium foil and shaken at 4h intervals for 24h at room temperature. These crude extracts were then filtered through Whatman No.1 filter paper. The supernatants were collected, covered, labelled and used for the preliminary Phytochemical screening.

Phytochemical screening

Preliminary qualitative phytochemical screening was carried out for the extract as per standard methods described by Trease and Evans, 1989; Sofowora, 1993; and Harbone 1998

Experimental Reagents

Test for Alkaloids

2ml of extract were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

(a) Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

(b) Wagner's test: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Test for Flavonoids

(a) Ferric Chloride Test

To 1ml of the extract, few drops of ferric chloride solution was added. A green colour indicates the presence of flavonoids.

(b) Shinoda test

To 1ml of the extract, 8 - 10 drops of concentrate HCl and a pinch of magnesium ribbon were added. Boiled for 10 to 15 minutes and cooled. A red colouration is obtained indicating the presence of flavonoids.

Test for Phenols

(a) Ferric chloride test: 1ml of the extract was treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

(b) Ellagic acid test: To 1ml of the extract added 0.5ml of 5% acetic acid and 0.5% of 5% sodium nitrate solution. Muddy or brown colour appeared which indicates the presence of phenols.

Test for Steroids

(a) Salkowskis Test: To 1 ml of extract 1-2 drops of concentrated sulphuric acid was added, shaken well and allowed to stand. Golden yellow colour appeared which indicates the presence of steroids.

(b) Libermann Burchard Test: To 1 ml of extract 2 drops of acetic anhydrine was added and boiled. After cooling concentrated sulphuric acid was added. Formation of brown ring was seen at the junction indicating the presence of steroids.

Test for Saponins

Foam Test: 1ml of extract was shaken in a test tube with a 2ml of water, the foam produced persists for 10 min, indicating the presence of saponins.

Test for Tannins

(a) Ferric chloride test: 2ml of extract was taken in a test tube and ferric chloride solution was added drop by drop. Blue black precipitate appeared which indicates the presence of tannins.

(b) Gelatin test: To 1ml of extract added few drops of 1% solution of gelatin containing 10% sodium chloride. White precipitate appeared which indicates the presence of tannins.

Test for Anthocyanins

Sodium hydroxide test: 0.2 gm of plant extract was weighed in separate test tube, 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at 100 ± 2°C. Bluish green colour observed indicating the presence of anthocyanin.

Test for lignins

(a) Labat test: Gallic acid is added to the extract, olive green colour indicating the presence of lignins.

(b) Furfuraldehyde test: Furfuraldehyde is added to the extract, red colour seen indicating the presence of lignin.

Tests for Glycosides

Kellar Kiliani test: 5ml of the extract is treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid. Brown ring was seen at the junction of liquids indicating the presence of cardiac glycosides.

RESULTS

This study has revealed the presence of phytochemicals which are considered as active medicinal chemical constituents. Important medicinal phytochemicals such as alkaloids, flavonoids, glycosides, tannins, saponins, and sterols were present in the different parts of *P.tirupatiensis* (Table 1, 2, & 3).

Leaf Extract

Phytochemical analysis of various solvent extracts of leaves of *P.tirupatiensis* revealed the presence of alkaloids, sterols, tannins, saponins, glycosides and flavonoids in high concentration, where as phenols, anthocyanidins and lignins in very low concentration. The aqueous extract of the leaf showed positive results for alkaloids, sterols, saponins, tannins and glycosides in high concentration, where as flavonoids, phenols, anthocyanidins and lignins in moderate and low concentration. The chloroform, petroleum ether and benzene extract revealed the presence of alkaloids, glycosides, sterols in high concentration where as flavonoids, tannins, lignins and anthocyanidins in moderate and low concentration. Anthocyanidins are absent in petroleum ether and benzene extract (Fig:2).

