IJMPR 2020, 4(1), 138-142

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

ANTIOXIDANT ACTIVITY OF THE LEAVES OF *SPHAGNETICOLA TRILOBATA* (L.)

A. K. Anjana[1], P. L. Rajagopal*[1], P. N. Sajith Kumar[1], I. Arthi[1], Meera B. Nair[1] and S. Aneeshia[2]

[1]Department of Pharmacognosy and Phytochemistry, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala, India.
[2]Government Institute of Paramedical Sciences, Government Medical College, Kannur, Kerala, India.

Received on: 22/01/2020
Revised on: 12/02/2020
Accepted on: 02//03/2020

***Corresponding Author**
P. L. Rajagopal
Department of Pharmacognosy and Phytochemistry, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala, India.

ABSTRACT

The *invitro* antioxidant properties of the whole plant were studied in ABTS ,NO and Superoxide dismutase models. The antioxidant study had been carried out with the leaves of the plant. The leaf extract of the plant showed significant antioxidant properties. The plant was also reported to contain secondary metabolites like phenolic compounds and flavonoids. The antioxidant property exhibited by the leaf extract of the plant could be due to the presence of these secondary metabolites.

KEYWORDS: Sphagneticola trilobata, Antioxidants.

INTRODUCTION

Antioxidants are substances that works by quenching free radicals that contains a highly reactive unpaired electron.[1] Antioxidants are pharmaceutical products whose deficiencies are associated with a number of diseases, namely cardiovascular diseases, diabetes, cataracts, rheumatoid arthritis, Alzheimer's and many more. Phytochemicals produce antioxidant activity by inhibiting the formation of reactive oxygen species or by directly eliminating free radicals. Some compounds may act to increase the level of endogenous antioxidant defense by excessively regulating the expression of genes encoding superoxide dismutase, catalase or glutathione peroxidase.[2]

Antioxidants are being efficiently used in food, cosmetic and therapeutic industry with a wide variety of applications. There are a variety of medicinal plants that act as potent source of antioxidants. For example: *Curcuma longa, Zingiber officinale, Citrus lemon, Eugenia caryophyllus, Elettaria cardamomum, Terminalia bellerica, Aloe vera, Ocimum sanctum* etc.[3,4]

Sphagneticola trilobata is a thick mat forming creeper plant belonging to the family Asteraceae. It is widely distributed over many places over the world including India, North and South America, Mexico, Brazil, Florida, South Africa etc. The plant is a weed that grows abundantly in wetlands, coastal areas, tropical and sub-tropical areas, waste lands, footpaths, forests, and waterways. The plant is also known as *Wedelia trilobata. S. trilobata* is known to possess different pharmacological activities.[5]

MATERIALS AND METHODS

Plant material

The leaves of the plant were collected from the waste lands of Cheruthazham Village of Kannur district, Kerala state in the month of July 2019. Its botanical identity was confirmed and then shade dried and the specimen bearing voucher has been deposited in the Department of Pharmacognosy, College of Pharmaceutical Sciences, GovernmentMedical College, Kannur, Kerala state.

Preparation of methanolic extract

The dried leaves of the plant were powdered and were extracted with 95% methanol in soxhlet apparatus for 6 hours. The total methanolic extract was then concentrated to a syrupy consistency and dried in vacuum desiccator.

Antioxidant Studies

ABTS scavenging activity

ABTS is chemically 2, 2 – Azinobis (3-ethyl BenzoThiazoline–6–Sulphonic acid. The reduction of free radicals by the test compound using ABTS is measured at 734 nm.The ABTS radical cation was prepared by the following method. ABTS 2mM was prepared in distilled water. Potassium per sulphate was prepared in distilled water. 200ml of potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. This solution is called as ABTS radical cation, which

was used for the assay. To 0.5 ml of various concentrations of the extract, 0.3 ml of ABTS radical cation and 1.7 ml of phosphate buffer, pH 7.4 were added. For control, instead of extract, methanol replaced the alcoholic extract and water for the aqueous extract. The absorbance was measured at 734 nm. The experiment was performed in triplicate.[6]

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Nitric oxide scavenging activity

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates NO_2, N_2O_4 and N_3O_4. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 546 nm.

