Somi. Tirupatirao. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2014; 5(5): 456-460.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

COMPARATIVE STUDIES ON THE *IN VITRO* ANTIOXIDANT PROPERTIES OF DIFFERENT LEAFY EXTRACTS FROM TINOSPORA AND TULASI

Somi. Tirupatirao*[1], D.Durga Rao[2], V.Sireesha[2], M.Rajya Lakshmi[2], G.Kasimary[2], K.Dhana lakshmi[2]

[1]Department of Pharmaceutical Biotechnology, [2]Department of Pharmaceutics, Vagdevi College of Pharmacy, Gurazala, Guntur Dt., Andhra Pradesh, India-522415.

ABSTRACT

The present study was undertaken to find the comparative studies on the *in vitro* antioxidant value of extracts from Tinospora and Tulasi leafs. Antioxidants have been reported to prevent oxidative damage caused by free radical. In this study, we assessed antioxidative properties of the Ethonolic, acetonic, water extracts of Tulasi, tinospora leafs. Antioxidant properties by different in-vitro experiments including DPPH radical assay, Hydrogen Peroxide Free Radical Scavenging**.** The present study revealed that tulasi extracts shows better anti-oxidant properties than Tinospora extracts**.** The results obtained in the present study indicate that leaves of tulasi plant materials have potent.

Key Words:

INTRODUCTION

5% or more than of inhaled oxygen is converted into reactive oxygen species (ROS) such as O_2, H_2O_2, OH. Oxygen free radical induces damage to bio membranes due to peroxidation and also to DNA which leads to cell damage that causes number of diseases (Vivek Kumar G and Surendra Kumar S, 2006). An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Synthetic antioxidant like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to ameliorate oxidative damages but they have been restricted due to their carcinogenic and harmful effect on the lungs and liver (Gokhan Z *et al*., 2011). Therefore, investigations of antioxidants are focused on naturally occurring Tinospora cordifolia an indigenous plant used in Ayurveda medicine belonging to the family Menispermaceae is a large spreading glabrous perennial deciduous twiner grown in India. Earlier studies reveal the antitumor, osteoporotic chemopreventive, hepatoprotective, Antihperglycaemic, immuno modulatory and antiallergic properties of Tinospora (Kapur P *et al*., 2008; Panchabhai TS *et al*., 2008; Bishayi B *et al*., ; Umamaheshwari S and Mainzen Prince PS, 2007).

Tulasi, the "Queen of Herbs", is the most sacred herb of India. Ocimum belongs to the Lamiaceae family, which has close to 252 genera and 6700 species (Mabberley DJ, 1997). There are three important varieties of Tulasi namely, Rama Tulasi (*Ocimum sanctum*), Krishna Tulasi (*Ocimum tenuiflorum*) and Vana Tulasi (*Ocimum gratissimum*). Basil is used as medicinal herbin medical treatments such as for headaches, coughs, diarrhea, worms, and kidney malfunctions. Basil essential oil has been utilized extensively in the food industry as a Flavoring agent, and in perfumery and medical industries (Simon JE *et al*., 1999). Numerous studies reported various

Corresponding Author

Somi. Tirupatirao
Email: tiru.somi@gmail.com

effects of *Ocimum* sp., including anti-inflammatory, antioxidative, chemopreventive, blood-sugar lowering, nervous system stimulation and radiation protection have been reported. In this screening project, comparative studies on the *in vitro* antioxidant properties of different leafy extracts from Tinospora and Tulasi were investigated to assess their antioxidant properties. In different antioxidant property determination assays include DPPH radical scavenging Method, Hydrogen Peroxide Free Radical Scavenging Activity. Total antioxidant activity, Total Phenol test and Total Flavonoids test were studied in this report. Ascorbic acid used as antioxidant standard compound respectively

MATERIAL AND METHOD

Plant Material

The plants Tulasi, Tinospora leafs were collected from Botanical garden of Vagdevi College of pharmacy of Gurazala, Guntur (dist.).

Preparation of crude plant extract

The fresh whole leafs collected from the Botanical garden of Vagdevi College of pharmacy of Gurazala, Guntur (dist.) and was shade dried at room temperature. The dried plant materials were powdered using a grinder. The extraction was done at room temperature. About 50 g of dried, ground plant materials were soaked in solvents separately (Ethanol, Water, and Acetone) for 5-7 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40^{C} and stored at 4^{C} for further use. The stock solution of crude extracts (mg/ml) was prepared by dissolving a known amount of dry extract in solvent. The working solutions (100, 200,300,400,500 and 1000µg/ml) of the extracts were prepared from the stock solution using suitable dilution

ANTI-OXIDANT ACTIVITY

DPPH radical scavenging activity

4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol. It was protected from light by covering the test tubes with aluminum foil. 150 ml DPPH Solution was added to 3ml methanol and absorbance was taken immediately at 517nmfor control reading. 50 ml of various concentrations of plant extracts as well as Standard compound (Ascorbic acid) were taken and the volume was made uniformly to150 ml using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 ml DPPH was added. Absorbance was taken after 15 min. at517nm using methanol as blank on UV-visible spectrometer (Ruch RJ *et al*., 1898).

