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PHARMACOGNOSTIC AND ANTIOXIDANT STUDIES OF *PYROSTEGIA VENUSTA* PRES. STEM

Md. Rageeb Md. Usman*, Neelesh Choubey[1]

[*1]Department of School of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Sehore, M.P, India.

Abstract:

The objective of present studies deals with the Pharmacognostic and antioxidant studies of stems of Pyrostegia venusta Pres. Some distinct and different characters were observed with section of young thin stems. Physiochemical parameter ash value and LOD of powder of stem was 1.85% w/w and 6.53 % w/w respectively. The phytochemical investigation of extracts of stem of Pyrostegia venusta shows the presence of sterols, triterpenes, flavonoids and tannins. Total phenolic content of total methanolic extract was determined by using folin Ciocalteu method. The total phenolic content in methanolic extract was found to be 5.55 % w/w equivalent to Tannic acid. Petroleum ether, ethyl acetate soluble, ethyl acetate insoluble and methanol exract was found to be scavenger of DPPH radical. The present study on Pharmacognostical investigation of Pyrostegia venusta Pers. stems might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Key Words: *Pyrostegia venusta Pers., Stems, Pharmacognostical, Physiochemical, Antioxidant, DPPH.*

Correspondence author:

Mohammed Rageeb Mohammed Usman,

Department of School of Pharmacy,

Sri Satya Sai University of Technology & Medical Sciences,

Sehore, M.P, India.

Email: rageebshaikh@gmail.com

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INTRODUCTION:

Humankind first utilized materials found in environment on an empirical basis to cure various ailments. Natural products from plants and animals traditionally have provided the pharmaceutical industry with one of its important sources of lead compounds in search of new drugs and medicines. The search for new pharmacologically active agents from natural resources such as plants, animals and microbes led to discovery of many clinically useful drugs [1]. Over the past two decades, researchers have also turned to many of the traditional folk medicines invariably a "cocktail" of natural products to uncover the scientific basis of their remedial effects, which improves the efficacy as to enhance modern medical practices [2]. The growing awareness of the harmful side effects of chemotherapy, has made people to explore the time tested remedies from traditional alternative medicine. India being a tropical country is blessed with vast natural resources and ancient knowledge for its judicious utilization. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and understand the mechanism of action [3]. There is a need for documentation of research work carried out on traditional medicines [4]. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [5]. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics [6].These standards are of utmost importance not only in finding out gentry, but also in detection of adulterants in marketed drug [7].

Pyrostegia venusta (Ker-Gawl) Miers (family, Bignoniaceae) is a neotropic evergreen vine that makes s a beautiful ornamental plant with cascades of orange flowers. It is commonly grown in tropical and subtropical areas, as well as in mild Mediterranean climates. The plants form dense masses, growing up trees, o n walls or over rocks, and are covered with flowers in the cool, dry season. Native Brazilians use decoction of aerial parts of P. venusta for the treatment of cough and flu. The general tonic control diarrhoea, vitilig o, and jaundice [8-10].

The present work was carried out on the Stem of *Pyrostegia venusta* (Bignoniaceae). Here more emphasis was given on pharmacognostic & antioxidant studies on stem of *Pyrostegia venusta.*

MATERIAL AND METHOD:

Plant material:

The plant specimens for the proposed study were collected from Maharashtra in the month of Oct. 2016 care was taken to select healthy plants and for normal organs [Figure 1]. The plant was authenticated by Dr. D. A. Dhale, PG and Research Department of Botany, SSVPS's L. K. Dr. P. R. Ghogare Science College Dhule, Maharashtra, India, identified the plant and Voucher R-002. The required samples of different organs were cut and removed from the plant and stain with different staining reagents [Table 1 and 2].

Fig. 1: Stem of *Pyrostegia Venusta*

Table 1: Transverse Section of Stem

Sr. No.	Test	Observation	Inference
1	Transverse section of stem + phloroglucinol + conc. HCL	Lignified fibers	Fibers present
2	Transverse section of stem + dil. Iodine solution	No blue colored Granules	Starch granules absent

Table 2: Powder Characterization of Stem

Sr. No.	Test	Observation	Inference
1	Powder + Few drops of water	No swelling	Mucilage absent
2	Powder + phloroglucinol + Conc. HCL	Lignified fibers	Fibers present
3	Powder + dil. Iodine solution	No blue starch grains observed	Starch grains absent
4	Powder + water + glycerin and observed under microscope	No yellow grains are observed	Aleurone grains are absent

Physicochemical Parameters:
Physicochemical parameter of stems of *Pyrostegia Venusta* Pers. were determined such as Total ash, Acid insoluble ash, Water soluble ash, Sulphated ash, moisture content etc [11-12].