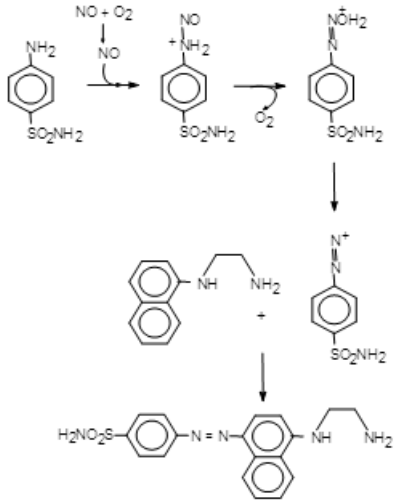

Fig. 1: Griess reaction.

Solution A - 1% sulphanilamide in 5% ortho phosphoric acid or 25% v/v hydrochloric acid; Solution B - 0.01% napthyl ethylene diamine in distilled water. It is known as Griess reagent. Solution A and Solution B were mixed in equal volumes within 12 hrs of use. Sodium nitroprusside 5mM (0.0373g in 25 ml) was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of the extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25°C for 5hr. After 5hrs, 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm. The experiment was performed in triplicate.[7]

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Superoxide dismutase scavenging activity

Alkaline DMSO is used as superoxide generating system. The generated superoxide will react with NBT to give coloureddiformazan. Diformazan being insoluble in water slowly precipitates out. Therefore, the spectral measurement must be done immediately after the reaction is carried out. In the presence of scavenger, reduction of NBT will occur which is measured at 560 nm.

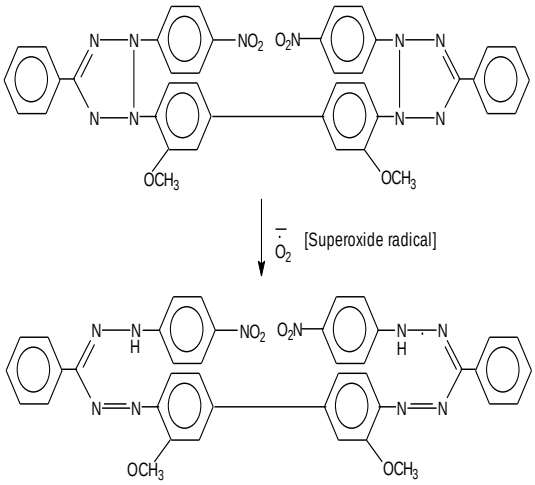

Fig. 2: Reduction of NB.

To 0.5 ml of different concentrations of extract, 1 ml alkaline DMSO and 0.2 ml NBT 20 mMwere added. The absorbance was measured at 560 nm. The experiment was performed in triplicate.[8]

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

In our study the alcoholic extract showed good free radical scavenging activity in various *in vitro* models viz. ABTS, Nitric Oxide and SOD. The major constituents reported from the leaf of the plant include flavonoids and phenolic compounds.[9,10] Flavonoids are well documented to have potent antioxidant activity.[11]

Table 1 Effect of methanolic extract of *Sphagneticolatrilobata*on ABTS scavenging.

Sl. No.	Conc. µg/ml	Ascorbic acid		Methanolic Extract	
		Absorbance	Percentage Scavenging	Absorbance	Percentage Scavenging
1	5	0.960	1.19	0.875	9.41
2	10	0.931	5.47	0.763	11.35
3	15	0.876	11.29	0.616	22.40
4	25	0.749	22.75	0.529	45.66
5	50	0.602	49.30	0.091	57.31
6	100	0.514	58.11	0.087	69.48
7	250	0.499	69.75	0.080	87.53
8	500	0.326	87.20	0.056	93.68
9	1000	0.111	104.29	0.031	101.55
10	Control	0.757		0.862	

Table 2 Effect of methanolic extract of *Sphagneticolatrilobata*on NO scavenging.

