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Preliminary phytochemical screening of some compounds from leaf and stem of *Putranjiva roxburghii* Wall

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Abstract
Medicinal plants have bioactive secondary constituents, which are used to control of various diseases. Medicinal plant *Putranjiva roxburghii* Wall were selected for the investigation. *P. roxburghii* were biologically active and were used for various types of ailments. Keeping in view their importance of this work was carried out to investigate the quantitative study of their crude phytochemicals. The quantitative determination of phytochemical (Alkaloids, caumarins, glycosides, tannins, phenol and reducing sugar) were determined in the afore mentioned tree. The phytochemicals including alkaloids, coumarins, glycosides, tannins, phenols were determine quantitatively use in standard literature method. *P. roxburghii* Wall. were studied with solvents *viz.* Ethanol, methanol were used to obtain crude extract from leaf and stem of produced plant. The plant was collected from western part of Satara District in M.S. India. Ethyl acetate was used as solvent. Alkaloid, coumarins, tannins, phenol, glycosides, reducing sugar was present in abundant amount whereas saponin & steroids was absent. This study could be of great value for ascertaining the medicinal role to the local practitioners and local people using this plant for remedy of various diseases.

Keywords: Phytochemical analysis, medicinal plant, secondary constituents, alkaloid, leaves

Introduction

Review of Literature

Study of Phytochemical analysis of stem and leaves of *Putranjiva roxburghii* was carried out for investigation in Western part of Satara District. Estimation of phyto- constituents of some medicinal plants is available but very scanty work is on *P. roxburghii* in botanical garden of Yashvantrao Chavan Institute of Science, Satara. The literature of phytochemical analysis is reviewed to view different roles of phyto-constituents present in different plants in India.

Sarath P. and Sudha Bai R. (2019) [18] studied a comparative evaluation of phytochemicals in bark, leaves and seeds of *Putranjiva roxburghii* Wall and he reported quantity of secondary metabolites was maximum in leaves while in bark and seeds are comparatively less.

Iqbal Husen and Jehangir Khan (2011) [9] investigated estimation of phytochemical analysis in selected medicinal plants and he exhibited scientific data of particular importance for the local people using these plants used for body disorders.

Extraction of phytochemicals from *Eucalyptus* Spp. and *Withania somnifera* are studied by Praveen Kumar and Ajit Kumar Srivastava (2018) [14] and reported *Withania somnifera* has potential antimicrobial, antioxidant anti- inflammatory and anticancer properties. Thilagavathi T and T. Arvindganth has explored preliminary screening of phytochemicals in different medicinal plants and exhibited medicinal plants have bioactive compounds which are used to cure of various diseases and secondary product are valuable for further analysis.

The phytochemicals are present in plants this investigation are most important for ascertaining the medicinal role of the plant explored by (Mudiganti and Selve Kumar 2017) [13] in analysis of phyto-constituents in herbal plant *Hygrophila auriculata.*

Extracts of the medicinal plant parts were analyzed for the presence of different phytochemicals like Alkaloids flavonoids, tannins, glycosides terpentenoids, were identified using phytochemical protocol.

Material and Methods

For the phytochemical analysis of *P. roxburghii* Wall for following chemicals are used Ethyl acetate, H_2SO_4, Chloroform, Methanol Wagners reagent, NaOH, HCl, Ethanol, Fehling's A & B, Glacial acetic acid, Benedict reagent. These chemicals were analytical grade and procured from Himalaya agency, Kolhapur.

Collection of plant material

The plant material of *P. roxburghii* Wall was collected from botanical garden, Department of Botany Yashawantrao Chavan Institute of Science, Satara (M.S.) India during second week of August 2019. Leaf and stem were procured for a comparative phytochemical analysis.

Authentification

The plant material collected and preserved with herbarium technique for authentication. Collected plant material identified from taxonomist in the department of Botany and well preserved in recognized research laboratory of department. Specimen voucher keep in the research lab for further procedure.

Methods

Preparation of extract

Plant part used in study were collected and washed thoroughly three times with tap water, dried and powdered into a fine powder and kept in zip lock plastic bag for further use. 5gm of dried powder of leaf and stem was extracted successively with 25ml of ethanol, methanol and distilled water using Soxhlet apparatus at 50-70 ^{o}C for 6-7 hours. The crude extract was stored in dark bottles in refrigerator at 4 ^{O}C.