Preliminary Phytochemical Parameters:
Preliminary phytochemical test of *Pyrostegia Venusta* Pers. were performed and the chemical constituents were detected [13-14].

Determination of Total Flavonoid Content (TFC):
The TFC in the extracts was measured by the $AlCl_3$ method as described by Misra et al. [15] using quercetin as the standard. The flavonoid content was expressed in quercetin equivalents (QE)/g of dry extract (dE). These experiments were run in triplicate.

Determination of Total Phenolic Content (TPC):
TPC in the extracts was measured using the Folin Ciocalteau method as described by Burgos et al [16]. The measurement was compared with gallic acid solution as the control. These experiments were run in triplicate. TPC in plant extracts was expressed in gallic acid equivalents (GAE).

DPPH Assay
The free radical scavenging activity of the extract fractions was measured in vitro according to Eniugha et al [17] using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay, and the inhibitory concentration (IC50) of the extract were estimated and calculated according to the equation:

RESULTS AND DISCUSSION:

Macroscopy:
Pyrostegia venusta is a fast-growing, evergreen woody vine that flourishes magnificent reddish-orange flowers. The compound leave s have 5.1-7.6 cm and are arranged in pairs opposite each other on the stem. Often, the center leaflet is modified into a coiled, three-parted tendril. F lame vine branches profusely and climbs by clinging with its tendrils. The tubular flowers are about 7.6 cm with the corolla in five lobes. Fruits are slender dry capsules [18].

Microscopy:
The Stem consists of pith that remains confined to the central part. The following sections were studied microscopically and microchemically.
TS: The TS of stem of *Pyrostegia venusta* revealed following elements

1.Xylem: Xylem consists of vessels and vascular tracheids. The primary xylem is confined toward pith. The secondary xylem consists of big vessels and xylem parenchyma. Xylem is found in the form of continuous cylinder traversed by narrow medullary rays

2.Phloem: The secondary phloem consists of sieve tubes, companion cells and phloemparenchyma. The primary phloem is also seen at certain places opposite primary xylem vessels in broken or crushed from.

3.Cambium: Below the secondary phloem, the cambial zone is present. The cambium consists of thin walled, rectangular cells arranged in radial rows. Actually the cambium is single layered, but it cut cells on both sides, and thus cambium zone is formed.
4.Pericyclic fibers: below the cortex consist of pericyclic fibers.
5.Pith: the pith is very small and remains confined to the central part of the stem. It consists of thin walled parenchyma cells having intracellular space among them.
6.Fibers: The supporting elements of xylem are fibers. This appears as thin walled lignified polygonal cells.
7.Medullary rays: Uni- or multiserate radial rays consist of thin walled parenchyma cells. The cells of medullary rays are with square or rectangular ends [Figure 2, 3 and 4].

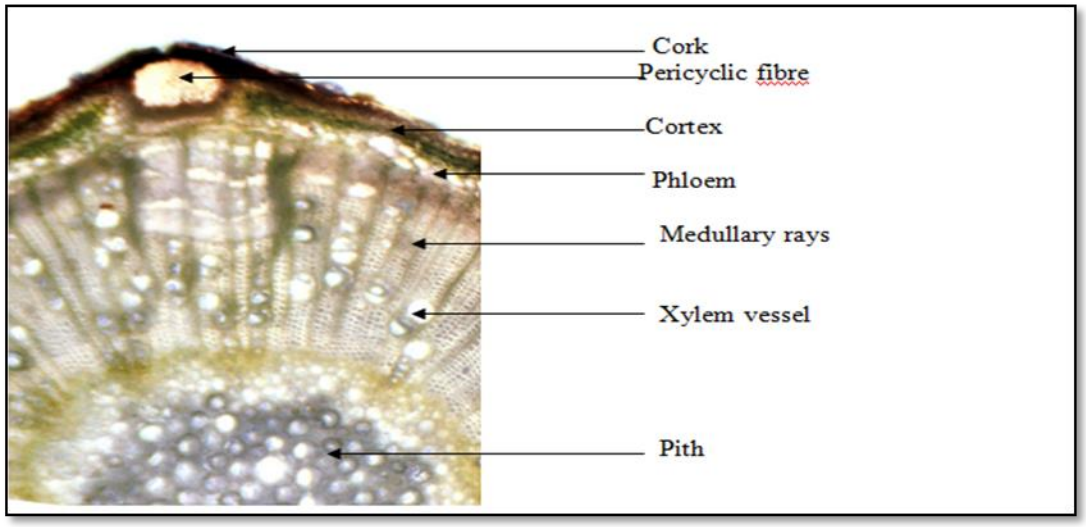

Fig. 2: T.S. of *Pyrostegia Venusta* Stem (Without Stain)

