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Studying invitro antioxidant effect of different extract of *Premna tomentosa (Linn)* aerial parts

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ABSTRACT

The genus Premna (Verbenaceae) comprises a group of more than 200 different trees, distributed in tropical and subtropical areas of the world. *P. tomentosa* (Verbanaceae) is a well-known medicinal plant used extensively for the treatment of various ailments. In the present study, the entire plant of different concentrates of aerial parts of *Premna tomentosa* was evaluated for its *in-vitro* anti-oxidant potential by superoxide scavenging activity, hydroxyl radical scavenging taking quercetin and ascorbate as the standard correspondingly. The results of antioxidant activity superoxide radical activity and hydroxyl potential were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. An IC_{50} value was recorded; methanolic concentrate of *Premna tomentosa* is more effective in superoxide radical activity, hydroxyl radical than that of EA and petroleum ether concentrate. The methanolic concentrate of *Premna tomentosa* and standard exhibited radical scavenging activity possessing IC_{50} values 225μg/mL and 60μg/mL (Superoxide Scavenging Activity), 288μg/mL and 65μg/mL (hydroxyl radical activity) correspondingly. All the above invitro studies clearly indicate that the methanolic concentrate of *Premna tomentosa* has better free radical scavenging activity. These *invitro* estimations point out that this methanolic concentrate of *Premna tomentosa* is a paramount source of expected antioxidant, which may be supportive in preventing the improvement of a variety of free radical induced diseases.

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INTRODUCTION

Free radicals induce lipid peroxidation and provokes damage in cell membrane. Incorporation of antioxidants in fats and oils or foods can prevent the deterioration of lipids in foods. Some of these compounds which retard lipid peroxidation are synthetic antioxidants whereas others occur as natural dietary constituents (Duh, 1999). The human body is in constant battle to keep from free radicals. The first line of defence is the preventive antioxidants which quench the free radicals generated in the body. An early stage Augmentation of antioxidant status should either prevent or greatly curtail tissue injury (Lobo *et al.*, 2010). Due to the adverse effects

of synthetic antioxidants on human health, restrictions have been imposed on their usage. Natural antioxidants have been greatly intensified as synthetic antioxidants exert carcinogenic effect (Bajpai *et al.*, 2014). Plants are enriched with bioactive ingredients which have most of the free radical scavenging and therapeutic function.

Premna tomentosa (Verbanaceae) is a glowing recognized herb used broadly for the cure of different disorders. *Premna tomentosa* was used for the cure the different disorders in India (Kubitzki and Kadereit, 2005). *Premna tomentosa* bark concentrate was used for treatment of hepatic disorders. *Premna tomentosa* leaves concentrates were used as diuretic (Krishnamurthi, 1969), *Premna tomentosa* was used for the treatment of lipid lowering (Devi *et al.*, 2004), antioxidant (Devi *et al.*, 1998), immune modulatory activities (Devi *et al.*, 2003) and mitochondrial dysfunction properties against acetaminophen induced rats (Devi *et al.*, 2005). Flavonoids, triterpenoids and steroids were isolated from *Premna* tomentosa (Chin *et al.*, 2006; Alam *et al.*, 1993). Still, no literature are available on the antioxidant activity of *Premna tomentosa* aerial parts. Thus, the present study to assess antioxidant activities of *Premna tomentosa aerial* parts.

MATERIALS AND METHODS

Gathering & Identification of Plant

The aerial parts of *Premna tomentosa* (family Verbenaceae) were gathered form Chenkottai, Tirunelveli District of Tamilnadu India. Plant recognition was made from Botanical investigation of India, Palayamkottai The *Premna tomentosa* were desiccated under shadowy, segregate, crushed through grinder (Sivakrishnan and KottaiMuthu, 2014).

Preparation of Concentrates

The pulverized materials were packed in muslin cloth and concentrated with pet.ether, ethyl acetate and methanol as solvents respectively according to the increasing order of polarity (Shajiselvin *et al.*, 2010) through hot constant percolation method in Soxhlet equipment (Harborne, 1984)for twenty-four hours. The concentrates were concentrated through rotational evaporator and subjected to solidify drying till dry powder was acquired by using lyophilizer. (SatheeshKumar *et al.*, 2010; Alagumanivasagam *et al.*, 2012).

