ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

In-vitro Acetylcholinesterase and antioxidant activity of *Ficus dalhousie* and *Melissa parviflora* Benth

Himabindu K*[1], Vijayalakshmi A[2]

[1]Research Scholar, Department of Pharmacology, Vels University, Pallavaram Chennai - 600 117, Tamil Nadu, India

[2]Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels University, Pallavaram - 600 117, Tamil Nadu, India

Article History:

Received on: 10 Jul 2020
Revised on: 14 Aug 2020
Accepted on: 10 Oct 2020

Keywords:

Acetylcholinesterase,
DPPH,
Hydrogen peroxide,
Scavenging activity,
Plant extracts

ABSTRACT

*Ficus dalhousie*and *Melissa parviflora* Benth both plants have been used as Tranquiliser, Relaxants, Nervine tonic and Calming aids throughout the world. The present study was aimed to identify the antioxidant potential of the Ethyl acetate and Hydro alcoholic extract of these plants by in vitro methods. Anti-Alzheimer activity of the plant extract were screened by Acetylcholinesterase (AChE) inhibition and antioxidant by DPPH and Hydrogen oxide. The results of the assays were calculated by the percentage inhibition of these free radicals. In Acetylcholinesterase (AChE) assay inhibitory potentials of *Ficus dalhousie* exhibited 73.34 ± 1.12%, whereas in *Melissa arviflora*it was 86.88± 2.12%. In DPPH radical scavenging assay the percentage inhibition was 77.87 ± 2.02% in *Ficus dalhousie*and 76.92± 1.32% in *Melissa arviflora.* Hydrogen peroxide scavenging assay the percentage inhibition was 86.56 ± 1.05% in *Ficus dalhousie*and 80.75± 1.92% was in *Melissa arviflora* . In all the research assays, the extract showed a concentration dependent increase in free radical scavenging activity. The results revealed that the antioxidant effects of the plant extract might be due to the presence of phenol and flavonoid compounds.

*Corresponding Author

Name: Himabindu K
Phone: +91 6304 844 859
Email: himabindu307@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i4.4817

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Medicinal plants are still important part of traditional medical system in the developing countries and well known to treat various disorders. Even day - today also, in developing countries medicinal plant playing a vital role in primary health care as therapeutic remedies (Rajamanikandan *et al.*, 2011). Present studies suggested that natural antioxidants obtained from medicinal plants not only protect from toxic radicals but also have anti Alzheimer potential (Bagchi and Puri, 1998). The presence of phytochemical constituents of such as Alkaloids, Flavonoids, Tannins, Terpenoids, and Glycosides are examples of secondary metabolites which is responsible for healing (Lovell *et al.*, 1995). Phenolic compounds have been integrated with antioxidant activity due to their free radical scavenging activity. Potentially active components of fruits and leaves have been studied systematically to avoid oxidative stress (Matanjun *et al.*, 2008). Antioxidants are the substances which prevent scavenging and useful for treatment of diseases such as Alzheimer, Atherosclerosis, Parkinson, Diabetes and Heart disease (Freeman and Crapo, 1982). The flavonoid's have been considered as great interest because of their beneficial effects on human health.

The current study was designed to assess the antioxidant effects potential and total flavonoid content in Ethyl acetate and Hydro alcoholic extract of *Melissa parviflora* and *Ficus dalhousie.* *Melissa parviflora Benth*., belongs to the family Lamiaceae, commonly called as lemon balm, is an aromatic perennial, erect, pubescent or glabrate herb ascending upto 1540 cm high. Its tonic effect towards the heart and circulatory system causing mild vasodilation of peripheral vessels, hence lowering blood pressure. A number of pharmacological potential like antifungal, anti-inflammatory, antipyretic, antiplasmodial, hypotensive, hypocholesterolemic, have been reported for Melissa parviflora. *Ficus dalhousie* belongs to the family Moracea which is a natural habitat of Tamilnadu. The fruit shows cardiotonic action, whereas the leaves and bark fights infections of the liver and skin. All the species of ficus are rich in source of sterols especially β- sitosterols and Ficus carica proved for its learning and memory enhancing activity on experimental study (Shi *et al.*, 1999).