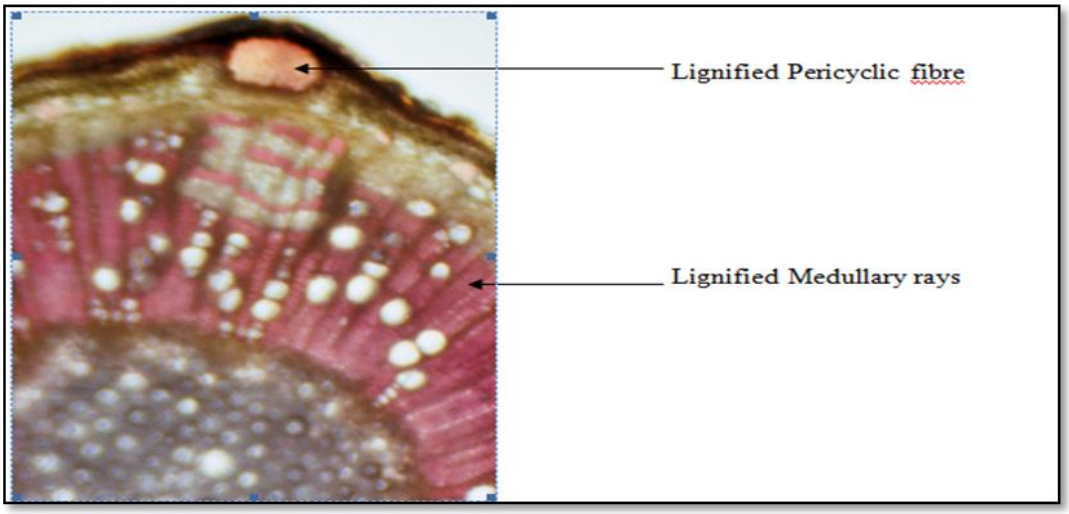

Fig. 3: T.S. of *Pyrostegia Venusta* Stem (Stain with Phloroglucinol: HCL)

