ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

COMPARATIVE PHYTOCHEMICAL INVESTIGATION AND DETERMINATION OF FLAVONOIDS, ALKALOID AND ANTIOXIDANT ACTIVITY OF LEAVES, STEM AND FLOWER EXTRACT OF *PYROSTEGIA VENUSTA*

Ravindra S. Chaudhary*[1], Amit Kumar Jain[1], Vivekanand K. Chatap[2]

[1]*B.R. Nahata College of Pharmacy (Department of Pharmacy), Mandsaur University, Mandsaur, Madhya Pradesh, India*

[2]*H.R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Tal. Shirpur, Dhule, Maharashtra, India*

**Corresponding author: rschaudhary2009@rediffmail.com*

ABSTRACT

Medicinal plants include bioactive substances used to treat different human diseases, and they often play a significant part in various treatment. Secondary materials include alkaloids, flavonoids, phenol, saponin, tannins, and steroids. Medicinal plants have properties such as anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammatory, etc. The growing interest of secondary metabolites in powerful biological activity outlined the need to determine their content in medicinal plants. *Pyrostegia venusta* (Bignoniaceae) is a neotropic evergreen vine growing a lovely ornamental plant with orange flowers cascades. This is usually present in tropical, subtropical and mild mediterranean climates. In the cool, dry season the plants form dense masses, growing trees, on walls or over rocks and are covered with flowers. Native Brazilians use decoction of *P. venusta* aerial parts in cough and flu treatment. The general diarrhoea, vitiligo, and jaundice control tonic. The purpose of this research is to investigate the phytochemical profile and antioxidant activity of the leaves, stems and flowers of *P. venusta*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total flavonoids and alkaloid content were determined by the well-known test protocols available in the literature. Using standard protocols, the *in vitro* antioxidant activity of ethyl acetate and methanol extract of the leaves, stems, and flower was evaluated against DPPH assay method. The preliminary phytochemical analysis showed the existence of various phytoconstituents in each extract. The present study concluded that *P. venusta* crude extract is a rich source of secondary phytoconstituents that provide substantial antioxidant capacity. The results from this research would be of assistance to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Pyrostegia venusta,* Physicochemical, Qualitative, Quantitative phytochemical, Antioxidant

1. INTRODUCTION

Plant medications are readily available, less costly, safe and efficient and seldom have side effects [1]. Medicinal plants should be the safest choice for providing a range of medicines, according to the World Health Organization (WHO). Large sections of the population in developing countries still rely to their primary care on traditional practitioners and herbal medicines [2]. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. It was also mentioned by the WHO Advisory Group which developed this concept that such a classification allows it possible to differentiate between medicinal plants whose therapeutic properties and constituents have been scientifically identified and plants which are considered medicinal but have not yet been thoroughly studied [3]. These plants will be studied in order to better grasp their products, their health and their efficacy. Many chemical constituents that induce some pharmacological activity on humans are responsible for the therapeutic effects of plants. For every form of medicinal plant study of the qualitative examination of a medicinal plant's phytochemicals is recorded as a crucial phase. Precise sampling of plant constituents may be performed using chromatographic techniques [4]. Quantification usually employs gravimetric and spectroscopic methods currently available with several advanced approaches [5]. Oxygen is necessary for the creation of energy to power biological processes in many living species. Nevertheless, the oxygen metabolism produces 'free radicals' that cause oxidative damage to biomacromolecules, including DNA,

proteins, membrane lipids, and carbohydrates [6]. Free radical stress is a prevalent phenomenon that underlies the etiology of many degenerative diseases [7]. Numerous diseases such as cancer, diabetes mellitus, atherosclerosis, cardiovascular disorders, aging, and inflammatory disorders are documented to contain free radicals [8-12]. Antioxidants are essential compounds, since they can shield the body from free radicals destruction. They exert their effect by scavenging the free radicals (i.e. reactive oxygen species (ROS) or reactive nitrogen species) universally present in biological systems [12]. Natural antioxidants (e.g. polyphenols (flavonoids and tannins)) found in plants used for medical and culinary purposes are progressively of concern, and may help reduce oxidative damage [13]. A number of synthetic antioxidants are very powerful, e.g. butylated hydroxyanisole. These may have other side effects, though, which are toxic to humans [14, 15]. Therefore, compounds (especially those from natural sources which can protect against ROS-mediated damage) may have potential applications in the prevention and/or cure of some human diseases. *Pyrostegia venusta* (Ker-Gawl) Miers (Bignoniaceae) is a neotropic evergreen vine widely distributed in southern Brazil. Native Brazilians use the aerial parts of *P. venusta* for the treatment of cough and flu. They administer its decoction orally as a general tonic and also as an infusion to treat diarrhoea, vitiligo and jaundice [16-18]. Tonics produced from this plant's stems are effective for diarrhoea recovery, while flower preparations have been shown to attenuate vomiting [18]. Chemical investigations have shown that methanolic extracts of the roots of *P. venusta* contain allantoin, steroids, flavonone hesperidin (4,7-O-b-D-rutinosil-3',5-dihydroxy-4'-methoxyiflavanona) and 3-b-b-D-glicopiranosilsitosterol[16]. Similar observations regarding the isolation of n-hentriacontan (n-C31H64) 7-O-b-D-glicopiranosilacacetina), meso-inositol (myo-inositol) as well as several amino acids and sugars have been observed in the flowers [18]. Based on the many ethno medicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition.

