Identification And Estimation Of Phytochemicals From The Plant Pedicularis Bicornuta Leaf Extract By Uv-Spectrophotometry

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Abstract

The soxhlet extracts of Pedicularis bicornuta using different solvents were investigated for their phytochemical screening, antioxidants. For phytochemical screening, some common & available standard tests were done. Phytochemical screening revealed the presence of phenolics, steroids & flavonoids. Plants have been known to revel various diseases in ayurveda.

Keywords Pedicularis bicornuta, phytochemical screening, antioxidants.

Introduction

Pedicularis bicornuta is a PERENNIAL. The flowers are hermaphrodite (have both male and female organs).The plant derives its name from the Latin word pediculus, which means a louse. In the western Indian Himalayas, the Tibetan women offer the flowers to the deity. The inhabitants consider the plant species to possess some sanctification value and use it in various social customs. The aerial parts of the plant are used for medicine and are edible.

The aerial parts including leaves are cooked as vegetable. The root is used for preparing liquor. The powdered aerial parts are given in a dose of 1 tablespoon a day to cure chest pain, backache and bleeding through the mouth. The locals use the leaf juice on cuts to control bleeding

Herbs perennial or annual, rarely biennial, hemi parasitic. Leaves alternate, opposite, or whorled, usually pinnati fid to 1- or 2-pinnatisect, rarely entire or dentate. Lower leaves usually long petiolate upper leaves often sessile. Inflorescences terminal or flowers auxiliary bracts usually leaf like. Calyx tubular to campanulate, often bilabiate, usually deeply cleft interiorly, (2) 5-lobed. Corolla purple, red, yellow, or white, strongly bilabiate upper lip (galea) hooded , enclosing anthers , laterally compressed , rounded or truncate , or terminating in teeth or in a beak ; lower lip 3-lobed, usually spreading , external to upper lip in bud. Stamens 4, didynamous filaments glabrous or pubescent; anthers mucronate or not. Stigma capitate. Capsule moderately compressed or not, loculicidal. Seeds numerous, reticulate or costate.

Himalayan genus Pedicularis L. consists of eighty three species in India of which, 81 species are distributed in the Himalayan range, stretching from Ladakh in the west, through the Sikkim Himalayas, reaching up to Arunachal Pradesh in the east. Two species, namely Pedicularis zeylanica Benth and P. perottettii Benth., are confined to the Nilgiri Hills of south India. The genus Pedicularis L. was first described by Linnaeus in 1737 and subsequently by many workers viz. Bunge (1846), Maximowicz (1882), Pennell (1943) and Prain (1889, 1890) and was recently revised (Husain and Garg, 2007). The generic name Pedicularis derives its origin from the Latin 'Pediculus' meaning louse, as its decoction was used against lice on domestic animals in some areas of central Europe. Hence, the members of this genus are commonly known as louseworts. Many species exhibit such natural variations and extreme adaptations in their floral dynamics which indicate their adaptive response to survive and perpetuate in the drastic climatic conditions native to them (Garg and Husain, 2003).

Chemicals and Reagents

All the chemicals used were of Analytical grade and were purchased from Merk chemicals private limited, Mumbai.

Collection of plant materials

The plant was collected from botanical garden, KBN College, Vijayawada. The collected plant was dried in shady conditions, the dried plant is taken and powered, the powdered plant is then stored in the suitable conditions (air tight, light resistant containers).

Extraction procedure

The powdered material was weighed in a selected quantity and is subjected to soxhelt extraction using Hexane, Acetone and Methanol in successive mode respectively for 48 h. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was further evoperated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical screening.

Preliminary phytochemical screening

The extracts of the powdered leaves of pedicularis bicornuta analyzed for the presence of various phyto constituents like steroids, tri terpenoids soponins Triterpenoidal soponins ,alkaloids ,carbohydrates, flavonoids ,glycosides and phenolic compounds were identified using standard phytochemical procedures as described below.

1. Test for steroidal soponins: The extract is hydrolyzed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

Test's for steroids:

Salkowski test: Few drops of conc.H_2SO_4 is added to the plant extract shaken and on standing if the lower layer turns red in color then it indicates the presence of steroids.

Libermann burchards test: To the chloroform solution of extract , few drops of acetic anhydride is added from the sides of test tube if a reddish brown ring is observed at the junction of the two layers indicates the presence of steroids.