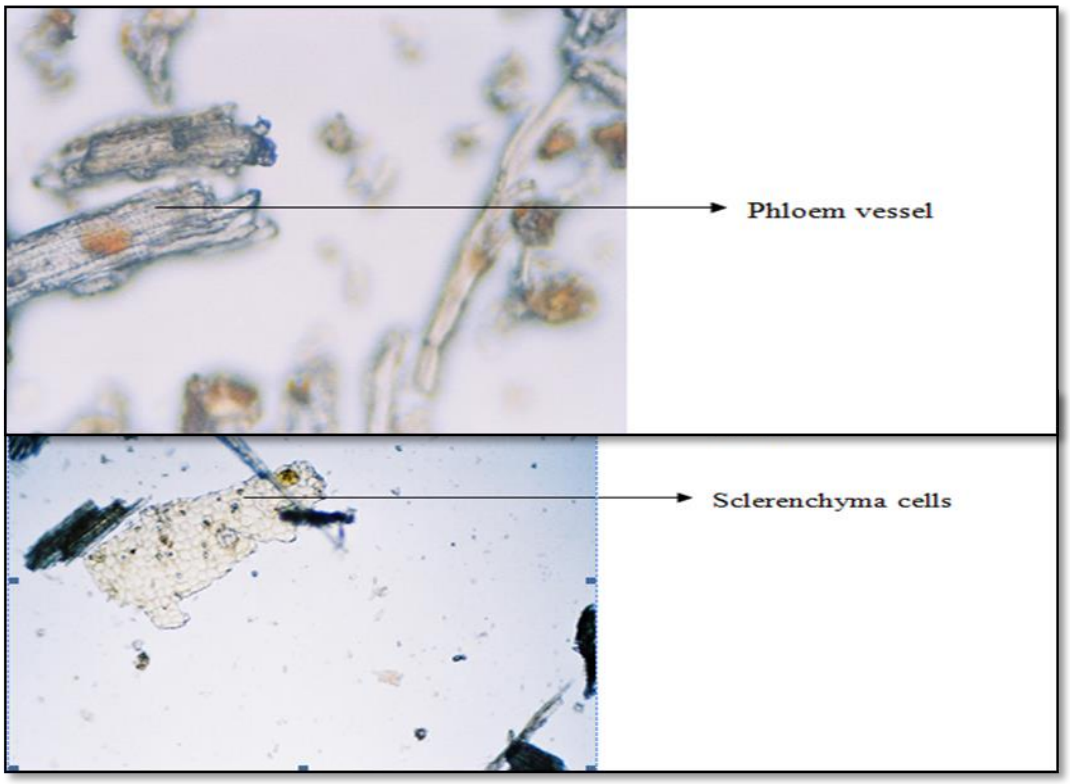

Fig. 4: Powder Characteristic of Stem

Physicochemical Parameters:

Physicochemical Parameters of stem of *Pyrostegia venusta* were shown in [Table 3 and 4].

Table 3: Extractive Values of Stem of *Pyrostegia Venust*

Sr. No.	Extractive	Color	Extractive Value (%w/w)
1	Methanol soluble extractive	Brown	6.12
2	Water soluble extractive	Brownish black	12.45
3	Petroleum ether	Yellowish brown	4.2

Table 4: Ash Values and Moisture Content

Sr. No.	Parameter	Result (%w/w)
1.	Total ash value	1.85
2.	Acid insoluble ash	0.31
3.	Water soluble ash	1.45
4.	Sulphated ash	3.4
5.	Moisture content	6.6

Preliminary Phytochemical Studies:

Methanolic extract and Pet. Ether of stems of *Pyrostegia venust* Pers. Showed the presence of various Phytoconstituents shown in [Table 5].

Table 5: Chemical Identification of Extracts of *Pyrostegia Venusta*

Sr. No.	Chemical Test	Extracts	
		Pet. ether	Methanol
1	**Test for Sterols** a. Salkowaski Test b. Liebermann Test	 + +	 – –
2	**Test for Glycosides**	-	-
3	**Test for Alkaloids** a. Dragendroff's Test	-	-
4	**Test for Triterpenoids** a. Liebermann-Burchard Test	+	-
5	**Test for Flavonoids** a. Shinoda test	-	+
6	**Test for Tannins** Ferric chloride Match stick	-	+
7	**Test for Carotenoids** a. Carr price reaction	-	-
8	**Test for Naphthaquinones** a. Juglone test	-	-

Table 6: Calibration Curve of Tannic Acid

Sr. No	Concentration (µg/ml)	Absorbance
1	25	0.087
2	50	0.17
3	75	0.255
4	100	0.331
5	125	0.416
6	150	0.498
7	175	0.57
8	200	0.674

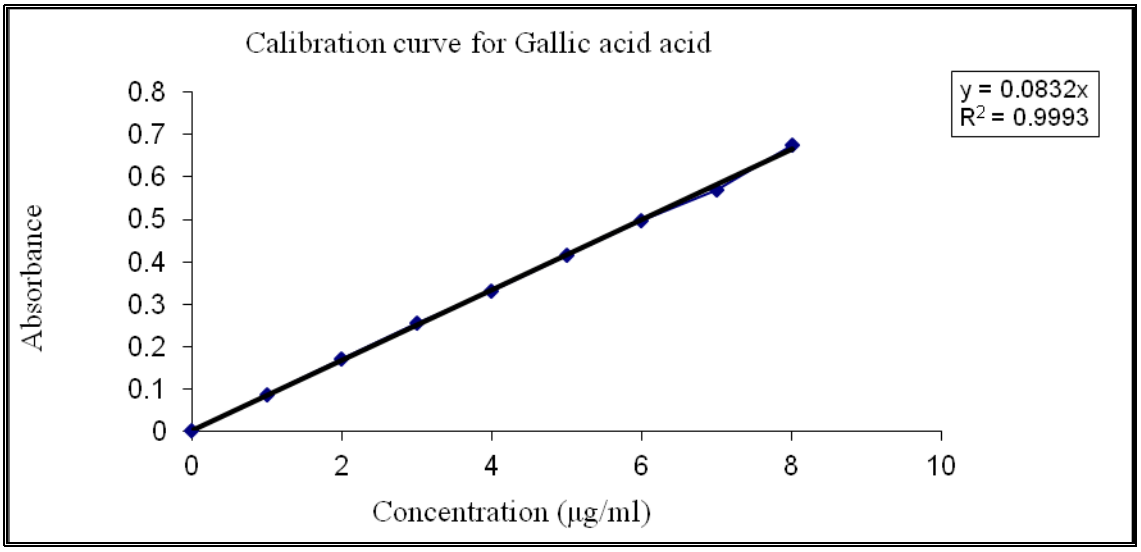

Fig. 5: Calibration Curve of Tannic Acid

Total Phenolic Content (TPC):

