WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 1066-1074. Research Article ISSN 2277 – 7105

PHYTOCHEMICAL ANALYSIS OF *Parthenium hysterophorus* L. LEAF

[1]Krishnaveni Marimuthu* and [2]Dhanalakshmi Ravi

[1]Assistant Professor, Department of Biochemistry, Periyar University, Salem- 636 011.

[2]M.Phil Student, Department of Biochemistry, Periyar University, Salem- 636 011.

Article Received on
16 June 2014,

Revised on 11 July 2014,
Accepted on 06 August 2014

***Correspondence for Author**
Krishnaveni Marimuthu
Assistant Professor,
Department of Biochemistry,
Periyar University, Salem- 636 011.

ABSTRACT

The phytochemical screening and its nutrients was done using standard methods. Qualitative analysis of aqueous Parthenium hysterophorus leaf extract was studied for the presence of carbohydrate, alkaloid, steroid, sterols, glycosides, tannin, phenolic compound, saponin, flavonoid, oil. Carbohydrate, protein, aminoacids were determined spectrophotometrically. The aqueous extract was studied for its fluorescence and yield. The behavior of Parthenium hysterophorus leaf powder (proceesed) was assessed for it behavior with different chemical reagents. The results showed that most of the phytochemicals were found to be present in aqueous Parthenium hysterophorus leaf extract, the percentage that has been recovered is 27.91. The nutrients such as carbohydrate, protein, aminoacid content was found to be 164.0±3.46mg/g, 4.17±0.15mg/g, 0.88±0.07mg/g. The behavior of aqueous leaf extract was found to be satisfactory showing presence for alkaloid, steroid, flavonoid, anthroquinone and protein.

KEY WORDS: Aqueous extract, Fluoresence, Nutrients, Phytochemicals, Yield.

INTRODUCTION

Parthenium hysterophorus L. (Asteraceae), also known as congress grass, is an annual herb, invasive weed throughout India and world. It is an aggressive colonizer of wastelands, pastures and roadsides in India. The leaf proteins are reported to be better than cereal and legume proteins. It is used as spices in many parts of the world. Parthenin free dried fibres of plants are used as cattle feed [1]. All parts of the plant are reported to be used as bitter tonic, febrifuge, emmenagogue, antidysenteric [2]. In Maharashtra and Gujarat (India) it is used in the treatment of diabetes mellitus [3], antibacterial [4], anti tumor activity [5] and also used as folk remedy for the treatment of infectious and degenerative diseases [6-8] and find application

in traditional, ethnomedicine for treatment of wounds, ulcerated sores, fever, anemia, heart troubles [9], fertility, fecundity and behavioral response [10] and also against inflammatory, skin, neural diseases and female reproductive problems [11, 12]. Hence, an initiative was taken to study the phytochemicals qualitatively and phytonutrients quantitatively in the shade dried leaf samples in order to study the nature.

MATERIALS AND METHODS

Sample collection

The sample Parthenium hysterophorus leaf were collected from Periyar University campus, Salem, Tamil Nadu, India. The collected leaves were cleaned thoroughly allowed to shade dry and ground to powder using blender for further use.

Aqueous extract preparation

Aqueous extract was prepared by dissolving 15g of powdered Parthenium hysterophorus leaf in 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage yield, behavior of leaf powder and the remaining marc left was extracted with water and used for qualitative analysis.

Phytochemical analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures [13,14] fluoresence analysis [15], behaviour of drugs powder with different chemical reagents [16].

Test for carbohydrate

Molisch's test: To the extract added few drops of alcoholic alpha napthol solution, few drops of concentrated Sulphuric acid along the sides of test tube. Positive result gives purple or violet colored ring at the junction.Fehlings test: To the extract added equal amount of Fehlings A and B solution, heat the tubes in a boiling water bath. Brick red precipitation of cuprous oxide is formed, if reducing sugar is present.Benedicts test: To the extract add Benedicts reagent, the tubes were heated in a boiling water bath. Red precipitation indicates positive result.