2. MATERIAL AND METHODS

2.1. Plant material

The plant material (Leaves, Stems, Flower) for the proposed study was collected from local area of Bhopal (M.P.) in month of Nov. 2019. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Career College Bhopal. A voucher specimen number Bot/2019/019 was kept in Department of Botany, Career College Bhopal for future reference. Plant material (leaves, stems and flower) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

2.3. Extraction by soxhletion method

80 gram of powdered stems, 46 gram of powdered leaves and 35 gram of powdered Flower of *Pyrostegia venusta* were exhaustively extracted with different solvent (n-Hexane, ethyl acetate and methanol) by Soxhlet extraction method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

2.4. Qualitative phytochemical analysis of plant extract

The *P. venusta* extracts obtained were subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [19, 20]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

2.4.1. Total flavonoids content estimation

The total content of flavonoids was determined using the Olufunmiso et al method [21]. 1 ml of 2% AlCl3 methanol solution was applied to 3 ml of extract or normal solution and permitted to stand at room temperature for 60 minutes; the absorption of the reaction mixture was calculated at 420 nm using UV / visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the

results were expressed as quercetin equivalent (mg/100mg).

2.4.2. Total alkaloids content estimation

The plant extract (1mg) was dissolved in methanol, 1ml of 2 N HCl was applied, and washed. This solution was transferred to a separate funnel, added 5 ml of green bromocresol and 5 ml of phosphate buffer. The mixture was shaken by intense shaking with 1, 2, 3 and 4 ml chloroform, then collected in a volumetric flask (10 ml) followed by dilution with chloroform to the volume. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

2.5. Antioxidant activity

2.5.1. DPPH free radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometer with slightly modification of method [21].

Stock solution of DPPH (6 mg in 100ml methanol) was prepared; adding 1.5 ml of this DPPH solution in 1.5 ml of methanol gave an initial absorbance (Absorbance of control). Different concentrations (10-100 µg/ml) of standard (Ascorbic acid) and extract (Hydroalcoholic bark extract of *P. venusta*) prepared and from this solution 1.5ml of each standard and extracts taken, add 1.5ml of DPPH solution and INCUBATE IN dark at room temperature for 15 min. Decrease in the absorbance was noted after 15 minutes at 517 nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control – absorbance of sample) /absorbance of control] × 100.

3. RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive soxhlation extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves, stem and flower of the plants using n-hexane, ethyl acetate, and methanol as solvents are depicted in the Table 1.

Table 1: Results of percentage yield of different extracts

S. No.	Extract	Stem	Leaves	Flowers
1.	n- Hexane	0.25	1.23	0.57
2.	Ethyl acetate	1.63	2.08	0.76
3.	Methanol	2.28	4.13	3.80

Table 2 Result of phytochemical screening of *Pyrostegia venusta*

Constituents	n- Hexane extract			Ethyl acetate extract			Methanol extract		
	Stem	Leaves	Flowers	Stem	Leaves	Flowers	Stem	Leaves	Flowers
Alkaloids Hager's Test:	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glycosides Legal's Test:	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Flavonoids Lead acetate Test:	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Diterpenes Copper acetate Test:	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Phenol Ferric Chloride Test:	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Proteins Xanthoproteic Test:	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
Carbohydrate Fehling's Test:	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Saponins Froth Test:	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve

The results of qualitative phytochemical analysis of the crude powder of leaf, stem and flower of *P. venusta* were as shown in Table 2. Extracts of *P. venusta* showed the presence of alkaloids, flavonoids, saponins, and diterpines.

