International Journal of Pharmaceutical Science and Practice.
Volume 7, Number 1 (2018) pp. 1-6

http://www.ripublication.com

Invitro Anti Oxidant Activity of Ethanol and Ethyl Acetate Extracts of *Leucas Urticaefolia*

A.Anton Smith[1], N.Balakrishnan[2], J.John Wesley*, S.Gopi Krishnan [2]

[1]Faculty of Engineering and Technology, Dept. of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, India.

[2]Department of Pharmacology, S.A.Raja Pharmacy College, Vadakkangulam, Tirunelveli District, Pin code-627 116, Tamilnadu, India.

Abstract

Plants are indispensible sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. There is considerable interest in identifying new antioxidants from plant materials. The investigation aim to evaluate the anti oxidant activity of ethanol and ethyl acetate extracts of *leucas urticaefolia*. The methods involved in this study are DPPH photometric assay, superoxide scavenging activity, nitric oxide scavenging activity. The ethanolic extract posses significant anti oxidant activity IC_{50} =420µg ml**,** In NBT dye reduction method[14] the IC50 values of ethanolic extract were found to have strong superoxide radical scavenging activity. Where as ethyl acetate extract showed weak activity when compared to the standard ascorbate . In Nitric oxide scavenging activity the ethanolic extract was found to be most effective in scavenging nitric oxide radicals than that of ethyl acetate extract.

Keywords: DPPH photometric assay, superoxide and nitric oxide radical scavenging.

INTRODUCTION:

Plants are indispensible sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs, Plants of genus Leucas (Lamiacae) have been widely employed by the traditional healers to cure

many diseased conditions which insinuated that this genus have immense potential for the discovery of new drugs or lead molecules. The human body has a complex system of natural; enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radials are responsible for causing a large number of diseases including cancer[5] (Kinnula and Crapo , 2004) cardiovascular disease[8] (Singh and Jialal , 2006) neural disorders[7] (Sas et al., 2007), Alzhemier's disease[9] (Smith et al., 2000) , mild cognitive impairment[3] (Guidi et al., 2006) , Parkinson's disease[2] (Bolton et al., 2000) alcohol induced liver disease[1] (Arteel, 2003) , ulcerative colitis[6] (Ramakrishna et al .m 1997) , aging[4] (Hyun et al., 2006) and atherosclerosis[10] (Upston et al ., 2003). Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery

PLANT INTRODUCTION.

Fig.1. *leucas urticaefolia*

Leucas urticaefolia belongs to the family lamiaceae is an annual erect herb 20 to 60 cm tall, pale yellowish found in shady habitat in waste lands and in the forests. Vernacular name (Tamil)- Perunthumbai. In India the plant is found in Tamilnadu, Andhra Pradesh, Madhya Pradesh, Rajasthan, Punjab, Haryana. The plant was collected from ettiayapuram, Tuticorin dist in the month September to October 2017. The plant was properly authenticated by a chief Botanist of Sidha medical college, Palayamkotai- Tirunelveli. Traditionally it is used in the treatment of fever, cold , cough and relieve swelling.

PREPARATION OF EXTRACT

(1) Preparation of Ethyl acetate Extract

For preparation of Ethyl acetate Extract Ethyl acetate obtained from S.D. fine

Chemicals of 2.5 litre. The Aerial parts of L. Utricaefolia (L,F, Fr) were collected, authenticated and shade dried were powered in a Electrical grinder and stored at room temperature until further used .

Ethyl acetate extract was prepared by mixing 10 gm of dry powder in 100 ml of ethyl acetate. The flasks were kept in shaken incubator overnight at 37^{0} C at 120 Rpm. Supernatant was filled with muslin cloth and filtered twice with whatman No. 1 filter paper. The supernatant collect was dried to form gell in a fan equipped incubator at 50^{0}c. The extract were stored at 4^{0} Untill used.

2) Preparation of Ethanol Extract :-

Ethanol extract was prepared by mixing 10 gm of dry powder in 100 ml of Ethanol. The flasks were kept in shaken incubator overnight at 37^{0} C at 120 Rpm. Supernatant was filled with muslin cloth and filtered twice with whatman No. 1 filter paper. The supernatant collect was dried to form gell in a fan equipped incubator at 50^{0}c. The extract were stored at 4^{0} Untill used.