Test for alkaloids

Wagners test: To the extract add few drops of iodine solution in potassium iodide. Reddish brown precipitate shows positive result.Hagers test: To the extract add few drops of saturated solution of picric acid. Yellow colour precipitation signifies positive result.

Test for steroids and sterols

Libermann-Burchard test: To the extract add 2ml chloroform, 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid. Bluish red to cherry red colour in chloroform layer shows positive result.Salwoski test: To the extract add few drops of chloroform, concentrated sulphuric acid. Bluish red to cherry red colour.

Test for Glycosides

Legal test: To the extract added pyridine, sodium nitroprusside. Positive result shows pink red colour.Baljet test: To the extract add picric acid. Appearance of orange color signifies positive result.

Test for saponins

Foaming test: Foams produces when the extract is shaked with water.

Test for flavonoids

Shinoda test: To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.Zinc hydrochloride test: To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.

Test for tannin and phenolic compounds

Ferric chloride test: To the extract add ferric chloride. Formation of greenish black colour shows positive result.Potassium dichromate test: To the extract add potassium dichromate solution. Positive result is confirmed by a formation of brown precipitate.Gelatin test: To the extract add 1% gelatin solution containing 10% sodium chloride gives white precipitation.

Test for protein and amino acids

Biuret test: To the extract added 4% sodium hydroxide, few drops of 15% copper sulphate gives purple colour. Ninhydrin test: Bluish violet colour forms when a solution of ninhydrin and extract mixture was heated.Heat test: Protein coagulation shows positive result when test solution is heated on a boiling water bath.

Test for fixed oil

Copper sulphate test: Blue colour forms when the extract is mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Quantitative analysis of phytonutrients

Total carbohydrates [17], proteins [18], aminoacids [19] were performed according to the standard prescribed methods.

Estimation of carbohydrate

The total carbohydrate was estimated by anthrone method**.** 1mg of Parthenium hysterophorus leaf powder was hydrolysed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N Hcl and cool to room temperature. After neutralizing, the contents were centrifuged and 0.1 ml of supernatant was used for the analysis. To the sample add 4ml of anthrone reagent and the contents were heated in a boiling water bath for 8 minutes. The tubes were cooled and read at 630nm using spectrophotometer Schimadzu Model - UV 1800. The standards were developed with glucose. Standard graph plotted was used to find out concentration of glucose present in unknown/ sample.

Estimation of Protein

The total protein was estimated by Lowry's method. To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of folin ciocalteau reagent (diluted in the ratio of 1: 2) was added and allowed for 30minutes incubation, then read at 660nm using spectrophotometer Schimadzu - Model UV 1800. The standards were developed with Bovine serum albumin. Standard graph plotted was used to find out concentration of protein present in unknown/sample.

Estimation of Aminoacids

The amino acid was estimated by Ninhydrin method. To 0.1 ml of sample added 1 ml of ninhydrin solution dissolved in ethanol. Cover the test tube with a piece of paraffin film to avoid the loss of solvent due to evaporation. With gentle stirring, react at 80-100ºC for 4-7 minutes. Cool the test tubes and the colour developed was read at 570nm. Tyrosine was used for developing standards.

Statistical Tool

Each experiments were carried out in triplicate and the results are given as the mean ± standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values), $s = \frac{\sqrt{\sum(x-M)^2}}{n-1}$

RESULTS AND DISCUSSION

The results obtained are shown in Table 1 to Table 5. The yield calculated was tabulated in Table.1. The results obtained for the analysis of leaf powder with different chemical reagents are shown in Table.2. The fluoresence analysis results are depicted in Table.3. Phytochemicals assessed qualitatively are expressed in Table.4. Table.5 shows the results of nutrients analysed.

Table.1 Percentage yield of Parthenium hysterophorus leaf aqueous extract

S.No	Name of the powder	Weight taken for extraction	Initial weight of the beaker (gm)	Final weight of the beaker (gm)	Weight of the extract Powder (gm)	Recovery (%)
1.	Parthenium hysterophorus leaf	15g in 200ml water	184.9340	189.1207	4.1867	27.91

The percentage recovery of the aqueous extract obtained was calculated and expressed in Table 1. The percent recovery was found to be 27.91 and the weight of the extract was 4.1867gm.