Test for Triterpenoidal soponins*:* The extract is hydrolyzed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for tri terpenoids.

2. Test's for tri terpenoids:

Salkowski test: Few drops of conc.H_2so_4 is added to the plant extract shaken and on standing if lower part turn golden yellow in color indicates the presence of tri terpenoids.

Libermannburchards test : To the chloroform solution of extract , few drops of acetic anhydride is added from the sides of test tube if a reddish brown ring is observed at the junction of the two layers indicates the presence of tri terpenoids.

Iscugajiu test: Excess of acetyl chloride & pinch of zinc chloride are added to the solution and kept a side for reaction to subside and warmed on water bath if cosion red color is produced indicates the presence of tri terpenoids.

Brickorn & brinar test*:* To the solution few drops of chlorosulfonic acid in glacial acetic acid (7:3) are added, if red color is observed indicates the presence of tri terpenoids.

3 .Test's for soponins:

Foam test: Small amount of extract is shaken with little quantity of water, and then if foam is produced and persists for 10 min. It confirms the presence of saponins.

4.Test's for alkaloids:

Mayers test: Mix the acid layer of the extract with potassium mercuric iodide solution creamy white ppt indicates the presence of alkaloids.

Dragendroffs test: The acid layer with few drops of dragendroffs reagent if reddish brown ppt indicates the presence of alkaloids.

Wagner's test: The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) if it gives brown to red precipitate indicates the presence of alkaloids.

Hager's test: The acid layer when mixed with few drops of Hager's reagent (saturated solution of pricric acid) if it gives yellow coloured precipitate indicates the presence of alkaloids.

5. Fehilings test: Test's for carbohydrates:
The extract when heated with Fehling's A & B solution if it gives an orange red ppt showing the presence of reducing sugar.

Molischs test: The extract is treated with molischs reagent and conc. H_2SO_4 along the sides of the test tube if a reddish violet ring is observed shows the presence of carbohydrates.

***Benedicts test*:** If the extract is heating with Benedict's reagent, if brown ppt is observed indicates the presence of sugar.

Barfords test: The extract on heating with Barfords reagent on boiling water bath for few min if reddish ppt is observed indicates the presence of carbohydrates.

6. Test's for flavonoids:
Shinoda test: If the alcoholic solution with few fragments of magnesium ribbon and conc. Hcl produced magenta color after few min is observed indicates the presence of flavonoids.

Ferric chloride test: Alcoholic solution of leaf extract react with freshly prepared $Fecl_3$ if it gives black fish green color indicates the presence of flavonoids.

Lead acetate test: Alcoholic solution of leaves extract with 10% lead acetate solution and gives white precipitate indicates the presence of flavonoids.

7. Test's for glycosides:
Anthraquinone test: if leaf extract powdered and extracted with either ammonia or caustic soda. If aqueous layer shows pink color indicates the presence of glycosides**.**

Keller-killiani test: This is for cardiac glycosides. Chloroform extract of plant and glacial acetic acid with ferrous chloride and 0.5 ml of conc. H_2SO_4. If acetic acid layer shows blue color indicates the presence of glycosides.

8. Test's for phenolic compounds:
Ferric chloride test: Treat the extract with ferric chloride solution if blue color appears then indicates the presence of hydrolysable tannins and green color appears indicates the presence of condensed tannins.
Gelatin test: To the solution add 1% gelatin solution containing 10% Nacl, and if ppt is formed indicates the presence of phenolic compounds.

Test for chlorogenic compounds: Treat the solution with aqueous ammonia and expose to air gradually, if green color is developed indicates the presence of phenolic compounds.

