Research Article

In Vitro Antioxidant Study of One Herbal Plant *Premna tomentosa*

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ABSTRACT

The present study deals with the antioxidant activity assays one plant extract, *Premna tomentosa,* a plant having many medicinal values, such as anti-inflammatory, hepatoprotective and antidoarrhial etc. It was observed that in all the three types of assays, namely, FRAP, DPPH and ABTS, the plant extract indicated good antioxidant activity. The antioxidant role of this plant could be one of the medicinal values as reported and claimed.

Keywords: *Premna tomentosa*, FRAP, DPPH, ABTS, Hepatoprotective, Anti-inflammatory.

INTRODUCTION

Premna tomentosa is a part of Sidha medicine, named "Pidangunari kudineer" which is used as potential hepato-protective formulation.[1] Ethno pharmacologically it is used to treat various ailments like diarrhea, for skin protection and in treatment of dropsy and sores. There are many research articles indicating its various medicinal roles such as anti inflamatory, immune modulatory, hepatoprotective and antimicrobial.[2, 3, 4] Devi *et al*, 1998 have shown the antioxidant activity of *Premna* on rats.[5] The present study deals with the in vitro antioxidant study of *Premna* by three different assays, namely, FRAP, DPPH and ABTS. The present study is in continuation of our reports on the antioxidant studies of various plants, Ayurvedic and Sidhha medicines.[6-18]

MATERIALS AND METHODS

The leaves of the plant, *Premna tomentosa* were collected locally and the ethanolic extract was processed for GC MS analysis, which was carried out by standard procedures. The plant was identified by Dr. S. Sankaranarayana, Asst. Professor and Head, Dept of Medicinal Botany, Govt. Sidha Medical College, Arumbakkam, Chennai-600106 with identification voucher no. GSMS/MB- Voucher Specimen No. 23/2017. The present study encompasses three different antioxidant assays, namely, ABTS, DPPH and FRAP. The FRAP assay was performed by Pulido *et al*, 2000), ABTS assay was done following the method of Re *et al*, (1999) and the DDPH assay was done by the method of Blios *et al*, (1958).[19-21]

FRAP Assay (Ferric Reducing/Oxidant Power)

The dried leave of *Premna tomentosa* were powdered the methanolic extract was taken for antioxidant study. Triplicates had been put for all the Processes.

Conc. = Concentration of the sample
OD = OD of the sample
Linearity (y) = mx + c
M = Slope
C = The point x crosses y axis
X = OD – c value / m value
mM Fe/mg = X value / concentration x 1000
Mean = Average of mM Fe/mg
STDEV = Standard Deviation for mM Fe/mg.

ABTS Assay

ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM respectively. These two solutions were mixed and the mixture allowed to stand in the dark at room temperature for 16 h before use in order to produce ABTS radical (ABTS•+). This was incubated with the Methanolic extract of *Premna tomentosa* leaves, at different concentrations and the reaction mixture which was blue became colourless due to the presence of antioxidants present in the medicine. This was change in colour was estimated spectrophotometrically.

DPPH Assay (1, 1-diphenyl-2-picrylhydrazyl)

The sample was dissolved in Methanol in 1mg/ml concentration and used as stock. From the stock, various concentrations (100, 200, 300, 400mg) were taken for further analysis.

Respective solvents were taken as negative control.

Conc. = Concentration of the sample
OD = OD of the sample
Neg. Control = The Solvent
Activity = Neg. Control – OD / Neg. Control

% of Activity = Activity/100
IC50 = 50 – c value / m value
IC50/ml = IC50/3 (3 ml of DPPH for the assay. To find the activity in 1 ml, the value had been divided by 3).

RESULTS AND DISCUSSION

Tables 1, 2 and 3 and Figures 1, 2 and 3 represent the antioxidant profiles of three assays namely FRAP, ABTS and DPPH, respectively for Methanolic extract of *Premna tomentosa*.