Analysis of Parthenium hysterophorus leaf powder for its behavior

The results obtained for the analysis of leaf powder with different chemical reagents are shown in Table.2.

Table.2 Behaviour of Parthenium hyleaf powder with different chemical reagents

S.No	Tests	Observation	Inference
1.	Powder+Picric acid	Yellow color	Presence of alkaloid
2.	Powder+Conc. H_2SO_4	Reddish brown color	Presence of steroids
3.	Powder+Aq. $FeCl_3$	Pale brown colour	Absence of flavonoids
4.	Powder+Iodine solution	Brown colour	Absence of starch
5.	Powder+Ammonia solution	Noblood red colour	Absence of anthroquinone
6.	Powder+Aqueous 5% KOH	Yellow color	Presence of anthroquinone
7.	Powder+NaOH	Yellow color	Presence of flavonoid
8.	Powder+ Aqueous $AgNO_3$	White precipitate	Presence of protein

The behaviour of Parthenium hysterophorus leaf powder with different chemicals showed positive result for alkaloids, steroids, anthroquinone, flavonoid and protein.

Fluoresence Analysis

Table.3 Fluoresence analysis of Aqueous Parthenium hysterophorus leaf extract

S.No	Name of the extract	Day light	UV light
1.	Aqueous	Green colour	Orange fluoresence

The fluoresence analysis results are depicted in Table.3. The Parthenium hysterophorus leaf powder extracted with water was green in color when observed in day light. The same when viewed under UV light it fluoresced orange in colour. (Table.3)

Phytochemical analysis

The results of qualitative phytochemical analysis are shown in Table.4.

Table.4 Qualitative analysis of phytochemicals in Aqueous Parthenium hysterophorus leaf extract

S.No	Name of the test	Results
1.	**Test for carbohydrate**	
	a)Molisch's test	+++
	b)Fehlings test	+++
	c)Benedicts test	+++
2.	**Test for alkaloids**	
	a)Wagners test	+++
	b)Hagers test	++
3.	**Test for steroids and sterols**	
	a)Libermann - Burchard test	+++
	b)Salwoski test	++
4.	**Test for Glycosides**	
	a)Legal test	+++
	b)Baljet test	++
5.	**Test for saponins**	
	Saponin test	+++
6.	**Test for flavonoids**	
	a)Shinoda test	+
	b)Zinc hydrochloride test	-
7.	**Test for tannin and phenolic compounds**	
	a)Ferric chloride test	++
	b)Potassium dichromate test	++
	c)Gelatin test	-
8.	**Test for protein and amino acids**	
	a)Biuret test	+++
	b)Ninhydrin test	++
	c)Heat test	-
9.	**Test for fixed oil**	
	a)Copper sulphate test	+++

+ Slight changes, ++ Moderate, +++ Stronger reactions

Results showed positive result for carbohydrate, alkaloids, steroids, sterols, glycosides, saponin, flavonoid, tannin, phenolic compound, ninhydrin, aminoacids, oil. (Table.4)

Phytonutrient Analysis

The phytonutrients estimated were tabulated in Table. 5.

Table.5 Phytonutrients in Aqueous Parthenium hysterophorus leaf extract

S.No	Phytonutrients	Calculated nutrient content
1.	Total carbohydrate	164.0±3.46mg/g carbohydrate
2.	Total protein	004.17±0.15mg/g protein
3.	Amino acids	000.88±0.07mg/g amino acids

Values are Mean ± SD for three experiments

The total carbohydrate content observed was 164.0±3.46mg/g carbohydrate. Likewise, the total protein, aminoacid content was found to be 4.17±0.15mg/g protein, 0.88±0.07mg/g amino acids. From, the observed result, the carbohydrate content was higher when compared to total protein and amino acid content. The Parthenium *hysterophorus* leaf was studied by Krishnaveni et.al for its air pollution tolerance index and antioxidant activities [20]

CONCLUSION

The present work established that Parthenium hysterophorus leaf contains most of the phytochemicals when tested qualitatively and also showed considerable amount of carbohydrate and protein. The percent yield was found to be moderate with Parthenium hysterophorus leaf aqueous esxtract.

