Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
3(4), 2014, 125-133

ISSN 2321 – 0958

Original Research Article

EVALUATION OF FREE RADICAL SCAVENGING POTENTIAL OF *TELOSMA PALLIDA*

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Abstract: In the present work antioxidant activity of *Telosma pallida* leaf and stem extract was evaluated. The leaf and stem parts were individually extracted with different solvents. Preliminary phytochemical screening of all the extracts was carried out. The present total phenolic contents were estimated by Folin- Ciocalteu reagent method and expressed as µg/mg of gallic acid equivalent. The antioxidant activity and reducing power of all the extracts were measured against DPPH as compared to standard ascorbic acid, and butylated hydroxy anisole respectively. The result data indicated that the phenolic contents were higher in methanolic extracts of leaf (60.13±0.28mg/g) followed by ethyl acetate (18.38±0.31mg/g), aqueous extract (18.08±0.45mg/g) and n-Hexane (4.11±0.18mg/g). The similar pattern in stem part was also observed i.e. methanolic extracts (77.10±0.28mg/g), ethyl acetate (28.00±0.38 mg/g), water (22.65±0.41mg/g) and n-Hexane (3.76±0.36 mg/g). The antioxidant capacity of methanolic extract of both the part i.e leaf and stem was found highest as IC50 values were 253.12±1.02, 158.43±0.48 respectively. The methanol extract of both parts also was found to exhibit maximum reducing capacity. The result data conclude that the higher antioxidant as well as reducing power may be due to present phenolic contents.

Keywords*:* *Telosma pallida*, Antioxidant, Reducing Power, DPPH, IC50

Introduction: The involvements of free-radicals in pathogenesis of various diseases have been well established. These free radicals are essential element to any biochemical process and also significantly involved in aerobic life and metabolism. Free radicals are being produced continuously through the respiration and some cell-mediated immune functions. At the same time they may be generated due to pollutants, automobile exhaust fumes, pesticides, smoking, radiations etc.[1], and become a part of our daily inhaling/ingesting life [2].

The antioxidants interact with these free radicals and terminate their action by

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Received on: September 2014
Accepted after revision: October 2014
Downloaded from: www.johronline.com

interrupting chain reaction, before vital molecules get damaged. The toxic profile of free radicals can be counteracted by the cellular antioxidant defense system of the body. Antioxidants are involved in prevention of cellular damages, and the pathway is common for ageing, cancer and a variety of diseases [3]**.** A wide range of antioxidants, both natural and synthetic are utilized in the treatment of diseases [4, 5]. Some enzymes such as superoxide dismutase, catalases, and glutathione peroxidase are involved in direct elimination of reactive oxygen species, while others like glutathione-S-transferase, glutathione reductase, and glucose-6-phosphate dehydrogenase helps in detoxification of reactive oxygen species by decreasing peroxide levels or by maintaining a steady supply of metabolic intermediates like NADPH and glutathione for other enzymes [6, 7]**.** Non-enzymatic antioxidants such as Vit.-E, β-carotene, Vit.-C, cysteine, and ceruloplasmin are also present [8]**.** Phytoconstituents like phenolics and flavonoids have been found to posses antioxidant capacity [9, 10, 11]**.** The plant containing these elements have been significantly found to exhibit anti-inflammatory, antibacterial, antiviral, antithrombic, antiallergic, and vasodilatory activities [12]**.**

The *Telosma pallida* **(**Asclepiadaceae) is also an important medicinal plant, and being used traditionally against various ailments (13). The plant has been found with presence of many phytoconstituents [14, 15, 16, 17]. Researchers have also been proved various biological activities on *Telosma pallida* [18, 19]. Still a lot of work is required to establish the pharmacological potential of the plant. The main objective of this study was to determine antioxidant and reducing power of the leaves and stem part of the *Telosma pallida* extracted successively with n-hexane, ethyl acetate, methanol and water.

Materials and methods:

Reagents and chemicals: All the chemicals used in the study were of analytical grade. The reagents used for preliminary phytochemical study were freshly prepared. Folin Ciocalteu reagent (Qualigens), Gallic acid (Fluka), DPPH (Sigma), Ascorbic acid, Iron chloride, Butylated hydroxy anisole (BHA), Trichloroacetic acid (Merck), Potassium ferricyanide (Thomas Baker) were incorporated in study.