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.040X + 0.009$, $R^2=0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance and Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $Y=0.007X+ 0.024$, $R^2=0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance (Table 3).

Table 3: Estimation of total flavonoids and alkaloid content of *Pyrostegia venusta*

Extract	Total flavonoids content (mg/ 100 mg of dried extract)			Total alkaloid content (mg/ 100 mg of dried extract)		
	Stem	Leaves	Flowers	Stem	Leaves	Flowers
n- Hexane	0.656	-	-	1.200	-	1.057
Ethyl acetate	3.418	8.331	2.577	5.985	0.971	1.300
Methanol	2.475	2.556	4.862	4.357	2.057	4.928

Table 4: DPPH assay of ascorbic acid and extracts

Conc. (µg/ml)	% Inhibition						
	Ascorbic acid	Stem		Leaves		Flowers	
		Ethyl Acetate	Methanol	Ethyl Acetate	Methanol	Ethyl Acetate	Methanol
10	23.44	3.76	13.91	30.83	17.18	0.72	27.11
20	47.66	20.12	18.24	32.24	18.83	0.93	29.47
40	58.93	22.94	18.99	34.59	26.12	9.45	31.94
60	68.11	23.13	22.94	35.77	32.95	11.61	39.43
80	81.48	24.07	25.94	36.24	42.36	16.02	42.82
100	90.98	25.76	50.94	36.95	56.48	16.43	47.13
IC 50	24.44	225.61	55.74	287.58	93.35	263.17	112.12

Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition, the better is the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 10 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed (Table 4).

4. CONCLUSION

Through this study, qualitative and quantitative analyzes of alkaloids and flavonoids from P. venusta's leaves, roots and flowers were first obtained. The amount of phytoconstituents detected showed P. venusta is a rich source of antioxidant compounds. Actually manufactured synthetic antioxidants are known to induce or trigger adverse health results, so there are heavy limitations on their use and there is an application to substitute them with natural antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant and others activity and to explore the existence of synergism if any, among the compounds.

5. REFERENCES

1. Yadav RNS, Agarwala M. *J Phytology* 2011; **3(12)**:10-14.
2. WHO. Traditional medicine-growing needs and potentials. WHO Policy Perspectives Med, 2002.
3. WHO. Traditional Medicine and Modern Health Care Progress Report by the Direct General World Health Organization, Document No A-144110, 1991.
4. Sneader W. The discovery of aspirin: A reappraisal, BMJ (Clinical research ed., 2000; 321(7276):1591-1594.
5. Aguoru CU, Ameh SJ, Olasan O. *European J Adv Res Biol Life Sci.,* 2014; **2(2)**:16-22.
6. Aiyegoro OA, Okoh AI. *BMC Comp Alter Med.,* 2010, **10**:21-28.

7. Halliwell B, Gutteridge JMC, Cross CE. *Lab Clin Med*. 1992, **119**:598-620.
8. Di Matteo V, Esposito E. *Curr Drug Target CNS Neuro Disor.,* 2003, **2**:95-107.
9. Geber M, Boutron-Ruault MC, Hercberg S, et al. *Bull Cancer.* 2002, **89**:293-312.
10. Kris-Etherton PM, Hecker KD, Bonanome A, et al. *Amer J Med.* 2002, **113**:71S-88S.
11. Serafini M, Bellocco R, Wolk A, Ekstrom AM. *Gastroenterol* 2002, **123**:985-991.
12. Wilson RL. Free radicals and tissue damage, mechanistic evidence from radiation studies. Biochemical mechanisms of liver injury Academy Press, New York; 1988, 123.
13. Ames BN, Gold LS, Willet WC. *Proc Natl Acad Sci USA* 1995, **92**:5258-5265.
14. Gülçin I, Bursa E, Şehitoğlu MH, et al. *Food Chem Tech.,* 2010, **48**:2227-2238.
15. Gülçin I, Topal F, Çakmakçı R, et al. J Food Sci., 2011, **76**:585-593.
16. Ferreira DT, Alvares PS, Houghton PJ, Braz-Filho R. *Quimica Nova* 2000, **23**:42-46.
17. Scalon SP, Vieira MC, Lima AA, et al. Rev. Bras. Plantas Med., 2008, **10**:37-42.
18. Velosoa CC, Bitencourta AD, Cabral LD, et al. *J Ethnopharmacol.* 2010, **132**:355-358.
19. Khandelwal KR, Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan; 2005.
20. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
21. Olufunmiso OO, Afolayan AJ. *BMC Comp Altern Med.* 2011; **11**:130.