Table 1: Shows the FRAP results of *Premna tomentosa* extract as compared to Ascorbic Acid

Control	0.416							
	Ascorbic Acid (Conc.)	**Conc.**	**Single**	**Doublet**	**Triplet**	**Mean**	**SD**	**SEM**
		5	0.469	0.454	0.472	0.465	.00964	0.00556
		10	0.483	0.491	0.487	0.487	0.004	0.00230
		20	0.542	0.553	0.538	0.544	0.0077	0.00448
		50	0.707	0.721	0.715	0.714	0.0072	0.00405
		100	0.992	0.998	0.987	0.992	0.0055	0.00318
		200	1.570	1.489	1.506	1.526	0.0427	0.02466
Sample	*Premna* extract							
		5	0.471	0.468	0.465	0.468	.0017	0.001732
		10	0.488	0.484	0.493	0.488	0.0026	0.002603
		20	0.518	0.525	0.531	0.524	0.0037	0.003756
		50	0.682	0.673	0.681	0.678	0.0028	0.002848
		100	0.876	0.881	0.869	0.875	0.0034	0.003480
		200	1.239	1.208	1.225	1.224	0.0089	0.008963
		400	1.868	1.854	1.880	1.861	0.0040	0.004041

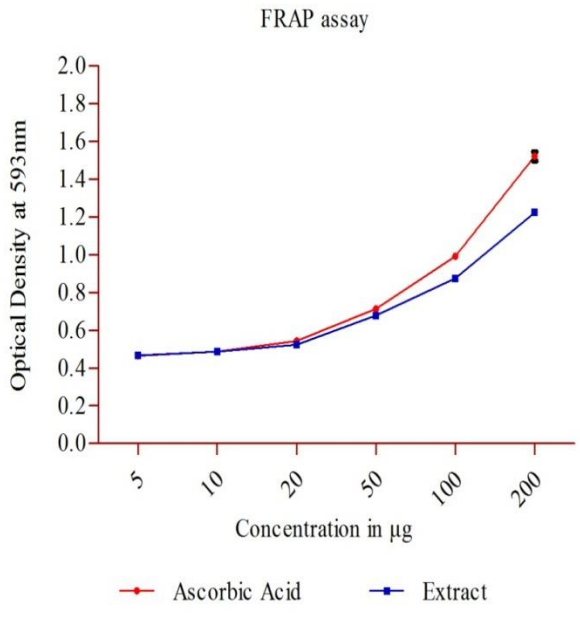

Figure 1: indicates the FRAP assay graph for *Premna tomentosa*

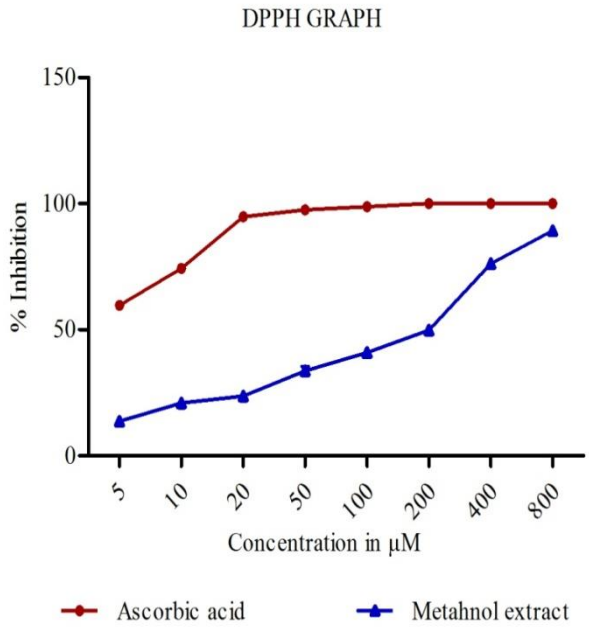

Figure 2: indicates the DDPH assay graph of *Premna tomentosa*.