Thus, the aim of the present study is to determine the free radical scavenging activity, and flavonoid content of selected plants.

MATERIALS AND METHODS

Plant Materials

Leaves of *Melissa parviflora* and Fruits of *Ficus dalhousie* were collected from various parts of Tirupati, Andhra Pradesh, India during the month of March. The Collected Plant materials were recognized and validated by a renowned Botanist Dr. Madhav Chetty, Asst. Professor Department of Botany, Sri Venkateswara University, Andhra Pradesh.

The plants were validated and the specimen kept in herbarium as *Melissa parviflora* Benth voucher No: 0046 and *Ficus dalhousiae* as voucher No: 0703 for future references.

Extraction Procedure

Leaves of *Melissa parviflora* and fruits of *Ficus dalhousie* were thoroughly washed, shaded dry at room temperature and then coarsely powdered. The Fine powder of crude drug used for extraction in different solvents by Continuous Hot Extraction (Soxhlet). Solvent used in extraction arranged according to their polarity from least polar to the most polar Like Petroleum Ether, Ethyl acetate and Hydro alcoholic extract.

The marc left after Ethyl acetate extraction was further used for Hydro alcoholic extraction. The extraction was performed with ethanol and water in the 70:30 v/v proportion, and then the mixture was stirred at room temperature (25ºC) for 24 h. Later, the mixture was filtered through vacuum filtrate. The 10 mL of ethanol were added to the solid residue,centrifuged at 3000 rpm for 5 min and re-filtered with help of the vacuum pump. The final residue was kept aside for further process (Tiwari *et al.*, 2011).

Acetylcholinesterase (AChE) inhibition assay

Acetylcholinesterase (AChE) is a cholinergic enzyme found at neuromuscular junction, mainly in muscles and nerves. Acetylcholinesterase breakdown or hydrolyze acetylcholine into acetic acid and choline and considered as the main therapeutic goal to control Alzheimer's disease (AD). Acetylcholine is involved in signal transmission in the synapse. Acetylcholinesterase enzymes inhibition is very important factor in treatment of AD and other neurological diseases (Mukherjee *et al.*, 2007; Mehta *et al.*, 2012).

The basic principle of the assays was based on the hydrolysis of acetyl thiocholine iodide with the respective AChE enzyme to the formation of 5-thio2-nitrobenzoate anion. The solution was maintained at 30°C for 15min using water bath by adding the substrate solution (5μl). A double beam spectrophotometer was used to measure the absorbance at 412nm (Ingkaninan *et al.*, 2003; Ellman *et al.*, 1961).

The Acetylcholinesterase enzyme inhibition was accessed by comparing control and tested samples from the rate of absorption with change in time (V=ΔAbs /Δt) as follow;

$$Enzyme\ \ inhibition\ \ (\%) = 100 - Percent\ enzyme\ activity$$

$$Enzyme\ activity\ (\%) = 100 \times V/Vmax$$

where (Vmax) is enzyme activity in the absence of inhibitor drug

Hydrogen peroxide scavenging (H2O2) assay

The scavenging ability of plant extracts estimated by hydrogen peroxide. A hydrogen peroxidesolution(40mM) was prepared with phosphate buffer (50 mM pH 7.4). The concentration was determined by absorption at230 nm using a spectrophotometer (Valentão *et al.*, 2002; Pal *et al.*, 2012). Extract (20-60μg/ml) was taken into distilled water and added into hydrogen peroxide solution for 10 min and check the absorbance at 230nm (Devasagayam *et al.*, 2004). The percentage inhibition of free radicals calculated by test sample using the formula:

$$Inhibitory\ ratio = (A0 - A1)\ X100/A0$$

Where, Ao = the absorbance of control;

A_1 = the absorbance with addition of test sample.

DPPH Scavenging assay

The DPPH scavenging assay is widely used to identify the similar research activity of natural compounds. It was calculated as per the procedure described by Kedare and Singh (2011). Firstly, 3 mL of 0.004% DPPH solution is mixed with ethanol and 0.1 mL of test sample at different concentrations. The solution was shaken vigorously and allowed to reach a constant state at room temperature for 30 min (Wettasinghe and Shahidi, 2000; Zhishen *et al.*, 1999). DPPH colorization was determined by measuring the absorbance at 517nm.