The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

Hydrogen Peroxide Free Radical Scavenging Activity

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method of Ruch et al. (1989) Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following Equation.

$S\% = [\ (A \text{ control} - A \text{ sample}) / A \text{ control}\ ] \times 100$

Where A control= absorbance of the blank control (containing all reagents except the extract Solution) and A sample= absorbance of the test sample.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Table-1 shows the result of the DPPH free radical scavenging activity of the Ethanol extracts of Tinospora, tulasi shows the highest DPPH scavenging activity with 72±0.40 at 1000µg/ml of the crude extracts respectively. In our experiment ascorbic acid which was taken as standard (Elina M *et al*., 2014). Table-2 shows the result of the DPPH free radical scavenging activity of the Water extracts of Tinospora, tulasi shows the highest DPPH scavenging activity with 50±0.40 at 800µg/ml of the crude extracts respectively (Ayoola GA *et al*., 2008).

Hydrogen peroxide scavenging activity assay

As shown in Table 4.5, Figure 4, 5, Tinospora and tulasi leaf demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H2O2 can probably react with Fe2+, and possibly Cu2+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the

amount of hydrogen peroxide that is allowed to accumulate. The decomposition of H2O2 by tinospora, tulasi may at least partly result from its antioxidant and free radical Scavenging activity.

Table-4 shows the result of the Hydrogen Peroxide Radical Scavenging Activity of the Ethanol extracts of Tinospora, shows the Hydrogen Peroxide Radical Scavenging with 54±0.40 at 1000µg/ml of the crude extracts respectively. In our experiment ascorbic acid which was taken as standard (Patel Rajesh M and Patel N, 2011). Table-5 shows the result of the Hydrogen Peroxide Radical Scavenging activity of the Water extracts of Tulasi shows the highest Hydrogen Peroxide Radical Scavenging with 71±0.61 at 500µg/ml of the crude extracts respectively. These results are in accordance with the reported values Bandyopadhyay *et al.* also observed. Tulasi leaf aqueous extract may be useful as a protective antioxidant supplement with promising antioxidant potential (Ayoola GA *et al*., 2008).

Table 1. DPPH free radical scavenging activity of ascorbic acid and Ethanol extract of tinospora

Conc of extract(µg/ml)	Antioxidant activity (%)		
	Ascorbic acid	Tinospora	Tulasi
12.5	26±2.46		
25	40±1.61		
50	64±1.91		
100	80±2.46	28±0.40	24±0.60
200		36±0.60	30±1.23
300		42±1.30	38±0.92
400		48±0.80	54±0.78
500		52±1.25	60±0.80
1000		64±1.34	72±0.40

Table 2. DPPH free radical scavenging activity of ascorbic acid and Water extract of Tinospora

Conc of extract(µg/ml)	Antioxidant activity (%)		
	Ascorbic acid	Tinospora	Tulasi
12.5	26±2.46		
25	40±1.61		
50	64±1.91		
100	80±2.46	3±0.42	24±1.30
200		4±0.51	32±1.25
300		6±0.51	40±0.80
400		7±0.84	48±0.72
500		10±1.23	50±0.40
1000		11±0.93	50±0.92

Table 3. DPPH free radical scavenging activity of ascorbic acid and Acetone extract of Tinospora

Conc of extract(µg/ml)	Antioxidant activity (%)		
	Ascorbic acid	Tinospora	Tulasi
12.5	26±2.46		
25	40±1.61		
50	64±1.91		
100	80±2.46	22±0.75	18±0.92
200	26±2.46	24±1.24	26±1.34
300		32±0.47	34±0.65
400		44±1.43	42±1.54
500		50±0.94	58±0.86
1000		54±1.32	63±1.46

Table 4. Percentage Inhibition of Hydrogen Peroxide Radical Scavenging Activity *In-Vitro* by *Tinospora* Ethanolic, Acetone ,Water extract

Conc of extract(µg/ml	Antioxidant activity (%)			
	Ascorbic acid	Tinospora leaf		
		Ethanol	Acetone	water
12.5	26±0.28			
25	40±1.21			
50	64±1.23			
100	80±0.23	14±0.48	10±0.84	06±0.64
200		21±0.94	18±0.68	09±0.84
300		34±1.32	26±1.46	13±0.48
400		42±1.48	30±0.94	16±0.58
500		54±1.62	38±0.74	20±0.64