Sl. No.	Conc. µg/ml	Ascorbic acid		Methanolic extract	
		Absorbance	Percentage Scavenging	Absorbance	Percentage Scavenging
1	5	0.797	2.59	0.714	5.12
2	10	0.609	3.18	0.655	12.25
3	15	0.599	6.25	0.616	29.37
4	25	0.502	17.39	0.577	47.14
5	50	0.497	39.25	0.498	59.25
6	100	0.310	68.10	0.425	78.30
7	250	0.289	89.57	0.389	94.32
8	500	0.177	98.10	0.212	112.51
9	1000	0.104	116.24	0.098	141.66
10	Control	0.855		0.798	

Table 3 Effect of methanolic extract *Sphagneticolatrilobata* on SOD scavenging.

Sl.No.	Conc. µg/ml	Ascorbic acid		Methanolic extract	
		Absorbance	Percentage Scavenging	Absorbance	Percentage Scavenging
1	5	0.879	3.99	0.992	12.50
2	10	0.805	12.45	0.910	29.99
3	15	0.796	27.30	0.879	46.75
4	25	0.714	46.15	0.816	66.12
5	50	0.685	69.25	0.794	74.11
6	100	0.600	93.30	0.630	87.55
7	250	0.587	112.59	0.541	99.10
8	500	0.410	137.61	0.445	121.25
9	1000	0.116	151.20	0.100	145.51
10	Control	0.874		0.797	

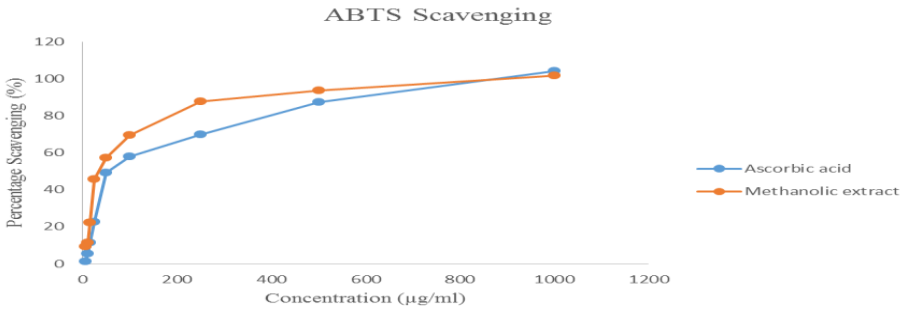

Fig. 1: Effect of methanolic extract of *Sphagneticolatrilobata*on ABTS scavenging.

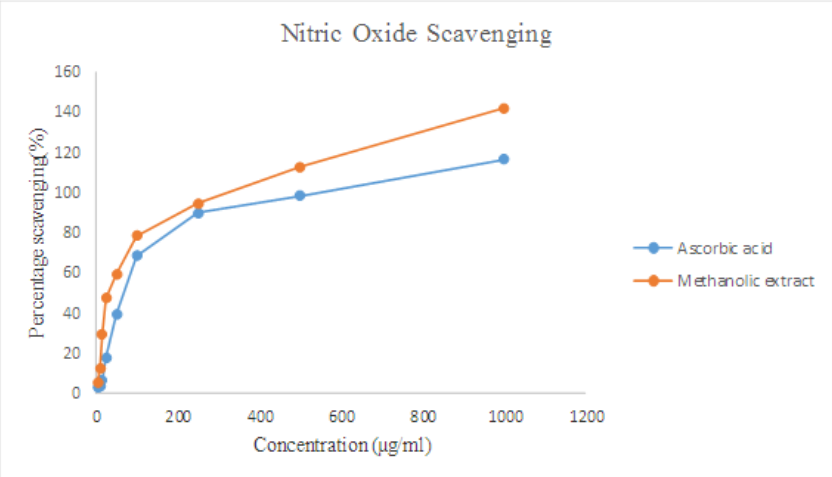

Fig. 2: Effect of methanolic extract of *Sphagneticolatrilobata*on NO scavenging.