Collection and authentification of plant parts: The leaves and stem of Telosma pallida was collected from tehsil Aamer, Jaipur (Raj.) India, in the month of September to November and was authenticated by Joint Director, Botanical survey of India, Jodhpur. Specimen samples are stored at herbarium of Maharishi Arvind college of Pharmacy, Ambabari, Jaipur.

Preparation of plant part extracts: The leaf and stem part was cleaned and shade dried. The samples were broken into small pieces with cutter mill, powdered and passed through sieve no. 44. The leaf and stem samples separately; were extracted successively using soxhlet apparatus with n-hexane, ethyl acetate and methanol. Finally remaining marc was extracted with water. The collected extracts were vaccume dried and were labeled as indicated in table 1.

Table 1. Labels of successive extracts of leaf and stem of *Telosma pallid*

Plant Part	n-Hexane	Ethyl Acetate	Methanol	Water
Leaf	TPLH	TPLEA	TPLM	TPLW
Stem	TPSH	TPSEA	TPSM	TPSW

Preliminary phytochemical screening of extracts: The collected extracts were subjected to preliminary phytochemical screening for qualitative determination of phytoconstituents [20, 21].

Estimation of total phenolic content: The total polyphenolic content of all extracts was measured by Folin- Ciocalteu reagent method. The absorbance was measured at 760 nm using UV spectrophotometer (Jasco V530 –

UV/VIS/NIR) and was expressed as µg/mg of gallic acid equivalent [22].

Antioxidant assay for plant extracts:

Estimation of free radical scavenging activity by DPPH method: Scavenging free radical potential of collected extracts was evaluated against a methanolic solution of 1, 1-diphenyl- 2- picryl hydrazyl (DPPH) as method describe by Cengiz 2008[23]. The 200µM methanolic solution of DPPH was prepared. 1ml of different concentration (10µg to 4 mg/ml) of extract solution and standard ascorbic acid solution (10- 60 µg/ml) were taken in different vials. To this 1 ml of methanolic solution of DPPH was added, shaken, and mixture was allowed to stand at room temperature for 20 min. A blank was also prepared in the similar way and the absorbance was measured at 517nm. Scavenging activity was expressed as the percentage inhibition calculated using the formula.

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs - Sample Abs}}{\text{Control Abs}} \text{ X } 100$$

Reducing power assay: The reducing capability was measured by the transformation of Fe^{3+}- Fe^{2+} in the presence of different extracts. Different concentrations of extracts (250-2500 µg) in 1ml of water were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50^0C for 20 min. 2.5 ml (10%) of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 RPM for 10 min. 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% Fecl3 solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power [24, 25]. BHA solution (1000 µg/ml) prepared in phosphate buffer was used as standard.

Result and Discussion:

% Yield of extracts: The extractive values of *Telosma pallida* with different solvents was found as shown in table 2.

Table: 2: % yield of extracts by successive extraction

Plant Part	Extract (% w/w)			
	n-Hexane	Ethyl Acetate	Methanol	Water
Leaf	5.32	2.24	2.64	3.96
Stem	2.16	1.36	5.19	9.94

Phytochemical screening results: The phytochemical screening results indicate that the Carbohydrates, Glycosides, Steroids Phenolic and Flavanoids are present in leaf and stem both. The alkaloid contents were present in stem part only. The saponin, amino acid and protein contents were not found in both extracts. The screening results are shown in table 3.

Total polyphenolic content determination: The methanolic extract of *Telosma pallid* was found to contain maximum amount of phenol than the other extracts (60.13±0.28 mg/g in leaf, and 77.10±0.28 mg/g in stem), while it was minimum in n- hexane extract. The estimated phenol contents are shown in table 4.