Assessment of Antioxidant potential through invitro methods

The variety of concentrate of *Premna tomentosa* were used as assessment of antioxidant activity by Winterbourn *et al.* (1975) method described for Superoxide radical (O_2^-) assay and Kunchandy and Rao (1990) method was adopted to determine the OH radical assay.

RESULTS AND DISCUSSION

Superoxide anion activity

Super-oxides might be produced in huge amounts by a variety of biological processes. It is recognized to be more harmful to cellular components as creator of the generally ROS, contributing to tissue injure and various disorders (Halliwell and Gutteridge, 1999). Superoxide radical potential of PE concentrates of *Premna tomentosa* appeared in Table 1. The more activity of PE concentrates of *Premna tomentosa* and quercetin at 1000μg/mL were recorded to be 57.68% & 99.12% correspondingly. The IC_{50} of PE concentrates of *Premna tomentosa* & quercetin were recorded as 762μg/mL and 60μg/mL correspondingly.

Superoxide radical potential of EA concentrates of *Premna tomentosa* appeared in Table 2. The more activity of EA concentrates of *Premna tomentosa* and standard at 1000 μg/mL was found to be 75.66% and 99.12% correspondingly. IC_{50} EA concentrates of *Premna tomentosa and* standard was recorded as 375μg/mL and 60μg/mL correspondingly.

Superoxide radical potential of Methanol concentrates of *Premna tomentosa* appeared in Table 3. The more activity of Methanol concentrates of *Premna tomentosa and* standard at 1000 μg/mL was found to be 78.68% & 99.12% correspondingly. The IC_{50} EA concentrates of *Premna t omentosa and* standard was recorded as 225μg/mL and 60μg/mL correspondingly.

IC_{50} values and percentage scavenging capacity, it was recorded that methanolic concentrates of *Premna tomentosa* had better activity in superoxide radical when compared EA and PE concentrates.

Hydroxyl radical scavenging activity

Hydroxyl radical activity was determined by generating the OH radicals using ascorbic acid. OH radicals were produced by the oxidation reaction with the Dimethylsulfoxide to give in HCHO, which provides an appropriate method to identify OH radicals by added with Nash reagent (Pavithra and Vadivukkarasi, 2015).

Hydroxyl radical activity of PE concentrate of *Premna tomentosa appeared* in Table 4. The hydroxyl radical activity of PE concentrate of *Premna tomentosa and* ascorbic acid *at* 1000 μg/mL was found to be 49.55% & 96.50 % correspondingly. The IC_{50} of PE concentrate of *Premna*

Table 1: Activity of PE concentrate of *Premna tomentosa* on Superoxide radical method

S.No	Concentrate (μg/mL)	% of activity(±SEM)*	
		PE concentrate	Quercetin
1	125	29.56±0.024	72.76±0.012
2	250	42.62±0.038	90.29±0.014
3	500	48.46±0.052	96.89±0.010
4	1000	57.68±0.069	99.12±0.018
		IC_{50} =762 μg/mL	IC_{50} = 60 μg/mL

* Every value was articulated as mean ± SEM for 3 experimentation

Table 2: Activity of EA concentrate of *Premna tomentosa* on superoxide radical method

S.No	Concentrate (μg/mL)	% of activity(±SEM)*	
		EA concentrate	Quercetin
1	125	23.54±0.024	72.76±0.012
2	250	37.25±0.045	90.29±0.014
3	500	56.80±0.052	96.89±0.010
4	1000	75.66±0.034	99.12±0.018
		IC_{50} = 375μg/mL	IC_{50} = 60 μg/mL

* Every value was articulated as mean ± SEM for 3 experimentation

Table 3: Activity of methanol concentrate of *Premna tomentosa* on Superoxide radical method