Preliminary Qualitative Analyses

The 25 ml extract (stalk) from every solvent prepared. 30 ml stock solution (mg/ml) of extract from each type of solvent was prepared using the mother solvent. These extracts along with blanks were analyzed qualitatively for the presence of various phytochemicals. Phytochemical examinations were carried out for all the extracts of leaf and stem, of *P. roxburghii* Wall as per the standard methods of (Harborne and J.B. *et al.*, 1998) [8]. The crude extract of leaf and stem of *P. roxburghii* tested for alkaloids, tannin, saponin, flavenoide, terpenoids, phenol, glycoside, and reducing sugar, anthraquinone. The qualitative analysis are recorded as (+) for the presence highly, moderately and (-) for the less or absence of a typical phyto-constituent.

Results and Discussion

Phytochemical screening

1. Test for alkaloids (Sofowara A 1993)

- **Mayers test**

One ml extract was taken in test tube and add in it few drops of Mayers reagent cream colour precipitate appears indicate presence of alkaloids.

Hagers test

2 ml of extract were taken in test tube and add it few drops of Haggers reagent appear formation of yellow coloured precipitate it shows presence of alkaloide.

Wagner's test

Additionof few drops of Wagner's reagent in 2 ml containing extract in test tube resulted formation of reddish brown precipitate it indicates the presence of alkaloids.

Dragendroff's test

Take few ml of sample and add 2 drops of Dragendroff's reagent it appears red precipitate indicates that reaction is positive (Sofowanra *et al.*, 1993).

2. Test for Tannins

- **Ferric chloride test**

For the test of tannins in 2 ml of extract add 10 ml of distilled water, filter it and in filtrate add FeCl3 (10%) appear blue black or green precipitate it indicates presence of tannins.

3. Test of Saponins: (Sofowara A. 1993)

- **Foam test**

Take 0.5 ml of test solution added 2 ml distilled water and shake the all tubes, if foam produced persist for 10 min, it resulted presence of saponins.

4. Test of Terpenoides: (Harborne JB 2004)

- **Salkowski test**

To the test solution added 2 ml of chloroform and 1 ml H_2SO_4, reddish brown colour at interface, indicate the presence of terpenoids.

5. Test for Phenols

Extract were treated with 3-4 drops of 10% (w/v) $FeCl_3$ solution resulted in greenish black color indicated the presence of phenol.

6. Test of steroids

- **Salkowski's test**

2 ml of extract was treated with 2 ml chloroform and 2 ml conc. H_2SO_4. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence which resulted the presence of sterols.

7. Test of Glycosides

- **Kellar Kiliani Test: (Trease GE and Evans WC 1989) [22]**

Take 2 ml of sample and add with 1 ml glacial acetic acid, one drop 5% $FeCl_3$ then add conc. H_2SO_4. (0.5ml) along with one side of test tube carefully. A brown ring of the interface indicated the presence of cardiac glycosides

8. Coumarine Test

2 ml of extract was treated with 3 ml of 10% NaOH solution. Yellow colouration indicated the presence of coumarins.

9. Test of Redusing Sugar: (Trease GE and Evans WC 1989) [22]

2ml of sample in test tube boiled on water bath with addition of Fehling reagent A and B, resulted in colored product it indicate presence of Sugar.

10. Saponnin

Taking 5 ml of extract in test tube and diluted with 10 ml of distilled water warm gently. It was shaken frequently for 5minutes formation of persistent froth indicates presence of saponine.

11. Test for proteins: (Kokate CK *et al.*, 2009) [12]

- **Ninhydrin test**

Taking test solution and added 1 ml of 0.2% ninhydrin solution in it, violet color indicates the presence of protein in sample.

12. Test for flavonoids (Khandelwal KR 2004) [10]

- **Alkaline reagent test**

In a 2 ml of sample added few drops of NaOH solution resulted intense yellow color, which turns to colorless on the addition of few drops of diluted acid, indicates presence of flavonoids.

- **Lead acetate test**

The extracts were treated with few drops of 10% lead acetate solution. The formation of precipitate confirmed the presence of flavonoids (Khandelwal KR. 2004) [10].

13. Anthraquinone Test
Few drops of sample, add a 1 ml of 10% KOH solution. The formation of red colour confirmed presence of anthraquinone.

Result and Discussion
The phytochemical test was carried by leaf and stem extract with three different solvents ethanol, methanol and aqueous extract were evaluated by colour test. The results are recorded in following the tables.