Table 2: Shows the DPPH results of *Premna tomentosa* methanolic extract as compared to Ascorbic Acid

Standard (Ascorbic Acid in µg)	Conc.	Single	Doublet	Triplet	Average	% Inhibition	Singlet	Doublet	Triplet	Mean	S.D	SEM
	5	0.348	0.354	0.362			58.290	67.570	56.610	57.4910	0.8418	0.48603
	10	0.211	0.210	0.223			74.710	74.830	73.272	74.2708	0.8670	0.50059
	20	0.041	0.045	0.044			95.086	94.606	94.726	94.8062	0.2495	0.14400
	50	0.022	0.010	0.021			97.963	97.722	97.483	97.5220	0.1830	0.10570
	100	0.001	0.009	0.011			98.800	98.921	98.681	98.8014	0.1198	0.06919
	200	0.002	0.001	0.003			99.760	99.880	99.640			
	Control	0.837	0.825	0.841	0.83433							
Sample												
	5	0.719	0.716	0.726			13.823	14.182	12.984	13.6636	0.6150	0.35510
	10	0.665	0.659	0.654			20.295	21.014	21.614	20.9748	0.6601	0.38118
	20	0.640	0.637	0.633			23.292	23.651	24.131	23.6915	0.4269	0.24301
	50	0.545	0.581	0.534			34.678	30.363	35.996	33.6795	2.9464	1.70113
	100	0.496	0.479	0.502			40.151	42.588	39.832	40.9908	1.4299	0.82556
	200	0.420	0.416	0.419			49.660	50.139	49.780	49.8601	0.2249	0.14404
	400	0.192	0.199	0.204			76.987	76.148	75.549	76.2285	0.7224	0.41711
	800	0.091	0.079	0.099			89.093	90.531	88.134	89.2528	1.2066	0.69658

Table 3: Shows the ABTS scavenging results of *Premna tomentosa* methanolic extract as compared to Ascorbic Acid

Standard (Ascorbic Acid in µg)	Conc.	Single	Doublet	Triplet	Average	% Inhibition	Singlet	Doublet	Triplet	Mean	S.D	SEM
	5	0.292	0.288	0.311			63.831	64.327	61.478	63.2122	1.522	0.878769
	10	0.023	0.021	0.022			97.151	97.398	97.274	97.2749	0.1238	0.071513
	20	0.009	0.008	0.012			98.885	99.009	98.513	98.8026	0.2578	0.148867
	50	0.003	0.001	0.002			99.628	99.876	99.752	99.7522	0.1238	0.071513
	100	0.001	0.001	0.001			98.876	100.000	99.876	99.9174	0.7151	0.041288
	Control	0.806	0.809	0.807	0.80733							
Sample												
	5	0.653	0.677	0.638			19.116	16.143	20.9744	18.744	2.4367	1.4068
	10	0.630	0.629	0.612			21.965	22.089	19.2402	21.0082	1.6102	0.9296
	20	0.563	0.542	0.579			30.264	32.865	28.2824	30.4706	2.2984	1.3270
	50	0.334	0.341	0.353			58.626	57.702	26.2758	57.5557	1.1902	0.6871
	100	0.066	0.074	0.052			91.824	90.834	93.5594	92.0726	2.3792	0.7963
	200	0.023	0.029	0.021			97.151	96.407	97.988	96.9859	0.5156	0.2977
	400	0.006	0.005	0.008			99.258	99.380	99.0090	99.2155	0.1892	0.1092
	800	0.001	0.001	0.002			99.876	99.876	99.7522	99.8348	0.0751	0.0428

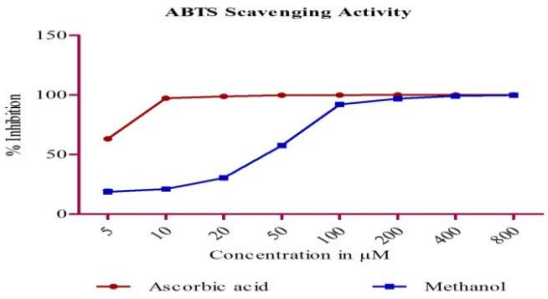

Figure 3: indicates the FRAP scavenging activity of *Premna tomentosa*.