RESULTS

Phytochemical screening of plant materials
The Phyto chemical screening of plant materials studied showed the presence of flavonoids, steroids & phenolic compounds

DISCUSSION

Phytochemical screening of the plants reveled some differences in the consistuents of the plant Pedicularis bicornuta. And also shows or exhibit potent antioxdent activity the presence of flavonoids in the plant is likely to be responsible for the free radical scavenging effects observed. Flavonoids, phenolic compounds & phenolics are a major group of compounds that act as primary antioxidants or free radicals scavengers
The Methanolic extract was tested for the quantitative estimation of the amount of steroids present in the sample by fallowing the standard procedure. Results indicate that the extract contain 13.36 mg amount of steroids in 200mg of the plant extract. Cycloartenol was considered as standard steroid. Results were expressed in terms of Cycloartenol equivalents. Results

of the standard graph were shown in Figure-1 and Table-1

Table-1

S.No	Concentration in µg/ml	Absorbance
1	4	0.229
2	8	0.351
3	12	0.459
4	16	0.583
5	20	0.696
6	24	0.81
7	28	0.955
8	32	1.066

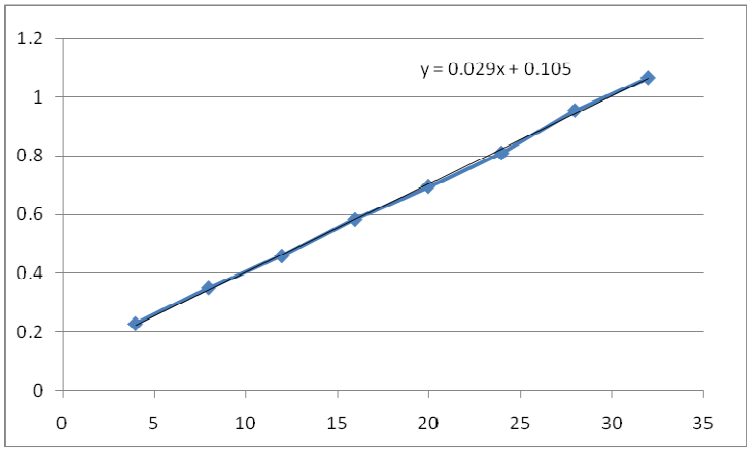

Fig-1 linearity graph for standard

The acetone extract was tested for the quantitative estimation of the amount of steroids present in the sample by fallowing the standard procedure. Results indicate that the extract contain 1.94 mg amount of steroids in 200mg of the plant extract. Cycloartenol was considered as standard steroid. Results were expressed in terms of Cycloartenol equivalents. Results of the standard graph were shown in Figure-2 and Table-2

Table-2

S.No	Concentration in µg/ml	Absorbance
1	4	0.229
2	8	0.351
3	12	0.459
4	16	0.583
5	20	0.696
6	24	0.81
7	28	0.955
8	32	1.066

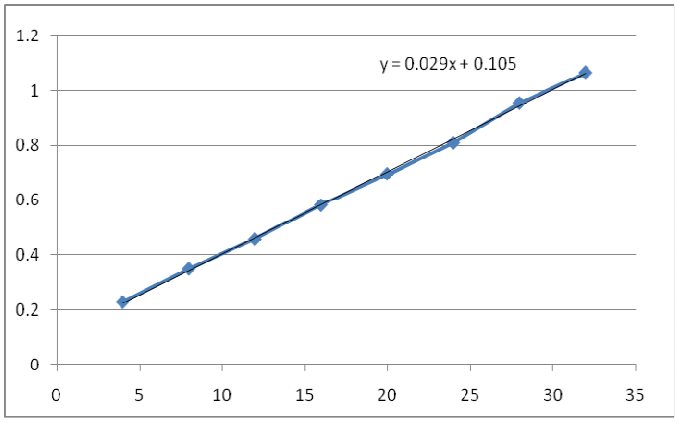

Fig-2 linearity graph for standard

The hexane extract was tested for the quantitative estimation of the amount of steroids present in the sample by fallowing the standard procedure. Results indicate that the extract contain 6.57 mg amount of steroids in 200mg of the plant extract. Cycloartenol was considered as standard steroid. Results were expressed in terms of Cycloartenol equivalents. Results of the standard graph were shown in Figure-3 and Table-3

Table-3

S.No	Concentration in µg/ml	Absorbance
1	4	0.229
2	8	0.351
3	12	0.459
4	16	0.583
5	20	0.696
6	24	0.81
7	28	0.955