Table 5. Percentage Inhibition of Hydrogen Peroxide Radical Scavenging Activity, In-Vitro by Ehanolic, Acetone ,Water extract of Tulasi

Conc of extract(µg/ml)	Antioxidant activity (%)			
	Ascorbic acid	Tulasi leaf		
		Ethanol	Acetone	Water
12.5	26±0.28	10±0.48	09±0.68	12±0.42
25	40±1.21	21±0.64	15±0.86	38±0.64
50	64±1.23	34±0.54	22±0.68	54±0.86
100	80±0.23	56±0.64	36±0.84	71±0.42

Fig 1 *In vitro* Concentration Dependent Percentage Inhibition of DPPH Radical Scavenging Activity by Ethanolic extract of *Tinospora and tulasi leaf* and Ascorbic acid.

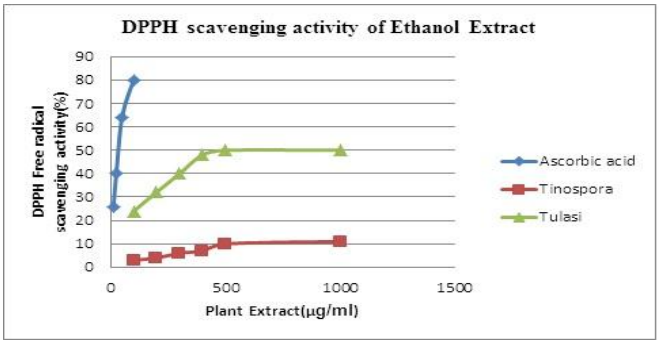


Fig 2. *In vitro* Concentration Dependent Percentage Inhibition of DPPH Radical Scavenging Activity by Water extract of Tinospora and Tulasi leaf and Ascorbic acid.

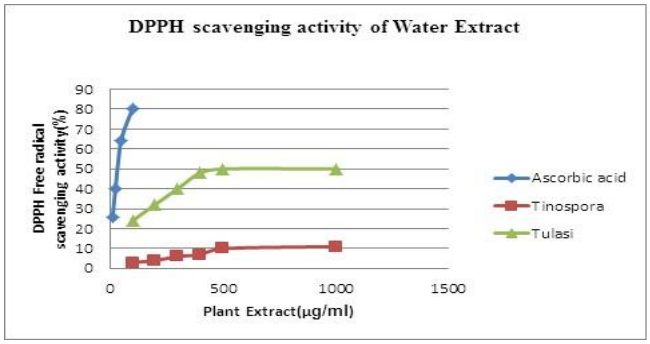


Fig 3. *In vitro* Concentration Dependent Percentage Inhibition of DPPH Radical Scavenging Activity by Acetone extract of Tinospora and Tulasi leaf and Ascorbic acid

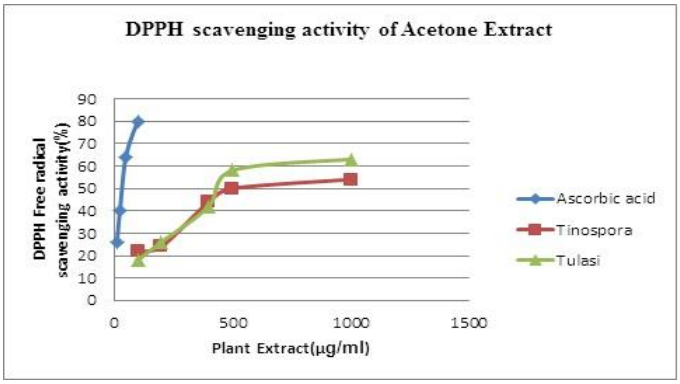


Fig 4. *In vitro* Concentration Dependent Percentage Inhibition of Hydrogen Peroxide Radical Scavenging Activity by different extracts of Tinospora leaf and Ascorbic acid