EVALUATION OF INVITRO ANTIOXIDANT STUDY

DPPH photometric assay

The free radical scavenging activity of various extracts was measured in turns of hydrogen donating a radical scavenging ability using the stable radical DPPH[11]. The percentage of DPPH radical scavenging activity of various extracts of Leucas Urticaefolia are presented in Table. The ethanolic extract of Leucas Urticaefolia was found to be more effective than ethyl acetate extract. The DPPH radical scavenging activity of the extract increases with increasing concentration. The IC_{50} of the ethanolic extract of Leucas Urticaefolia and Rutin were found to be 420µg/ ml and 470 µg/ml respectively.

Table 01. Effect of various extracts of Leucas Urticaefolia on DPPH assay

Concentration (µg/ml)	**% of activity (± SEM)***		
	Ethyl acetate Extract	**Ethanol Extract**	**Standard (Rutin)**
100	13.71± 0.62	39.49± 0.46	18.73±0.21
200	17.43± 0.19	47.25±0.30	23.08±0.57
400	28.16±0.41	52.20±0.20	52.41±0.70
800	37.30±0.44	65.853±0.40	70.60±0.17
	IC_{50} =1230 µg/ml	**IC_{50} =420 µg ml**	**IC_{50} =470 µg ml**

* All values are expressed as mean ± SEM for three determinations

SUPEROXIDE SCAVENGING ACTIVITY

NBT dye reduction method

The percentage scavenging of superoxide anion[12]. examined at different concentrations of various extracts of Leucas Urticaefolia (100, 200, 400 , 800 µg/ml) presented in Table . The IC_{50} values of ethanolic extract of Leucas Urticaefolia were found to have strong superoxide radical scavenging activity whereas, ethyl acetate extract showed weak activity when compared to that of standard Ascorbate. The IC_{50} of the ethanolic extract of Leucas Urticaefolia and Ascorbate were found to be 170µg/ ml and 70µg / ml respectively.

Table 02. Effect of Various extracts of Leucas Urticaefolia on Superoxide anionscavenging activity.

Concentration (µg/ml)	**% of activity (± SEM)***		
	Ethyl acetate Extract	**Ethanol Extract**	**Standard (Ascorbate)**
100	21.30± 0.66	43.20± 0.28	74.60±0.35
200	38.44± 0.60	60.20±0.20	90.18±0.35
400	53.60±0.22	74.80±0.63	93.20±0.30
800	75.50±0.22	80.25±0.55	97.30±0.26
	IC_{50} =320 µg/ml	**IC_{50} =170 µg ml**	**IC_{50} =70 µg ml**

* All values are expressed as mean ± SEM for three determinations.

NITRIC OXIDE SCAVENGING ACTIVITY

The reduction of nitric oxide[13] radical by the various extracts of Leucas Urticaefolia and ascorbate was noted to be concentration dependent and was illustrated in Table. The ethanolic extract of Leucas Urticaefolia was found to be most effective in scavenging nitric oxide radicals than that of ethyl acetate extract . But when compared to both extracts with Ascorbate (standard) , the ethanolic extract of the Leucas Urticaefolia showed significant result. The IC_{50} of the ethanolic extract of Leucas Urticaefolia and Ascorbate were found to be 180µg/ ml and 410 µg/ ml respectively.

Table 03. Effect of Various extracts of Leucas Urticaefolia on Nitric oxide scavenging activity

Concentration (µg/ml)	% of activity (± SEM)*		
	Ethyl acetate Extract	**Ethanol Extract**	**Standard (Ascorbate)**
100	41.25± 0.42	38.76± 0.20	27.15±0.25
200	51.10± 0.15	58.70±0.54	31.12±0.30
400	58.65±0.80	63.18±0.65	60.23±0.12
800	64.21±0.44	71.15±0.90	56.41±0.60
	IC_{50} =260 µg/ml	**IC_{50} =180 µg ml**	**IC_{50} =410 µg ml**

* All values are expressed as mean ± SEM for three determinations

RESULTS AND DISCUSSION

In DPPH photometric assay the ethanolic extract of L. Urticaefolia was found to be more effective than ethyl acetate extract. In NBT dye reduction method[14] the IC50 values of ethanolic extract were found to have strong superoxide radical scavenging activity. Where as ethyl acetate extract showed weak activity when compared to the standard ascorbate . In Nitric oxide scavenging activity the ethanolic extract was found to be most effective in scavenging nitric oxide radicals than that of ethyl acetate extract.