Stem Extract

Phytochemical analysis of all the solvent extracts of stem of *P.tirupatiensis* revealed the presence of alkaloids, flavonoids, sterols, saponins, tannins and glycosides in high concentration, where as phenols, anthocyanidins and lignins in very low concentration. The aqueous and chloroform extract of the stem showed positive results for all tests. In petroleum ether and benzene extract revealed the presence of alkaloids and glycosides in high concentration, sterols, tannin, flavonoids, saponins in moderate and low concentration. The benzene extract, sterols, flavonoids, saponins are present in moderate and low concentration. Phenols, anthocyanidins, and lignins were absent in both petroleum ether and benzene extract. Tannins were present in petroleum ether but absent in benzene extract (Fig:3)

Root Tuber Extract

Different solvent extracts of root tuber of *P.tirupatiensis* revealed the presence of alkaloids, flavonoids, glycosides, sterols, saponins, tannins in high concentration, where as phenols, anthocyanidins and lignins in very low concentration. The aqueous extract of root tuber of *P.tirupatiensis* gave positive results for all tests. In chloroform extract, alkaloids, tannins and glycosides are present in high concentration and flavonoids, sterols, saponins in moderate and low concentration, remaining phenols, anthocyanidins and lignins are absent. In petroleum ether extract, alkaloids, tannins and glycosides are present in high concentration, and phenols, sterols, flavonoids and saponins are moderate and low concentration, alkaloids, tannins, saponins and sterols were present in moderate and low concentration and flavonoids, phenols, anthocyanidins and lignins are absent. (Fig:4).

Table:1 Phyto Chemical Anlaysis of *Pimpinella tiruaptiensis* – Leaves.

Name of The Test		**Aqueous Extract**	**Chloroform Extract**	**Petroleum Ether Extract**	**Benzene Extract**
Test for Alkaloids	A) Mayers Reagent Test	+++	+++	+++	+++
	B) Wagners Reagent Test	+++	+++	+++	+++
Test for Flavonids	A) Shinodas Test	++	+	+	+
	B) Ferric Chloride Test	++	++	++	+
Test for Phenols	A) Ferric Chloride Test	+	+	+	+
	B) Ellagic Test	++	+	----	----
Test for Steriods	A) Salkowski Test	+	+	+	+
	B) Liebermanns Test	+++	+++	++	++
Test for Saponins	Foam Test	+++	+	+	+
Test for Tannins	A) Gelatin Test	+++	++	+	+
	B) Ferric Chloride Test	+++	++	+	+
Test for Anthocyanidins	Sodium Hydroxide Test	++	+	----	----
Test for Lignins Test	A) Lignin Test	+	+	-----	----
	B) Labat Test	+	++	+	+
Test for Glycosides	Keller Kilani Test	+++	+++	+++	+++

Phytochemicals present in high (+++), moderate (++), less concentration (+) and absent (-)

Table: 2 Phyto Chemical Anlaysis of *Pimpinella tiruaptiensis* – Stem.

Name of The Test		Aqueous Extract	Chloroform Extract	Petroleum Ether Extract	Benzene Extract
Test for Alkaloids	A)Mayers Reagent Test	+++	+++	+++	+++
	B)Wagners Reagent Test	+++	+++	+++	+++
Test for Flavonids	A)Shinodas Test	+++	+	----	----
	B)Ferric Chloride Test	+++	++	+	+
Test for Phenols	A) Ferric Chloride Test	+++	+	---	---
	B)Ellagic Test	+++	+	---	---
Test for Steriods	A)Salkowski Test	++	+	+	++
	B)Liebermanns Test	+++	+	++	++
Test for Saponins	Foam Test	+++	+	+	+
Test for Tannins	A) Gelatin Test	++	+	+	----
	B)Ferric Chloride Test	+++	+	++	+
Test for Anthocyanidins	Sodium Hydroxide Test	++	++	---	----
Test for Lignins Test	A) Lignin Test	++	+	-----	----
	B)Labat Test	+	+	----	-----
Test for Glycosides	Keller Kilani Test	+++	+++	+++	+++

Phytochemicals present in high (+++), moderate (++), less concentration (+) and absent (-)

Table: 3 Phyto Chemical Anlaysis of *Pimpinella tiruaptiensis* - Root Tuber.