ACKNOWLEDGEMENT

The author wishes her thanks to Honorable Vice-chancellor Dr. C. Swaminathan Avl and Registrar Dr. K. Angamuthu Avl, Periyar University, Salem for their administrative support and excellent infrastructure facilities provided and also thank Dr. V. Raj, Professor and Head, Department of Chemistry, Periyar University, Salem for his help.

REFERENCES

1. Narasimhan TR, Murthy BS, Rao PV. Nutritional evaluation of silage made from toxic weed Parthenium hysterophorus in animals. Food Chem Toxicol, 1993; 31(7): 509-515.

2. Oudhia P. Medicinal uses of Congress weed Parthenium hysterophorus L.: A Review. Raipur, India: Society for Parthenium hysterophorus L Management (SOPAM); 2001.
3. Patel VS, Chitra V, Prassanna PL, Krishnaraju V. Hypoglycemic effect of aqueous extract of Parthenium hysterophorus L. in normal and alloxan induced diabetic rats. Ind J Pharmacol, 2008; 40: 183-185.
4. Pandey AK. Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed Parthenium histerophorus an in vitro study. Natl Acad Sci Lett, 2007; 30: 383-386.
5. Reddy DM, Qazi NA, Sawant SD, Bandey AH, Srinivas J, Shankar M. Design and synthesis of spiro derivatives of parthenin as novel anti-cancer agents. European J Med Chem, 2011; 46: 3210- 3217.
6. Knox J, Jaggi D, Paul MS. "Population dynamics of Parthenium hysterophorus Asteraceae) and its biological suppression through Cassia occidentalis (Caesalpiniaceae)." Turkishournal of Botany, 2011; 35(2): 111–119.
7. Kumar S, Mishra A, Pandey AK."Antioxidant mediated protective effect of Parthenium hysterophorus against oxidative damage using in vitro models." BMC Complementary and Alternative Medicine, 2013; 13: 120-130.
8. Pandey AK. "Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed Parthenium hysterophorus: an in vitro study." National Academy Science Letters, 2007; 30(11-12): 383–386.
9. Das B, Venkataiah B, Kashinatham A. "(+)-Syringaresinol from Parthenium hysterophorus." Fitoterapia, 1999; 70(1): 101–102.
10. Kumar S, Singh AP, Nair G. "Impact of Parthenium hysterophorus leaf extracts on the fecundity, fertility and behavioural response of Aedes aegypti L." Parasitology Research,2011; 108(4): 853–859.
11. Recio MC, Giner RM, Uriburu L, Manez S, Cerda M, De la fuente JR, Rios JL. "In vivo activity of pseudoguaianolide sesquiterpene lactones in acute and chronic inflammation." Life Sciences, 2000; 66(26): 2509–2518.
12. Ramos A, Rivero R, Visozo A, Piloto J, García A. "Parthenin, a sesquiterpene lactone of Parthenium hysterophorus L. is a high toxicity clastogen." Mutation Research, 2002; 514(1-2): 19–27.
13. Harborne JB. Phytochemical methods, 2nd edition, Chapman and Hall, New York,1984.
14. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosyy, 3rd edition, Nirali Prakashan, Pune, 1995.

15. Kokoshi CL, Kokoshi RJ, Sharma FJ. Fluoresence of powdered vegetable drugs under UV Radiation. J Am Pharm Assoc, 1958; 47: 715-717.
16. Chase CR, Pratt RJ. Fluoresence of powder drugs with particular reference to development of a system of identification. J Am Pharm Asso, 1949; 38: 324-331.
17. Hedge JE, Hofrelter BT. In: Carbohydrate chemistry, 17 Eds. Whistler RL, Be Miller JN, Academic press, New York, 1962.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol Reagent. J Biol Chem, 1951; 193: 265-275.
19. Yemm EW, Cocking EC , Ricketts RE. The determination of amino acids with ninhydrin. Analyst, 1955; 80: 209-214.
20. Krishnaveni M. Air pollution tolerance index and antioxidant activity of Parthenium hysterophorus. Journal of Pharmacy Research, 2013; 7: 296-298.