It was observed that results of FRAP study (Table 1, Figure 1) indicated that *Premna tomentosa* extracts show a similar antioxidant activity when compared to ascorbic acid up to a concentration of 50 µg whereas at 100 and 200 µg concentration, ascorbic acid was showing higher activity as compared to *Premna*. The results clearly indicate that *Premna* indicated very good antioixidant activity in terms of FRAP study.

The DDPH antioxidant study (Table 2 Figure 2) indicated that the *Premna* does show this activity but when compared to Ascorbic acid the activity was low.

The ABTS antioxidant activity of *Premna* (Table 3 Figure 3) shows almost similar results as that of Ascorbic acid at higher concentrations of 100, 200, 400 and 800 µg, whereas its activity was quite low at lower concentrations. Pawar *et al,* 2016, have reported the antioxidant and gastro proetective potential of *Premna*.[22] Rajalakshmi *et al*, 2016, have reported the antioxidant activities of *Premna* using ethyl acetate extracts.[23]

The above results clearly indicated that *Premna tomentosa* plant has good antioxidant properties which could be one of the factors for its medicinal value.

CONCLUSION

The above study clearly indicates that *Premna tomentosa* has a powerful antioxidant activity which could be one of the main reasons of its medicinal value.

REFERENCES

1. Thanigavelan V, Kaliyamurthi V, Pitchiah Kumar M, Elansekaran S, Victor Rajamanickam G. An overview of the Herbs in a Siddha Polyherbal decoction-Pidangunaari Kudineer indicated for Hepatomegaly. Journal of Applied Pharmaceutical Science, 2 (7), 2012, 8-14.
2. Alam M, Joy S, Susan T, Usman Ali S. Antiinflammatory activity of *Premna tomentosa* Willd. in albino rats. Ancient Sciences of Life, 13, 1993, 85-188.
3. Devi, KP, Sairam M, Sreepriya M, Ilavazhagan G, Devaki T. Immunomodulatory effects of *Premna tomentosa* extract against Cr (VI) induced toxicity in splenic lymphocytes – an *in-vitro* study. Biomedicine and Pharmacotherapy, 57, 2003, 105-108.
4. Devi KP, Sreepriya M, Balakrishna K, Devaki T. Protective effect of *Premna tomentosa* extract (*Verbenaceae*) on membrane-bound phosphatases and inorganic cations transport in acetaminophen-induced hepatotoxicity rats. Journal of Ethnopharmacology, 93, 2004, 371-375.
5. Devi KP, Anandan R, Devaki T, Apparanantham T, Balakrishna K. Effect of *Premna tomentosa* on rat liver antioxidant defense system in acetaminophen intoxicated rats. Biomed Res, 19, 1998, 339–342.
6. Rao MRK, Saikumar P, Prabhu K, Arul Amutha Elizabeth, Sumathi, Lakshmi Sundaram, Sruthi Dinakar, Kumari Sangita Singh, Ayub Alam. The GC-MS and Antioxidant Study of an Ayurvedic Medicine Ayaskriti. IJPSRR, 42(1), 2017, 15-19.
7. Nirupa, Mudiganti Ram Krishna Rao, Prabhu K, Kaliaselvi VS, Kumaran D, Sivaram E, Sruthi Dinakar. Antioxidant study of one Ayurvedic medicine, "Sukumara Kashayam". IJPSRR, 42(1), 2017, 35-41.
8. Sivasankar Reddy Konda, Mudiganti Ram Krishna Rao, Minu Priya, Prabbu K, Kalaivani VS, Kumaran D, Ayub Alam, Kumari Sangeeta Singh, Lakshmi Sundaram. "The antioxidant study of An Ayurvedic medicine, Balarishtam". IJPSRR, 42(1), 2017, 29-34.