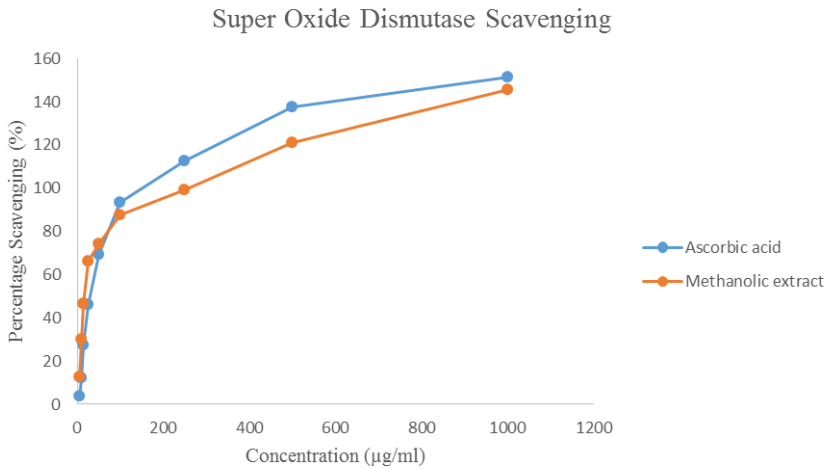

Fig. 3: Effect of methanolic extract *Sphagneticolatrilobata* on SOD scavenging

Flavonoids have the ability to directly scavenge the reactive oxygen species. They can also chelate free radicals by donating a hydrogen atom or by single-electron transfer. Flavonoids can also act as an intracellular antioxidant through inhibition of free radical generating enzymes like xanthine oxidase, lipoxygenase, protein kinase etc.[12]

Phenolic compounds possess redox properties that allow them to act as potent antioxidants. The free radical scavenging abilityof phenoilic compounds are facilitated by their hydroxyl groups hence the total phenolic concentration could be used as a basis for screening of antioxidant activity.[13]

Hence the antioxidant potential of the methanolic extract of the leaf of *Sphagneticolat rilobata* could be due to the presence of flavonoids and phenolic compounds which might be responsible for the various pharmacological activity of the plant.

ACKNOWLEDGEMENT

The authors are thankful to **Kerala State Council for Science, Technology and Environment**, Sasthrabhavan, Pattom, Thiruvananthapuram, Kerala for providing necessary helps to complete this research work.

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology. 7th Edition. Jaypee Brothers Medical Publishers (P) LTD. 2013: 912.
2. Rangari VD. Pharmacognosy and Phytochemistry. 2nd Edition. Volume II. Career Publications. 2009:104.
3. Sindhi V, Gupta V, Sharma K, Bhatnagar S,Kumari R, Dhaka N. Potential applications of antioxidants : A review. Journal of Pharmacy Research. 2013; 7: 828-835.
4. Mandal S, Yadav S, Yadav S, Kumar RN. Antioxiants-A review. Journal of Chemical and Pharmaceutical Research, 2009, 1 (1):102-104.
5. Balekar N, Nakpheng T and Srichana T. *Wedeliatrilobata* L.: A phytochemical and

pharmacological review. Chiang Mai J Sci. 2014; 41(3): 590-605.
6. Miller N J. Total antioxidant status in plasma and body fluids. Methods enzymol. 1994; 234: 279-293.
7. Sreejayan, N, Rao M. N. A. Nitric oxide scavenging by curcuminoids. J. Pharm Pharmacology. 1997; 49: 105.
8. AlamMdN, Bristi NJ, Rafiquzzaman Md. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J. 2013; (2):143–152.
9. Verma N, Khosa RL. Chemistry and biology of genus *Wedelia jacq*: A review. Indian Journal of Natural Products and Resources. 2015; 6(2): 71-90.
10. Sushama RRV. Primary phytochemical investigation of *Sphagneticola trilobata* (L.) Puruski. Journal of Pharmacognosy and Phytochemistry 2019; 8(1): 968-971.
11. Rajnarayanan K et.al Bioflavonoids, classification, pharmacological, biochemical, effects and therapeutic potential, Indian Journal of Pharmacology. 2001; 33: 2-16.
12. Prochazkova D, Bousova I, Wilhelmova N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011; 82(4): 513-23.
13. TulikaTyagi and Mala Agarwal. Antioxidant Properties and Phenolic Compounds in Methanolic Extracts of *Eichhornia crassipes*. Res. J. Phytochem. 2017; 11(2): 85-89.