Table 3: Preliminary Phytochemical screening of *Telosma pallida*

Sr. No.	Test	***Telosma pallid***							
		Leaf				**Stem**			
		n-Hexane	**Ethyl Acetate**	**Methanol**	**Water**	**n-Hexane**	**Ethyl Acetate**	**Methanol**	**Water**
Alkaloids	Dragendroff's test	-	-	-	-	-	-	+	-
	Mayer's test	-	-	-	-	-	-	+	-
	Hager's test	-	-	-	-	-	-	+	-
	Wagner's test	-	-	-	-	-	-	+	-
Carbohydrates	Molish test	+	-	+	-	+	+	+	+
	Fehling's test	+	-	+	-	+	+	+	+
	Barfoed's test	+	-	+	-	+	+	+	+
Glycosides	Kellar-Killani test	+	-	+	+	-	-	+	-
	Borntrager's test	-	-	-	-	-	-	-	-
	Legal's test	-	-	-	-	-	-	-	-
Flavonoids	Shinoda test	-	-	+	-	-	-	+	-
Saponin	Foam test	-	-	-	-	-	-	-	-
	Haemolytic test	-	-	-	-	-	-	-	-
Sterols/ Steroids	Salkowaski reaction	+	-	-	-	-	+	+	-
Tannins and Phenolic compounds	5% Ferric chloride solution	-	+	+	+	+	+	+	+
	Lead acetate solution	-	+	+	+	+	+	+	+
	Dil. Potassium permanganate solution	-	+	+	+	+	+	+	+
	Bromine water	-	+	+	+	+	+	+	+
Amino acids	Ninhydrin test	-	-	-	-	-	-	-	-
Proteins	Biuret test	-	-	-	-	-	-	-	-

Table 4: Total polyphenolic content in plant extracts

Plant Part	**Total phenolic Content mg/g equivalent to Gallic Acid**			
	n-Hexane	**Ethyl Acetate**	**Methanol**	**Water**
Leaf	4.11±0.18	18.38±0.31	60.13±0.28	18.08±0.45
Stem	3.76±0.36	28.00±0.38	77.10±0.28	22.65±0.41

Antioxidant assay of extracts:

Results for DPPH Free Radical Scavenging Activity: Scavenging activity of various extracts and ascorbic acid was studied against DPPH radicals. All the samples were analysed in triplicate.

The DPPH assay of TPLH extract found to produce no response (% inhibition) even at concentration 4000µg/ml. The TPLEA, TPLM and TPLW extract at the level of 350µg/ml, 300µg/ml and 500µg/ml showed > 50% inhibition respectively, along with concentration dependent response. The methanolic extract of *Telosma pallida* leaves exhibited a maximum DPPH scavenging activity. IC50 value was 253.12±1.02 µg/ml followed by the ethyl acetate and water whose scavenging activities (IC50) were 323.34±0.49 and 481.40±0.70 µg/ml, respectively. The IC50 value of standard Ascorbic acid was also 36.14±0.26.

TPSH extract also produce similar results upto concentration 4000µg/ml as of TPLH extract i.e. no response was generated. The TPSEA, TPSM, TPSW extract, at the concentration level of 210µg/ml, 180µg/ml and 300µg/ml was found to exhibit > 50% inhibition, which was a concentration dependant response. The IC50 value of methanolic extract of stem part was, 158.43±0.48 µg/ml, followed by ethyl acetate and water, whose IC50 values were 202.45±0.42, and 279.89±0.92 µg/ml respectively.