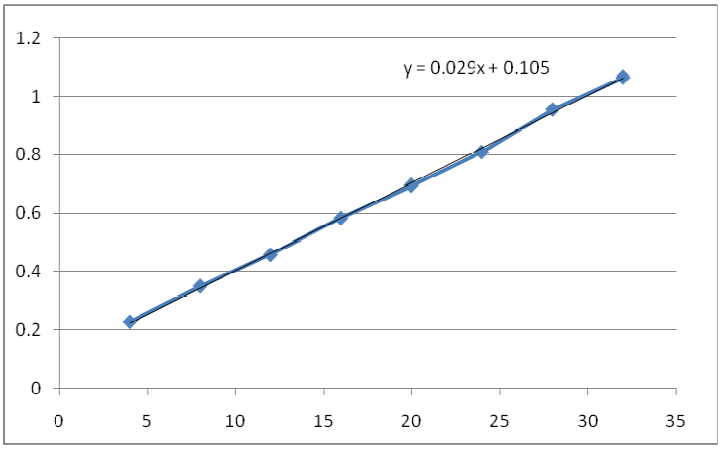

Fig-3 linearity graph for standard

By substituting the absorbance of the sample in the standard equation, it was found that the sample contain 19.85 mg amount of steroids in 200mg of dry weight of the plant extract.

The amount of Flavanoids present in the Methanolic extract was estimated by Alluminium Chloride method. Rutine was used as standard Flavanoid and results were expressed in routine equivalents. Standard values were shown in Table-4 same procedure was applied for the sample extract. Results of the extract analysis shows that the extract contain 19.09 mg

amount of Flavanoids in the methanolic extract of the leaf of pedicularis bicornuta.

Table-4

S.No	Concentration in µg/ml	Absorbance
1	2	0.221
2	4	0.359
3	6	0.502
4	8	0.669
5	10	0.812
6	12	0.975

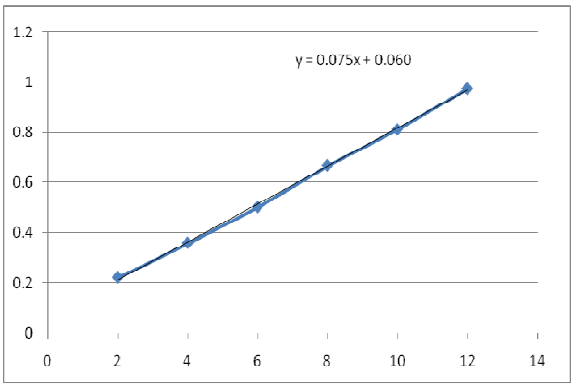

Fig-4 linearity graph for standard

The amount of Flavanoids present in the hexane extract was estimated by Alluminium Chloride method. Rutine was used as standard Flavanoid and results were expressed in routine equivalents. Standard values were shown in Table-5 and Figure-5 same procedure was applied for the sample extract. Results of the extract analysis shows that the extract contain 17.53 mg amount of Flavanoids in 200 mg of the methanolic extract of pedicularis bicornuta.

Table-5

S.No	Concentration in µg/ml	Absorbance
1	2	0.221
2	4	0.359
3	6	0.502
4	8	0.669
5	10	0.812
6	12	0.975

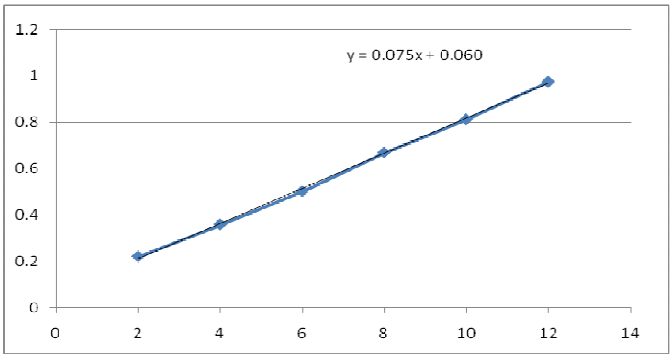

Fig-5 linearity graph for standard

The amount of Flavanoids present in the acetone extract was estimated by Alluminium Chloride method. Rutine

was used as standard Flavanoid and results were expressed in routine equivalents. Standard values were shown in Table-6 and Figure-6 same procedure was applied for the sample extract. Results of the extract analysis shows that the extract contain 15.94 mg amount of Flavanoids in 200 mg of the methanolic extract of the leaf of pedicularis bicornuta.