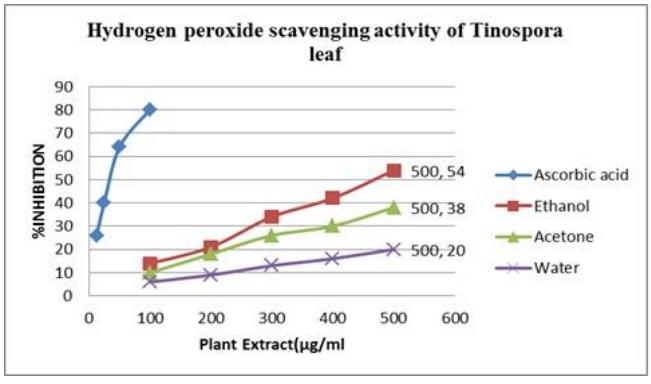
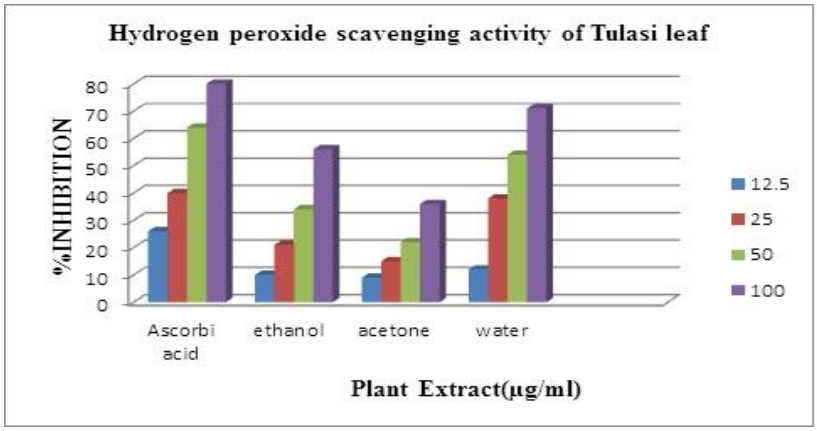


Fig 5. *In vitro* Concentration Dependent Percentage Inhibition of Hydrogen Peroxide Radical Scavenging Activity by different extracts of Tulasi leaf and Ascorbic acid



CONCLUSION

Present study concludes that Tinospora and Tulasi leaf Ethanolic, Acetone, Water extracts posses antioxidant activity. The active constituents alone or in combination may be responsible for the antioxidant activity. Ethanolic extract of tulasi leaf shows high DPPH free radical scavenging activity and Water extract of tulasi leaf shows high Hydrogen peroxide radical scavenging activity. The providing data can enrich the existing comprehensive data of free radical scavenging activity of plants materials.

ACKNOWLEDGEMENTS

Authors are grateful thank to Vagdevi College of Pharmacy management and staff and non-teaching staff members for providing all necessary facilities to carry out the work

REFERENCES

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia ECA. Phytochemical screening and antioxidant activities of some selected medicinal plants used formalaria therapy in southwestern Nigeria. *Trop J Pharm Res.* 2008; 7(3): 1019-1024.

Bishayi B, Roychowdhury S, Ghosh S and Sengupta M. Hepatoprotective and immunomodulatory properties of Tinospora cordifolia in ccl.

Elina M, Debosree G, Arnab Kumar G, Anjali B, Aindrila C, Sanjib Kumar P, Santanu D, Debasish B. Aqueous Tulasi leaf (ocimum sanctum) extract possesses antioxidant properties and protects against cadmium-induced oxidative stress in rat heart. *Int J Pharm Pharm Sci.* 2014; 6(1): 500-513.

Gokhan Z, Abdurrahman A, Gokalp Ozmen G, Yavuz Selim C, Evren Y. Antioxidant Properties of Methanolic Extract and Fatty acid Composition of *Centaurea urvillei* DC. Subsp. Hayekiana Wagenitz. Rec. *Nat. Prod*. 2011; 5: 123-132.

Kapur P, Jarry H, Wuttke W, Pereira BM and Seidlova-Wuttke D. Evaluation of the antiosteoporotic potential of Tinospora cordifolia in female rats. *Maturitas.* 2008; 59(4): 329-338.

Mabberley DJ. The plant Book: A portable Dictionary of the Higher plants of genera and families of angiosperms. *J. California Native Plant Soc.* 1997; 30(2): 1-36.

Panchabhai TS, Ambharkhane SV, Joshi AS, Samant BD and Rege NN. Protective effect of Tinospora cordifolia, Phyllantus emblica and their combination against antitubercular drugs induced hepatic damage: an experimental study. *Phytother Res.* 2008; 22(5): 646-650.

Patel Rajesh M and Patel N. *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *Journal of Advanced Pharmacy Education & Research.* 2011; 1: 52-68.

Ruch RJ, Cheng SJ, Klaunig JF. Prevention of cytotoxicity and inhibition of intracellularCommunication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis.* 1989; 10: 1003–1008.

Simon JE, Quinn J, Murray RG. Basil: a source of essential oils. In: Janick J, Simon JE. (Eds).Advanced in New Crops. Timber Press, Portland, OR. 1999; 484- 489.

Umamaheshwari S and Mainzen Prince PS. Antihyperglycaemic effect of logen- Excel, an ayurvedic herbal formulation in steptozotocin - induced diabetes mellitus. *Acta Pol Pharm.* 2007; 10: 1375-1386

Vivek Kumar G and Surendra Kumar S. Plants as a antioxidants. *Natural product radiance.* 2006; 5(4): 326-334.