A control sample was prepared using 0.1mL of respective vehicle in the place of test sample.

The percentage inhibitor of free radicals was identified by the test sample and calculated using the formula:

$$(A0 - A1)\ X100/A0$$

Where, Ao = the absorbance of control;

A1 = the absorbance with addition of test sample.

Estimation of Total flavonoids

Estimation of total flavonoid content done by using Aluminium Chloride method. 2 mL of the test solution, added into 10mL of NaNO2 (5%) solution, mix up and then allowed to stand for 6 min (Fernando and Soysa, 2014; Adebiyi *et al.*, 2017). After that, 0.5 mL of the Al(NO3)3 (10%) solution was added into the volumetric flask, shake, and kept for 6 min. Finally 3.0 mL of the NaOH (4.3%) solution added to the volumetric flask, followed by addition of water to the scale, shake, and left for 15 min. The colorization was determined by measuring the absorbance at 510nm to determine the content of flavonoids present in the test sample by using ultraviolet–visible detector (Saeed *et al.*, 2012). The results were expressed as quercetin equivalents gram per 100 gram of dry weight (QE g/100g DW). Three readings were taken for each sample and the results were averaged.

The total flavonoid content was calculated using the formula:

$$y = mx + c$$

Where, y= Instrument Response,

m = represent the sensitivity

x= constant that describe the back ground

c = Analyte Concentration of unknown sample

Statistical Analysis

All tests results done in triplet and presented as mean ± SD. F- and t-tests (p-value <0.001) were used to get significant relationships between parameters used for assessment and regression analysis.

RESULTS AND DISCUSSION

The leaves of *Melissa parviflora* extracted with Petroleum ether, Ethyl acetate and Hydro alcoholic solution and percentage yield was found to be 17.23 %, 9.14 % & 11.38 %w/w respectively. The qualitative analyses revealed the presence of Flavonoids, Alkaloids, Glycosides, terols, Tannin, and Terpenoids. However, Carbohydrate, Resin and Saponins were found to be absent.

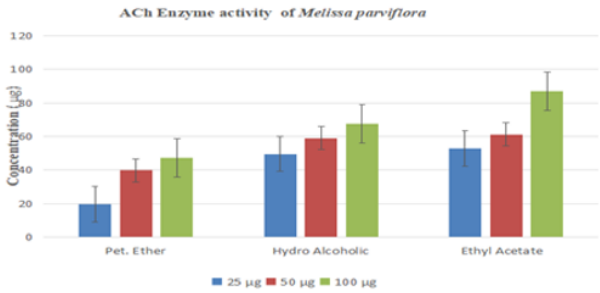

Figure 1: In-vitro inhibitory potentials of *Melissa parviflora* leaves extracts against AChE Enzyme activity (%)

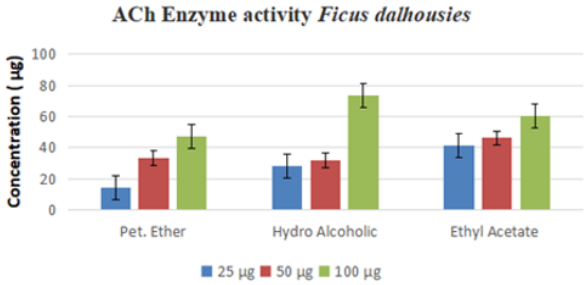

Figure 2: In-vitro inhibitory potentials of *Ficus dalhousie* Fruits extracts against AChE Enzyme activity (%)

The fruits of *Ficus dalhousie* extracted with Petroleum ether, Ethyl acetate and Hydro alcoholic solution that percentage yield was found to be 13.58 %, 8.57 % & 10.12 %w/w respectively. The qualitative analyses revealed the presence of Carbohydrates, Flavonoids, Tannins, Terpenoids,Glycosides, Resins and sterols. However, Alkaloids, Fat & oils & Amino acids & Proteins were found to be absent.

Acetylcholinesterase (AChE) inhibition assay

Acetylcholinesterase is the major enzyme which play a crucial role in AD. To reverse the cholinergic deficiency in AD Acetylcholinesteraseinhibitors required, which increase transmission of cholinergicwith modest and transient therapeutic effects.