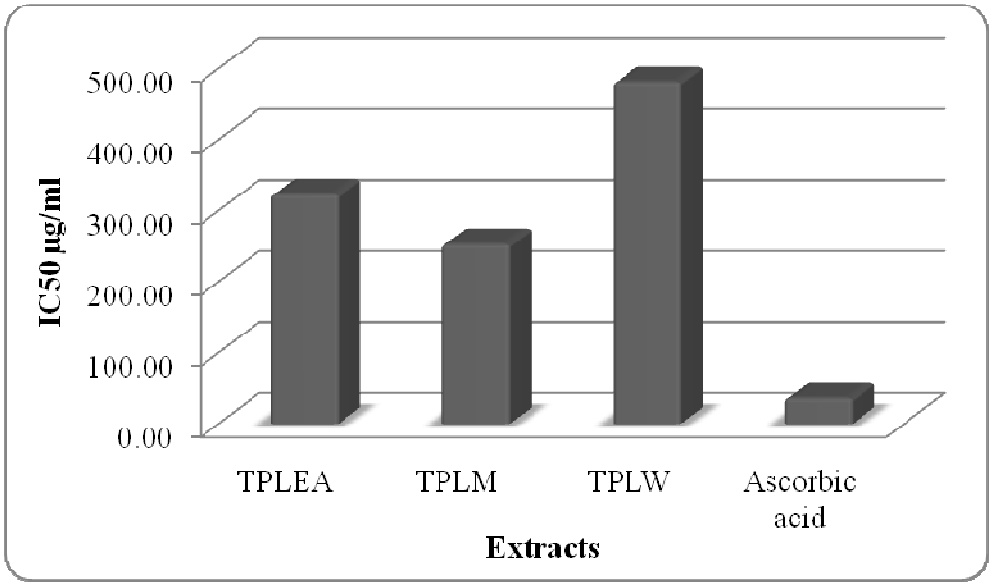

Figure 1: IC 50 of *Telosma pallida* leaves extracts

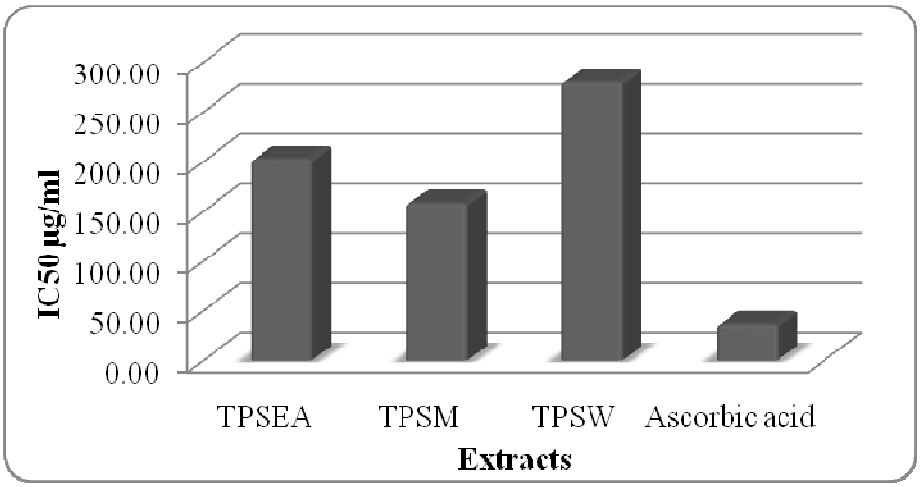

Figure 2: IC 50 of *Telosma pallida* stem extracts

Results for Reducing Power Activity: Result of reducing power assays for antioxidant activity have been recorded for *Telosma pallida* leaves extracts and compared with standard drug Butylated Hydroxy Anisole (BHA). TPLEA, TPLM and TPLW generated responses were quite good, and were maximum with methanolic extract. Although TPLH also produced somewhat reducing effect, yet it was not significant. The similar finding was also observed with different extracts of *Telosma pallida* stem. The result data were found as in table 5 and 6.

Table 5: Reducing power of *Telosma pallida* leaves extracts

S.No.	Conc (µg/ml)	BHA	TPLH	TPLEA	TPLM	TPLW
1	31.2	0.306±0.001	0.004±0.001	0.020±0.001	0.028±0.001	0.010±0.001
2	62.5	0.551±0.002	0.007±0.002	0.042±0.002	0.063±0.002	0.017±0.002
3	125	0.985±0.001	0.010±0.001	0.144±0.002	0.185±0.001	0.080±0.002
4	250	1.631±0.001	0.015±0.002	0.421±0.003	0.490±0.002	0.282±0.002
5	500	2.603±0.002	0.015±0.002	0.576±0.002	0.674±0.002	0.279±0.002

n=3, Data are given as Mean± S.D.

Table 6: Reducing power of *Telosma pallida stem* extracts

S.No.	Conc (µg/ml)	BHA	TPSH	TPSEA	TPSM	TPSW
1	31.2	0.306±0.001	0.004±0.001	0.042±0.002	0.049±0.001	0.027±0.002
2	62.5	0.551±0.002	0.008±0.002	0.085±0.002	0.089±0.002	0.059±0.002
3	125	0.985±0.001	0.012±0.001	0.178±0.002	0.226±0.002	0.178±0.002
4	250	1.631±0.001	0.016±0.001	0.377±0.002	0.547±0.002	0.467±0.002
5	500	2.603±0.002	0.017±0.002	0.524±0.002	0.805±0.003	0.633±0.002

n=3, Data are given as Mean± S.D.