Natural phenolics have strong antioxidant properties since these molecules are able to terminate the generation of free radical chain reactions in the presence of hydroxyl groups which act as reducing agents [19]. All phenolic samples, including flavonoids, anthocyanins, and non-flavonoid phenolic compounds, were measured by the Folin-Ciocalteu method [19-20].

The TPC in the extracts was calculated from the regression equation (y = 0.0832x; R2 = 0.9993) of the calibration curve. Total Phenolic content in total methanolic extract of Stem of *P. venusta* was found to be 5.55 % w/w equivalent to Tannic acid.

Absorbance of *Pyrostegia venusta* stem extract = 0.185 [Table 6 and Figure 5].

DPPH Assay:

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of phytoconstituents. It has also been used to quantify antioxidants in complex biological systems in recent years. The assay is based on the treatment of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity of extracts (PTM, PPE. PC, PM) were examined in- vitro using DPPH induced free radicals. The different concentrations of extracts (150 µg/ml – 25 µg/ml) were treated with same concentration of DPPH for 5minutes. The reaction mixture consisted of 1ml of 0.1mM DPPH in ethanol, 1ml of ethanol and varying concentration of extract. The absorbance of the mixture was measured at 517nm exactly 30 seconds after adding DPPH. The experiments were performed in triplicate and percentage of scavenging activity was calculated using formula 100- [100 / blank absorbance X sample absorbance]. PE (132.178 µg/ml), ETS (64.95 µg/ml), ETI (71.76 µg/ml) and ME (60.23 µg/ml) showed free radical scavenging effect of DPPH. The ME showed more scavenging activity than other extracts [Figure 6, 7 and Table 7].

Table 7: Dpph Assay of Stem Extract of *Pyrostegia Venusta*

Test component	Conc. (µg/ml)	% inhibition	IC_{50} (µg/ml)
Petroleum ether Extract (PE)	25	15.6	132.178
	50	26.12	
	75	32.13	
	100	40.41	
Ethyl acetate soluble fraction (ETS)	25	23.17	64.95
	50	41.17	
	75	58.19	
	100	71.15	
Ethyl acetate insoluble fraction (ETI)	25	19.15	71.76
	50	35.17	
	75	51.49	
	100	69.45	
Methanol Extract (ME)	25	21.5	60.23
	50	44.55	
	75	63.52	
	100	77.17	
Ascorbic acid (ASS)	5	15.64	13.83
	10	34.51	
	15	51.45	
	20	73.87	
	25	91.93	

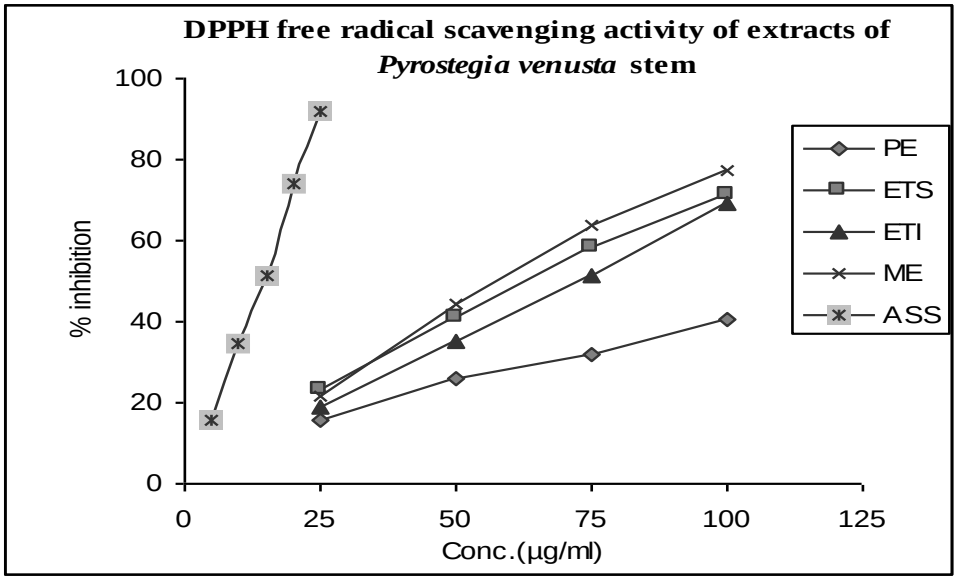

Fig. 6: DPPH Free Radical Scavenging Activity of Extract of *Pyrostegia Venusta* Stem