9. Mudiganti Ram Krishna Rao, Bidita Chattrerjee. Preliminary Phytochemical, antioxidant and antimicrobial activities of different extracts of *Cassia tora* and *Trichodesma indicum.* International Journal of Pharmacy and Technology, 8(2), 2016, 12578-12597
10. Lenin, Mudiganti Ram Krishna Rao, Prabhu K, Bindu, Amutha Elizabeth AR, Sruthi Dinakar. "The Study of antioxidant activities of an Ayurvedic medicine Ayaskriti". Der Pharmacia Lettre, 8 (6), 2016, 203-211
11. Sivakumaran G, Mudiganti Ram Krishna Rao, Prabhu K, Kalaiselvi VS, Sumathi Jones, Johnson WM, J Antony. Preliminary GC-MS Anlaysis and Antioxidant Study of One Ayurvedic Medicine "Manasa Mitra Vatakam". Int. J. Pharm. Sci. Rev. Res., 37(1), 2016, 190-199
12. Mudiganti Ram Krishna Rao, Aparna Ravi, Shridhar Narayanan, Prabhu K, Kalaiselvi VS, Shruthi Dinakar, Guru Rajan, Kotteeswaran N. Antioxidant Study and GC MS Analysis of an Ayurvedic Medicine 'Talisapatradi Choornam'. Int. J. Pharm. Sci. Rev. Res., 36(1), 2016, 158-166.
13. Aparna Ravi, Hassan Mohammad, Rao MRK, Prabhu K, Hari Babu, Shridhar Narayanan, Guru Rajan, Sanjay Singh. "Antibacterial, antioxidant activity and GC MS analysis of a sidha medicine "Neerkovai tablets". International Journal of Phamacy and Technology. IJPT, 7 (3), 2015, 10091-10112.
14. Mudiganti Ram Krishna Rao, Hassan Mohammad, Sridhar Narayanan, Prabhu K, Kalaiselvi VS, Aparna Ravi, Hari Babu, Guru Rajan, Suganya S. Antioxidant assay and GC MS analysis of one Sidha medicine Swasa Kudori tablets. Int. J. Pharm. Sci. Rev. Res., 37(1), 2016, 19-25.
15. Mudiganti Ram Krishna Rao, Selva Kumar S. Preliminary phytochemical analysis And antioxidant properties of *Gynandropsis pentaphyla*. Der Pharmacia Lettre, 7(12), 2015, 20-24.
16. Sadhanandham S, Narayanan G, Mudiganti Ram Krishna Rao, Prabhu K, Sumathi Jones, Aparna Ravi, Shruthi Dinakar. GC MS Analysis and Antioxidant studies of An Ayuredic drug, Partharishtam. Int. J. Pharm. Sci. Rev. Res., 34(2), 2015, 273-281.
17. Chandrasekar T, Mudiganti Ram Krishna Rao, Vijaya Kumar R, Prabhu K, Nandha Kumar S, Divya D. GC-MS analysis, antimicrobial, antioxidant activity of an Ayurvedic medicine, Nimbapatradi Choornam. Journal of Chemical and Pharmaceutical Research, , 7(8), 2015124-136.
18. Mudiganti Ram Krishna Rao, Sanitha Philip, Muttevi Hyagreva Kumar, Saranya Y, Divya D, Prabhu K. GC-MS analysis, antimicrobial, antioxidant activity of an Ayurvedic medicine, *Salmali Niryas.* Journal of Chemical and Pharmaceutical Research, 7(7), 2015, 131-139.
19. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 29, 1958, 1199-1200.
20. Pulido R, Bravo L, Sauro-Calixo F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agri Food Chem, 48, 2000, 3396-3402.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice- -Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med, 26, 1999, 1231-1237
22. Pawar SD, Kusu Susan Cyriac, Meena N, Shirolkar AR, Gaidhani SN, Veluchamy G . Evaluation of Antioxidant and Hepatoprotective Potential of *Premna Tomentosa* L. In Albino Wistar Rats. RJPBCS, 7(4), 2016, 2990-2298.
23. Rajalakshmi P, Pugalenthi M and Vadivel V. *In vitro* and inhibitory activity of pathogens on leaves of *Argemone mexicana* L. and *Premna tomentosa* L. International Journal of Herbal Medicine 4(5), 2016, 84-90.

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