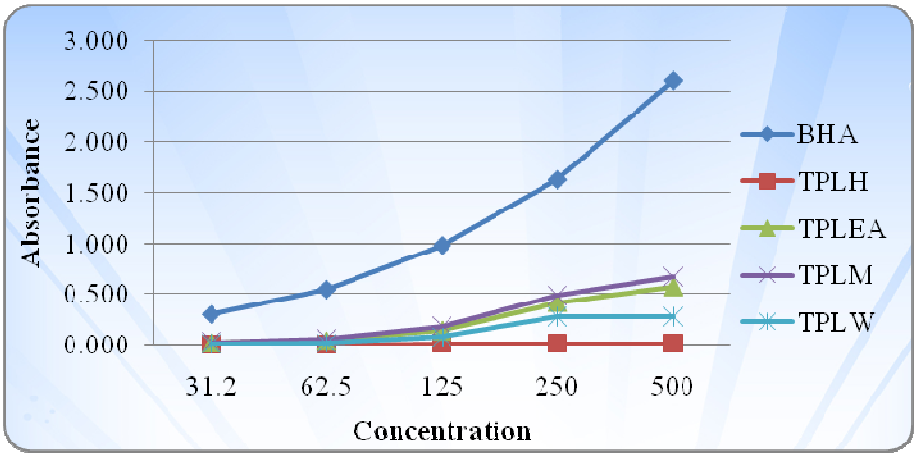

Figure 3. Reductive potential of different concentrations of *Telosma pallida* leaves extracts

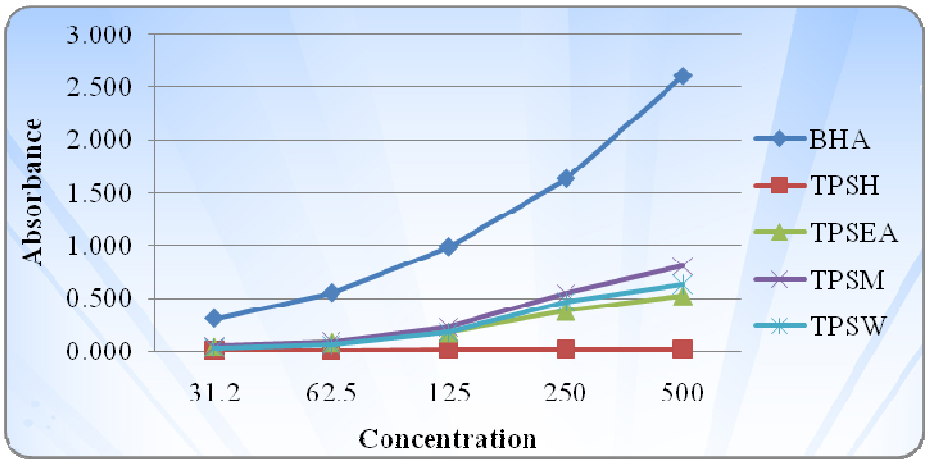

Figure 4. Reductive potential of different concentrations of *Telosma pallida* stem extracts

Conclusion:

The free radicals have been find as major cause for many diseases, and the use of antioxidant materials to resolve the diseased condition or to prevent prognosis of diseases have also been reported well. A number of synthetic as well as herbal origin elements have been used as antioxidants. Although focus is being made on plant derived materials, as they are less or non-toxic, easily available and of low cost.

In the same course of study *Telosma pallida* was evaluated for antioxidant capacity to establish its therapeutic values, as it is being used in many traditional system of medicine.

The leaf and stem part of the plant were extracted successively using different solvents. The extracts were subjected to phytochemical screening to determine presence of phytoconstituents and secondary metabolites. In terms of present total phenolic contents, the methanol extract of both the part was found abundant, as compared to other extracts.

The free radical scavenging activity against DPPH, and reducing power of the methanol extract was found best for methanol extract, than the others. At the same time the results also demonstrate that the IC50 values of different extracts may also be co-related with present phenolic content as it was in proportion to that of concentration of the extract and present phenolic moieties. Furthermore, the antioxidant property of the leaf extracts was

also better than the stem extracts. From the result data it may also be concluded that the higher total phenolic content of plants extracts resulted in higher antioxidant activity as similarly reported by earlier [26, 27, 28]. Although the results of the present work significantly establishing therapeutic value of *Telosma pallida*, Still; further preclinical as well as clinical work is required to establish proper mechanism of action at molecular level, and to prepare suitable dosage form.

Acknowledgement: The authors are thankful to School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan (India) for providing necessary support and facilities to carry out this research work.

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