Table 1: IC_{50} values of Ethyl Acetate and Hydro Alcoholic extracts for Hydrogen peroxide scavenging assay

Extract	IC_{50} values
Ficus dalhousie Ethyl Acetate extract	86.56
Ficus dalhousie Hydro Alcoholic Extract	51.17
Melissa parviflora Ethyl Acetate extract	53.82
Melissa parviflora Hydro Alcoholic Extract	80.75

Table 2: IC_{50} values of Ethyl Acetate and Hydro Alcoholic plant extracts for DPPH scavenging assay

Extract	IC_{50} values
Ficus Dalhousie Ethyl Acetate extract	77.87
Ficus Dalhousie Hydro Alcoholic extract	38.88
Melissa parviflora Ethyl Acetate extract	45.49
Melissa parviflora Hydro Alcoholic extract	76.92

Table 3: Total flavonoid content in μg/100g of extract

Plant	Part	Extract	Total flavonoid content in μg/100g of extract (in QE)
Ficus dalhousie	Fruit	Hydro Alcoholic Extract	4.30 $\pm$0.02
Ficus dalhousie	Fruit	Ethyl Acetate Extract	2.16$\pm$0.32
Melissa arviflora	Leaves	Hydro Alcoholic Extract	0.86 $\pm$0.18
Melissa arviflora	Leaves	Ethyl Acetate Extract	3.22 $\pm$0.42

Cholinesterase inhibitors act on many therapeutic targets and prevent formation of β-amyloid plaquesand modulation of APP processing. Many plants have been studied with thisconcept to identifythe new AChE inhibitors. Percentage Inhibitory potentials of *Melissa arviflora* leaves and *Ficus dalhousie* fruit extract represented in Figures 1 and 2.

Hydrogen peroxide scavenging (H2O2) assay

Hydroxyl radicals are the most potent reactive oxygen species that can interact with all the biological molecules. They cause lipid peroxidation, protein damage and membrane disruption. Hydroxyl radicals are very dangerous as the cells have no enzymatic mechanism for their removal. They can attack DNA and form protein cross links that cannot be easily repaired, hence causing cell death. Hydroxyl radical scavenging activity is determined by measuring the inhibition of degradation of 2-deoxyribose by the radicals generated by Fenton reaction.

In the present study *Ficus dalhousie* Hydro Alcoholic Extract has more H_2O_2 scavenging activity when compared to its Ethyl Acetate extract. In *Melissa arviflora* Ethyl Acetate Extract shown more H_2O_2 scavenging activity when compared to Hydro Alcoholic Extract.IC_{50} values of Ethyl Acetate and Hydro Alcoholic extracts for Hydrogen peroxide scavenging assay represented in Table 1.

DPPH Scavenging assay

In the present study, the Hydro Alcoholic extract of *Ficus dalhousie* emitting significant effects on the DPPH radical that shows the increasing percentage inhibition of the radical with concentration. This suggests that the plant extract has a dose dependent effect on the DPPH radicals. *Ficus dalhousie* Hydro Alcoholic extract has more antioxidant activity in comparison to Ethyl acetate Extract. Whereas in *Melissa arviflora* Ethyl acetate Extract shown more antioxidant activity in comparison to Hydro AlcoholicExtract. IC_{50} values of Ethyl Acetate and Hydro Alcoholic plant extracts for DPPH scavenging assay represented in Table 2.

Estimation of Total F lavonoids

The total flavonoid contents (TFC) of *Ficus dalhousie* and *Melissa arviflora* represented in Table 3. The Hydro Alcoholic extract of *Ficus dalhousie* and Ethyl Acetate extract of *Melissa arviflora* exhibited significant presenceof Flavonoids. Due to the presence of Flavonoids, these extracts have more antioxidant

activity in comparison to other extracts.