Name of The Test		Aqueous Extract	Chloroform Extract	Petroleum Ether Extract	Benzene Extract
Test for Alkaloids	A)Mayers Reagent Test	+++	+++	+++	++
	B)Wagners Reagent Test	+++	+++	+++	+
Test for Flavonids	A)Shinodas Test	+++	++	+	---
	B)Ferric Chloride Test	+++	+	+	---
Test for Phenols	A)Ferric Chloride Test	++	---	---	---
	B)Ellagic Test	++	---	++	+
Test for Steriods	A)Salkowski Test	+++	+	++	+
	B)Liebermanns Test	+++	++	++	+
Test for Saponins	Foam Test	+++	+	+	+
Test for Tannins	A) Gelatin Test	++	+++	+++	++
	B)Ferric Chloride Test	++	+	++	+
Test for Anthocyanidins	Sodium Hydroxide Test	++	---	---	---
Test for Lignins Test	A) Lignin Test	+	---	---	---
	B)Labat Test	+	---	---	---

Test for Glycosides	Keller Kilani Test	+++	+++	+++	+++

Phytochemicals present in high (+++), moderate (++), less concentration (+) and absent (-)

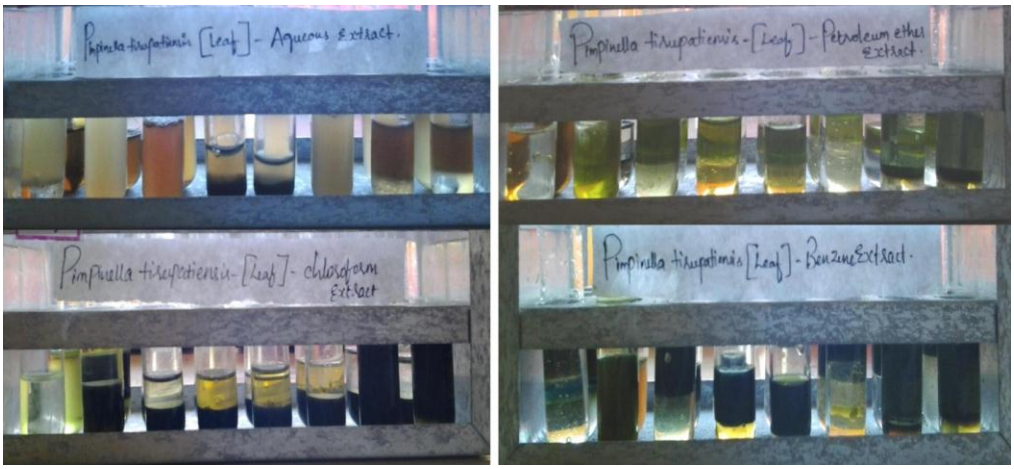

Fig 2: Preliminary phytochemical screening of Aqueous, Chloroform, Petroleum Ether, and Benzene extract of *Pimpinella tiruaptiensis*-Leaf.

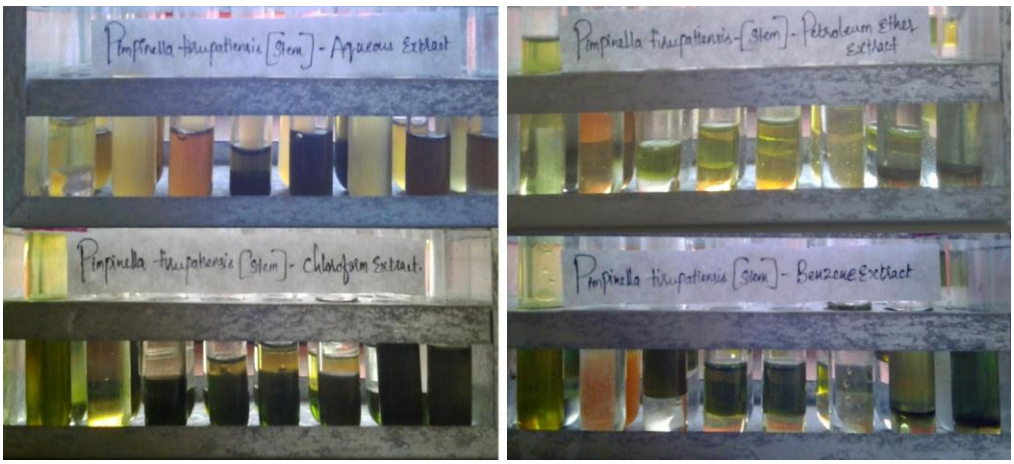

Fig 3: Preliminary phytochemical screening of Aqueous, Chloroform, Petroleum Ether, and Benzene extract of *Pimpinella tiruaptiensis*-Stem.