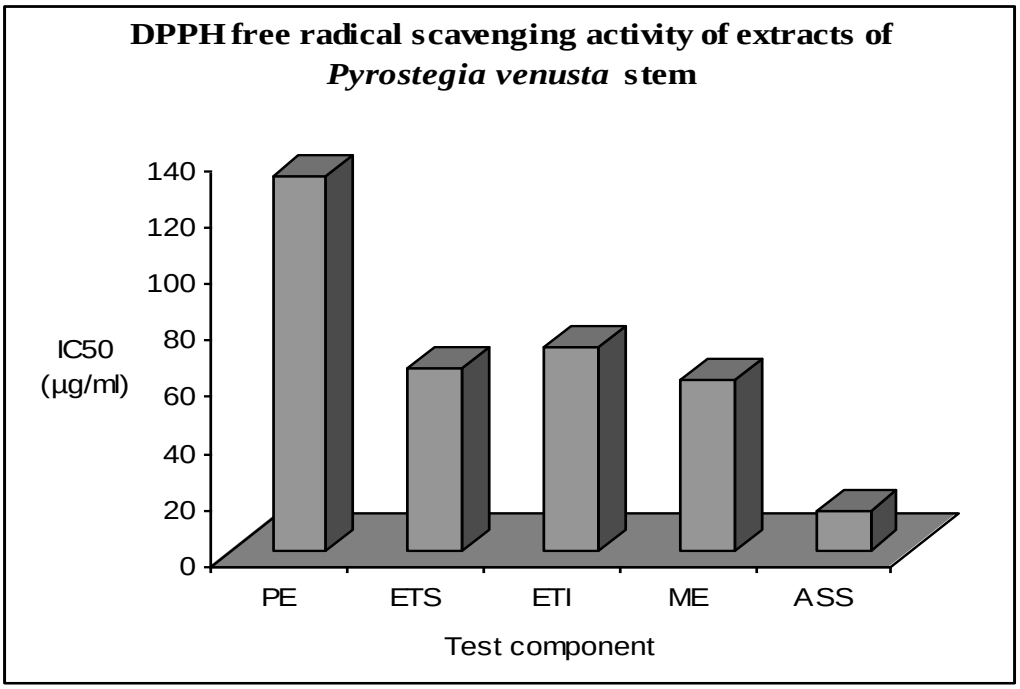

Fig. 7: DPPH Free Radical Scavenging Activity of Extract of *Pyrostegia Venusta* Stem

CONCLUSION:

The present work was carried out on the Stem of *Pyrostegia venusta* (Bignoniaceae). Here more emphasis was given on pharmacognostic, phytochemical & pharmacological studies on stem of *Pyrostegia venusta*. The stem of *Pyrostegia venusta* was studied for its microscopy, stem shows presence of vessels, xylem parenchyma, fibers, pericyclic fibers, medullary rays and phloem. Standardization of *Pyrostegia venusta* stem was carried out. It includes determination of ash value, LOD and extractive value. The percentage ash value of powder of *Pyrostegia venusta* stem was 1.85%w/w, the percentage loss on drying was found to be 6.53% w/w. Using different solvents the extraction of stem powder was carried out. The extractive value for Petroleum ether4.2% w/w and for methanol was 12.45%w/w.

Different chemical tests were performed on the extracts find out the nature of chemical constituents. The phytochemical investigation of extracts of stem of *Pyrostegia venusta* shows the presence of sterols,

triterpenes, flavonoids and tannins. Total phenolic content of total methanolic extract was determined by using folin Ciocalteu method. The total phenolic content in methanolic extract was found to be 5.55 % w/w equivalent to Tannic acid. Petroleum ether, ethyl acetate soluble, ethyl acetate insoluble and methanol exract was found to be scavenger of DPPH radical.

CONFLICT OF INTEREST:

Authors have no conflicts of interest to declare.

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