CONCLUSION

Hence the plant extracts having potential capability of free radical scavenging activity and known to ha antioxidants properties along with rich phytoconstituents. These medicinal plants are rich sources of antioxidants as they could have very good therapeutic action against prevention or delaying the progress of aging and age associated related degenerative diseases. Due to their anti-oxidant properties the plant extracts were subjected to analysis to estimate the total bio active compounds responsible for therapeutic efficacy. These plant material are a commonly essential for relaxants, nervine sleeping aids and its hytoconstituents revealed that Flavonoids are the major components of the extract. Flavonoids exhibit various biological activities including Anti-oxidant, Anti-alzheimer and anti-Parkinson activities.

ACKNOWLEDGEMENT

The authors acknowledge the recognition of Vel's University to provide the facility to carry out this work. We also want to support our department Faculties who provide their continuous support during the study.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Adebiyi, O. E., Olayemi, F. O., Ning-Hua, T., Guang-Zhi, Z. 2017. In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of Grewia carpinifolia. *Beni-Suef University Journal of Basic and Applied Sciences*, 6(1):10–14.

Bagchi, K., Puri, S. 1998. Free radicals and antioxidants in health and disease: A review. *EMHJ-Eastern Mediterranean Health Journal*, 4(2):350–360.

Devasagayam, T. P. A., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S., Lele, R. D. 2004. Free radicals and antioxidants in human health: current status and future prospects. *The Journal of the Association of Physicians of India*, 52:794–804.

Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7(2):88–95.

Fernando, C. D., Soysa, P. 2014. Total phenolic, flavonoid contents, in-vitro antioxidant activities and hepatoprotective effect of aqueous leaf extract of Atalantia ceylanica. *BMC Complementary and Alternative Medicine*, 14(1):395–395.

Freeman, B. A., Crapo, J. D. 1982. Biology of disease: free radicals and tissue injury. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, 47(5):412–426.

Ingkaninan, K., Temkitthawon, P., Chuenchom, K., Yuyaem, T., Thongnoi, W. 2003. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology*, 89(2-3):261–264.

Kedare, S. B., Singh, R. P. 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4):412–422.

Lovell, M. A., Ehmann, W. D., Butler, S. M., Markesbery, W. R. 1995. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology*, 45(8):1594–1601.

Matanjun, P., Mohamed, S., Mustapha, N. M., Muhammad, K., Ming, C. H. 2008. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *Journal of Applied Phycology*, 20(4):367–373.

Mehta, M., Adem, A., Sabbagh, M. 2012. New Acetylcholinesterase Inhibitors for Alzheimer's Disease. *International Journal of Alzheimer's Disease*, 2012:1–8.

Mukherjee, P. K., Kumar, V., Mal, M., Houghton, P. J. 2007. Acetylcholinesterase inhibitors from plants. *Phytomedicine*, 14(4):289–300.

Pal, R., Hooda, M., Bhandari, A., Singh, J. 2012. Antioxidant potential and free radicals scavenging activity by pod extracts of acacia senegal willd. *IJPCBS*, 2(4):500–506.

Rajamanikandan, S., Sindhu, T., Durgapriya, D., Sophia, D., Ragavendran, P., Gopalakrishnan, V. K. 2011. Radical scavenging and antioxidant activity of ethanolic extract of Mollugo nudicaulis by invitro assays. *Indian Journal of Pharmaceutical Education and Research*, 45(4):310–316.

Saeed, N., Khan, M. R., Shabbir, M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC*

Complementary and Alternative Medicine, 12(1).

Shi, H., Noguchi, N., Niki, E. 1999. Comparative study on dynamics of antioxidative action of α-tocopheryl hydroquinone, ubiquinol, and α-tocopherol against lipid peroxidation. *Free Radical Biology and Medicine*, 27(3-4):334–346.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H. 2011. Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia*, 1(1):98–106.

Valentão, P., Fernandes, E., Carvalho, F., Andrade, P. B., Seabra, R. M., Bastos, M. L. 2002. Antioxidative Properties of Cardoon (Cynara cardunculusL.) Infusion Against Superoxide Radical, Hydroxyl Radical, and Hypochlorous Acid. *Journal of Agricultural and Food Chemistry*, 50(17):4989–4993.

Wettasinghe, M., Shahidi, F. 2000. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chemistry*, 70(1):17–26.

Zhishen, J., Mengcheng, T., Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4):555–559.