S.No	Concentrate (μg/mL)	% of activity(±SEM)*	
		Methanolic concentrate	Quercetin
1	125	40.34±0.045	72.76±0.012
2	250	54.42±0.056	90.29±0.014
3	500	69.44±0.031	96.89±0.010
4	1000	78.68±0.022	99.12±0.018
		IC_{50} =225 μg/mL	IC_{50} = 60 μg/mL

* Every value was articulated as mean ± SEM for 3 experimentation

Table 4: Activity of PE concentrate of *Premna tomentosa* hydroxyl radical method

S.No	Concentrate (μg/mL)	% of activity(±SEM)*	
		PE concentrate	Ascorbate
1	125	20.22±0.064	70.34±0.38
2	250	29.22±0.034	85.54±0.15
3	500	38.68±0.022	90.23±0.24
4	1000	49.55±0.064	96.50±0.16
		IC_{50} = 1038 μg/mL	IC_{50} = 65 μg/mL

* Every value was articulated as mean ± SEM for 3 experimentation

Table 5: Activity of EA concentrate of *Premna tomentosa* hydroxyl radical method

S.No	Concentrate (μg/mL)	% of activity($\pm$SEM)* EA concentrate	(Ascorbate)
1	125	20.32$\pm$0.045	70.34$\pm$0.38
2	250	44.89$\pm$0.042	85.54$\pm$0.15
3	500	53.78$\pm$0.024	90.23$\pm$0.24
4	1000	62.88$\pm$0.016	96.50$\pm$0.16
		IC_{50} =464 μg/mL	IC_{50} = 65 μg/mL

* Every value was articulated as mean $\pm$ SEM for 3 experimentation

Table 6: Activity of Methanolic concentrate of *Premna tomentosa* hydroxyl radical method

S.No	Concentrate (μg/mL)	% of activity($\pm$SEM)* Methanolic concentrate	Ascorbate
1	125	43.66$\pm$0.032	70.34$\pm$0.38
2	250	49.28$\pm$0.046	85.54$\pm$0.15
3	500	67.46$\pm$0.048	90.23$\pm$0.24
4	1000	70.34$\pm$0.056	96.50$\pm$0.16
		IC_{50} = 288 μg/mL	IC_{50} = 65 μg/mL

* Every value was articulated as mean $\pm$ SEM for 3 experimentation

t omentosa and ascorbic acid were found to be 1038μg/mL and 65μg/mL correspondingly.

Hydroxyl radical is the most ROS and causes severe injure to adjacent biomolecule. Hydroxyl radical activity of EA concentrate of *Premna tomentosa* and ascorbic acid were showed in Table 5. The hydroxyl radical activity of EA concentrate of *Premna tomentosa and* ascorbic acid *at* 1000 μg/mL was found to be 62.88% & 96.50 % correspondingly. The IC_{50} of EA concentrate of *Premna tomentosa and* ascorbic acid were found to be 464μg/mL and 65μg/mL correspondingly.

Hydroxyl radical scavenging potency of methanolic concentrate of *Premna tomentosa* and ascorbic acid were showed in Table 6. The hydroxyl radical activity of methanol concentrate of *Premna tomentosa and* ascorbic acid *at* 1000 μg/mL was found to be 70.34% & 96.50 % correspondingly. The IC_{50} of methanol concentrate of *Premna tomentosa* and ascorbic acid were found to be 288μg/mL and 65μg/mL correspondingly.

Based on the above report the methanolic concentrate of *Premna tomentosa* (IC_{50} = 288 μg/ml) was found more effective than that of PE(IC_{50} = 1038 μg/ml) and EA concentrates (IC_{50} = 464 μg/mL). The methanolic concentrate of *Premna tomentosa exhibited* better hydroxyl radical scavenging activity compared to other concentrates. The scavenging of the hydroxyl radicals possibly may be presence of hydrogen donating ability flavonoids from methanolic concentrates.

CONCLUSION

From this investigation, it is concluded that aerial parts of methanolic concentrate of *Premna tomentosa* is established to have valuable potential than PE and EA concentrates. Thus, present data suggest that methanolic concentrate of *Premna tomentosa* is a remarkable resource of natural antioxidant, which might be helpful in preventing the improvement of a variety of free radical induced diseases.

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