Table 1: Phytochemical analysis of leaves

Sr. No.	Phytochemical test	Ethanol	Methanol	Aqueous
1.	Alkaloids	++	+++	-
2.	Tannin	+++	-	+++
3.	Saponin	-	+++	+++
4.	Terpenoids	++	++	-
5.	Phenol	+	+++	++
6.	Steroids	+	+	+
7.	Glycosides	+	++	-
8.	Coumarine	+	+	++
9.	Reducing sugar	++	+++	+++
10	Saponin		+++	
11	Protein	--	--	--
12	Flavenoides		++	+++
13	Anthraquinone	-	++	++

(+++ High, ++ Moderate and + Low)

Table 2: Phytochemical analysis of Stem

Sr. No.	Phytochemical test	Ethanol	Methanol	Aqueous
1.	Alkaloids	-	+++	+
2.	Tannin	+	+	++
3.	Saponin	-	+++	+++
4.	Terpenoids	++	++	+
5.	Phenol	++	+	++
6.	Steroids	-	-	++
7.	Glycosides	+	+	-
8.	Coumarine	+	++	++
9.	Reducing sugar	+	+	+
10	Saponin	-	+++	+++
11	Protein	-	-	+
12	Flavenoides	+++	++	++
13	Anthraquinone		-	++

(+++ High, ++ Moderate and + Low)

The results of phytochemical analysis of *Putranjiva roxburghii* leaf, stem sample in ethanol, methanol and aqueous extract showed alkaloids, tannin, saponine, flavenoide, terpenoids, phenol, glycoside, and reducing sugar while protein, anthraquinone were not detected. From leaf, stem of *P. roxburghii* the ethanolic extract which exhibited alkaloid, tannin and terpenoid while protein, steroid, were reported to be absent in methanol while tannin, saponine, flavenoides were presented in aqueous extract.
The phytochemical analysis of the ethanolic extracts in the leaf and stem sample of *P. roxburghii* resulted in presence of tannin, terpenoid and phenol but anthraquinone, protein, steroid was absent (Table. 1). The phytochemical analysis of stem extract showed presence of saponin, alkaloid and tannin while steroids, protein were present moderate or absent. There are certain phytochemical constituent such as alkaloid, saponin which are similar in aqueous and methanolic extract of *P. roxburghii.* Polyphenol were strongly supported to contribution for prevention of cardiovascular diseases. This paper mainly revealed that the phytochemical as secondary metabolites and they are used in pharmaceutical industry for producing efficient drug. (Thilagavathi *et al*., 2015) [21].
Alkaloids and their derivatives are very important and used in analgesic, antiapasmodic and bactericidal activities. However, alkaloids are mainly found in large amount in all flowering plants and they have an important physiological effect on human being. Quinine and Morphine are the major types of alkaloids which are used for narcotic analgesic as well as anti-tussive agent. (Stary *et al*., 1998) [20].
Flavenoides are water soluble phytochemical and an important plant phenolic. It shows antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. Leaves and stem are conventionally used for freeing muscle sprain and curing arthralgia in Thai medicine and the total plant issued to treat sickness and hemorrhoids. Woman munches the nuts of *P. roxburghii* orally to influence the birth of a male kid (Khare C.P. 2007) [11], (Farquar *et al*., 1996) [5].
P. roxburghii exhibited more amount of phenol in methanolic extract in leaves. Phenolic compounds in plant which are may be used as anti – microbial agents (Table 1) recorded high amount of phenol. Due to this reason leaves are used for skin diseases. Phenol and phenolic compounds are used in skin infections and other wound treatments and also for healing. (Okwu *et al*., 2001).
The phytochemical analysis of plant parts recorded in high amount of saponine (Table 2). Saponin in the plant used for wound healing and bleeding treatments. It has properties of precipitating and coagulation in red blood cells and having cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo *et al.*, 2000). Present investigation highlighted that *P. roxburghii* have high amount of phytochemicals such as alkaloids saponin, tannin, flavenoides, phenols, coumarine and reducing sugar. As per earlier discussion the presence of these phytochemicals exhibited this plant are used as basic medicinal agent such as rheumatic, ayurveda, unani, ophthalmic emetic, anti-seditious, diuretic and aphrodisiac. It is used in tribal in treating number of health problem (Padal *et al.*, 2013).

Conclusion
Estimation of phytochemical analysis of *Putranjiva roxburghii* showed maximum amount of phytoconstituents. The plant has highest therapeutic efficiency by pharmaceutical field. Screening of phytochemicals in given plant showed more positive result in methanolic plant extract, and other extract have resulted positive result. The plant extract were to determine the presence of phyto-constituents. It is also highlighted the medicinal value as well as the commercial usage of *P. roxburghii* can be used as biofuel. Trypsin inhibitor, antifungal, antipyretic and antidiabetic agent.
The plant used in treatments of so many diseases and their medicinal role of these plants have such a secondary metabolites and identified bioactive compounds. This paper revealed that above given plant gives a basis of its use in medicine and develop to further drug in pharmaceutical area and secondary products are used for further investigation. It is concluded that, instead of cultivation of decorative trees as garden trees, we can plant *Putranjiva roxburghii* as a gigantic as banyan tree having qualities of also being a good garden tree that can provide a dense shadow and cool air.

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