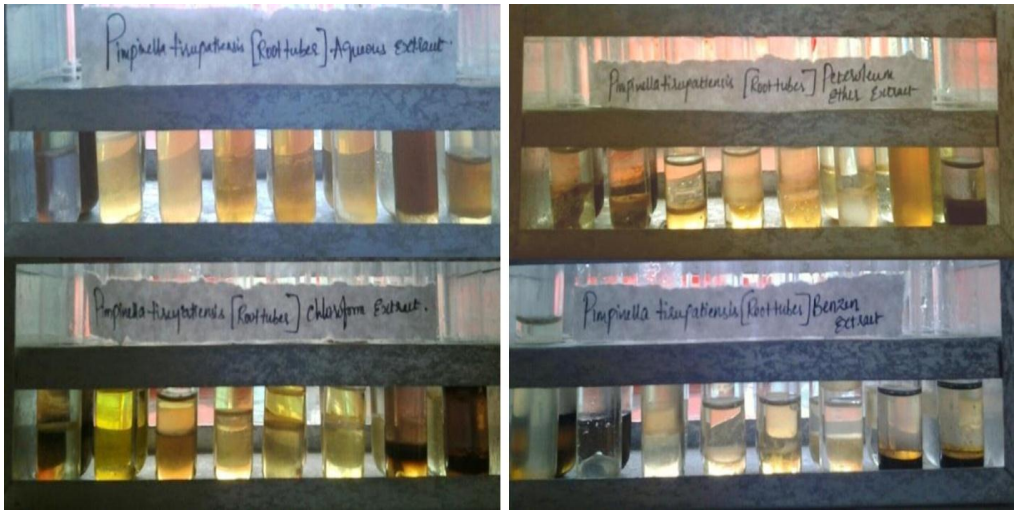

Fig:4 Preliminary phytochemical screening of Aqueous, Chloroform, Petroleum Ether, and Benzene extract of *Pimpinella tiruaptiensis*-Root tuber.

DISCUSSION

The leaf, stem, and root tuber extracts of the *P. tirupatiensis* analyzed were rich in phytochemical constituent's i,e., alkaloids, flavonoids, glycosides, tannins, saponins and sterols. Presence of these important secondary metabolites are responsible principles for medicinal values of the respective plant. Similar results were observed by Sudhakar *et.al.,* (2011) who have carried out Preliminary phytochemical test on root tuber of *P. tirupatiensis.* Rich content of Cardiac glycosides was found in various solvent extracts of *P. tirupatiensis*. Cardiac glycosides have been used for over two centuries as stimulant, in case of cardiac failure (Olayinki *et.al.,* 1992; Trease and Evans, 1998).

The biological functions of flavanoids are antioxidant properties, protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins, viruses and tumors (Barakat *et. al.,* 1993).

Tannins have cytotoxic and antineoplastic agents (Aguinaldo *et.al.,* 2005) and antimicrobial, antioxidant activities (Rievere *et. al.,* 2009). Tannins possess analgesic and anti-inflammatory activities and also contribute the property astringency (Okwu and Josiah, 2006).

Steroids are found in various solvent extracts of *P. tirupatiensis*. The steroidal compounds are important in pharmacy since they are associated with sex hormones (Anubha Arora, 2013).

Saponins are mild detergents used in intracellular histochemistry staining to allow antibody access to intercellular proteins. The saponins are used in hypercholestrolaemia,

hyperglycemic, antioxidant, anti cancer, anti inflammatory activity and weight loss (Manickam murugan *et.al.*, 2014).

Alkaloids posses antibacterial and antidiabetic properties (Akinyeye *et.al.*, 2014).

These photochemicals are potential to cure many aliments due to presence of various important compounds which play vital role for good health.

CONCLUSION

The present study concludes that the leaves, stem and root tuber of *P. tirupatiensis* have the potential to act as a source of useful drugs because of presence of various phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, saponins, and sterols in high concentrations. These phyto constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health.

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