Table-6

S.No	Concentration in µg/ml	Absorbance
1	2	0.221
2	4	0.359
3	6	0.502
4	8	0.669
5	10	0.812
6	12	0.975

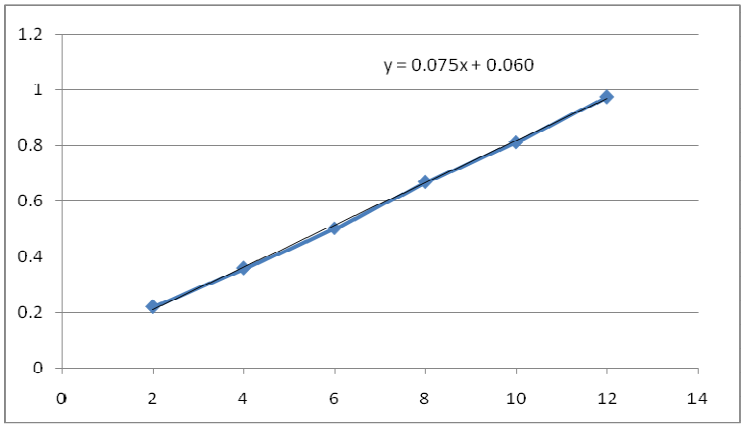

Fig-6 linearity graph for standard

By substituting the absorbance of the sample in the standard equation, it was found that the sample contain 5.24 mg amount of Flavanoids in 200mg of dry weight of the plant extract.

By fallowing the FC reagent method for the estimation of Phenolic compound in the leaf extract, it was found that in 200mg of the leaf extract in methanol shows 45.16 mg amount of phenolic compounds in terms of catechol standard. Results were shown in figure-6 and Table-6

Table-6

S.No	Concentration in µg/ml	Absorbance
1	15	0.087
2	30	0.175
3	45	0.261
4	60	0.354
5	75	0.457
6	90	0.558

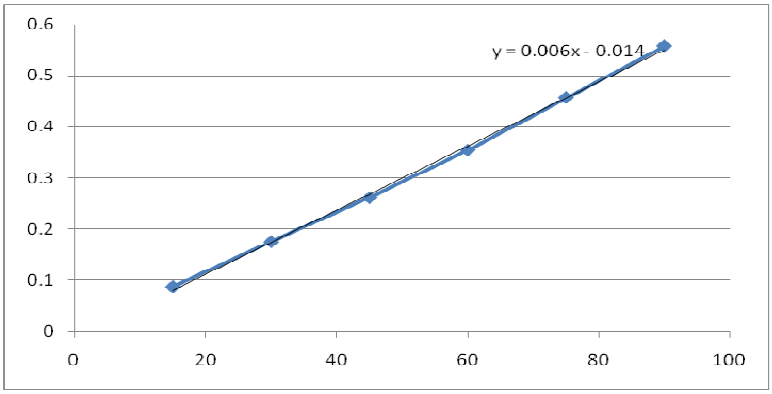

Fig-7 linearity graph for standard

Results of the quantitative estimation of phyto-constituents present in the leaf extract of pedicularis bicornuta show high amount of chemical constituents. Among the chemicals under the study steroids were found to be high amount in 200 mg of hexane extract. Summary results of the quantitative study were shown in Table-6.

Table-7

S.No	Name of the Phyto-constituent	Standard Compound	Amount found in 200mg of the extract
1	Flavonoids	Rutine	5.24
2	Phenolic Compounds	Catechol	4.51
3	Steroids	Atropine	19.85

The obtained yield of the plant extracts has been presented in the Table

Table-8

Plant part	solvents	Yield in 200 mg
	Methanol	7.74
leaves	Hexane	8.30
	Acetone	3.53

Table-9

s.no	Test	Pedicularis bicornuta
1	Reducing sugar	-ve
2	Antharaquinone	-ve
3	Terpenoids	-ve
4	flavonoids	+ve
5	Saponins	-ve
6	Tannins	-ve
7	Alkaloids	-ve
8	Phenolic compounds	+ve
9	steroids	+ve

CONCLUSION

The selected plant Pedicularis bicornuta is the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, ect. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anti-cancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The previous phytochemical analysis and present studied show nearly the similar results due to the presence of the phytochemical constituents. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. Further studies are therefore suggested to ascertain their antimicrobial, antiplasmodic and antihelminthic activities for the plant Pedicularis bicornuta. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

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