STATISTICAL ANALYSIS :-

Datas were expressed as means (±) SEM for three determination.

CONCLUSION

Reports Suggest that the invitro antioxidant study of L. Urticaefolia of ethanolic extract was found to be more effective than ethyl acetate extract. This study is focused on invitro methods of antioxidant evaluation. It was prepared based on Literature research presently 19 Invitro and 10 invivo methods are being used for antioxidant evaluation purpose. DPPH method is most frequently used one for *Invitro* antioxidant evaluation. Ethanol extract was found with the highest frequency for anti oxidant study. This article will be a comprehensive ready reference for those who are interested in antioxidant study.

REFERENCES

[1] Arteel, G.E., Oxidants and antioxidants in alcohol induced liver disease. *Gastroenterol* 2003. 124: 778-790.

[2] Bolton , J.L., Trush , M.A., Penning , T.M. Dryhurst, G., Monks, T.J. . Role of quinones in toxicology . *Chem .Res. Toxicol* . 2000. 13: 135- 160.

[3] Guidi. I. Galimberti, D.. Lonati, S., Novembrino, C., Bamonti.F., Tiriticco, M. Fenoglio, C., Venturelli, E., Barlton, P.Bresolin N., Oxidative imbalance in patients with mild cognitive impairment and Alzeimer's disease. *Neurobiol, Aging* 2006 27: 262- 269.

[4] Hyun , D.H. Hernandez, J.O., Mattson , M.P. de Cabo, R. The plasma membrance redox system in aging. *Aging Res. Rev*. 2006 5: 209- 220.

[5] Kinnula, V.L.m Crapo, J.D., Superoxide dismutases in malignant cells and human tumors. *Free Radic. Biol. Med.* 2006 36: 718-744.

[6] Ramakrishna , B.S., Varghese, R., Jayakumar. S., Mathan . M. Balasubramanian, K.A., Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. *J. Gastroenterol, Hepatol* 1997 12: 490 – 494.

[7] Sas, K., Robotka , H., Toldi , J., Vecsi, L. Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorders. *J. Neurol. Sci.* 2007 257: 221-239.

[8] Singh, U., Jialal, I., Oxidative stress and atherosclerosis, *pathophysiology* 2006 13: 129 -142.

[9] (9 Smith , M.A. Rottakamp , C.A., Nunomura , A., Raina , A.K. Perry ,G. Oxidative stress in Alzheimer's disease. *Biochim. Biophys Acta* 2000 1502: 139-144.

[10] Upston, J.M.Kritharides, L., Stocker, R., The role of vitamin E in atherosclerosis. *Prog. Lipid Res.* 2003 42: 405-422.

[11] Mensor, L.L., Meneze, F.S., Leitao, G.G., Reis, A.S., Dos santor , J.C., Coube, C.S., and Leitao, S.G. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother .Res* ., 2001, 15: 127-130.

[12] Winterbourne , C.C., Hawkins, R.E., Brain , M and Carrel, R.W. The estimation of red cell superoxide dismutase activity *J. Lab. clin. Med*., 1999, 85: 337-341.

[13] Garrat, D.C. The quantitative analysis of drugs, Champman and Hall, Japan, 1964 3: 456-458.

[14] Hasan Ms, Ahmed MI, Mondal S. Uddin SJ, Masud MM, Samir Kumar S.et al. Antioxidant antinociceptive activity and general toxicity study of Den – Dropthoe falcata and isolation of quercitin as major component. *Orient pharm Exp med* 2006: 6: 355-60.