



Antioxidant activity of methanol extract leaves of *Melaleuca Alternifolia* (Maiden & Betche) Cheel

Gagan Shah ^{*1}, R.K. Dhawan ¹, Stalinjit singh², U.S. Baghel ³

¹Department of pharmacy, Khalsa college of pharmacy, Amritsar, Punjab, India

²Department of pharmacy & Technology, Khalsa college of pharmacy & Technology, Amritsar, Punjab, India

³Department of Pharmaceutical sciences, Kota University, Kota, Rajasthan, India

Received 17 Oct 2019,
Revised 05 Nov 2019,
Accepted 06 Nov 2019

Keywords

- ✓ *Melaleuca alternifolia*,
- ✓ DPPH
- ✓ Antioxidant activity,
- ✓ Phytochemical screening,

gaganshah83@gmail.com

Phone: +919814616627

Abstract

Melaleuca alternifolia family Myrtaceae also called Tea Tree oil. In traditional system of medicine the plant have various uses for aromatherapy, skin problems, inflammation hair care, stress reduce, and anxiety. Hence the present studies were designed to evaluate the antioxidant activity of methanol extract of *Melaleuca alternifolia* leaves. Antioxidant activity carried out using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), NO (Nitric oxide) and SO (Super oxide) assay and phytochemical screening of the methanol leaves extract were carried out. The results shows that *Melaleuca alternifolia* methanol extracts of leaves shows significant antioxidant activity which is higher when compared with ascorbic acid. In DPPH of IC₅₀ value of methanol extract of leaves is 67.69 µg/ml. In superoxide assay IC₅₀ value of methanol extract of leaves is 89.73 µg/ml ABTS assay shows the IC₅₀ of methanolic extract of *M. alternifolia* of leaves 51.70 µg/ml and, Nitric oxide assay shows IC₅₀ of methanolic extract of *M. alternifolia* of leaves is 56.11. The methanol extract of leaves shows the existence of various phytoconstituent such as terpenoids, phenolic compounds, flavonoids and steroids. Thus the study suggest that methanol extract of *Melaleuca alternifolia* leaves shows strong antioxidant activity might a possible source.

1. Introduction

Human use natural products from history times such as animals, plants, marine and microorganisms in order to treat several diseases [1]. The use of traditional drugs increase day by day because of its availability and lower side effects. As indicated by World health organization (WHO) more than half population depend upon herbal drugs [2]. Many traditional drugs are antioxidant as they contain large amount of phenolic compounds i.e flavonoids, carotenoids, vitamin C [3-4]. These traditional drugs are effective to prevent oxidative stress [5].

The term oxidative stress use to describe the steady state level of oxidative damage in tissue, organ and cell and free radical production is increase and low antioxidant activity [6-8]. The low and moderate concentration of Reactive nitrogen species (RNS) and Reactive oxygen species (ROS) play an important role in cell life and death, pathogen protection and cellular pathways. But when the concentration of ROS and RNS is high the antioxidant mechanism in the body is low and cause oxidative stress [9-11]. Oxidative stress cause many disease such as cancer, Parkinson, diabetes, atherosclerosis and aging [12-15]. Therefore, antioxidant prevent oxidative stress and control the various diseases.

Tea tree oil also known as *Melaleuca alternifolia* (Maiden and Betche cheel) belonging to the family myrtaceae. This plant is grown in Australia, found in new south wales grown above 300 m sea level. In ayurveda system of medicine the Tea tree oil from leaves have been used for, wounds treatment, fungal disease [16], sore throats, skin diseases [17] and acne [18]. *Melaleuca alternifolia* contain large number of terpenes hydrocarbons [19] arjunic acid, betulinic acid and melaleulic acid and fatty acid mixtures [20].

The Tea tree oil (TTO), has been found to have several medicinal effects as an anti-inflammatory effect, antibacterial, onychomycosis, candidiasis, clearance of bronchial congestion; effective in asthma, coughs, sinusitis, whooping cough, antioxidant, antifungal, antianxiety but there is no work done on extract of leaves. Therefore keeping the application of the oil the present study evaluate antioxidant, activity of methanol extract of leaves.

2. Material and Methods

2.1 Plant material

The *Melaleuca alternifolia* (Myrtaceae) leaves were collected from Nilgiris hills in Tamil Nadu in April 2016. The healthy plant material was prepared and Prof Madhava Chetty, Botany Department S.V university, which is situated in Tirupati, A.P, India identify the plant species. The Voucher specimen no is 1241,28 May,2016. A plant material of Voucher specimen stored in Khalsa college of pharmacy, Amritsar, Punjab, India.

2.2 Extraction

1600 gm leaf powder was taken in round bottom flask and subjected to soxhlet extraction with methanol solvent for 12hour. The methanol extract was concentrated under reduced pressure at 50-60°C until the extract is complete drying. The extract yield is 347 gm and stored in vessel 4°C.

2.3 Antioxidant activity

2.3.1 DPPH radical scavenging activity [21]

1. Prepare 0.1mM DPPH solution (4mg/100ml) in methanol.
2. Prepare different concentration of extract with methanol.
3. Add 2ml of extract and 1ml of DPPH solution.
4. Incubated at room temperature for 10 min.
5. Take absorbance at 515 nm against blank (methanol).
6. Calculate % Inhibition = $[(AC\ 515\ nm - AS\ 515\ nm) / AC\ 515\ nm] \times 100$ (AC=Absorbance control, AS=Absorbance sample).
7. Plot a curve for % Inhibition and concentration and using line of regression estimate IC₅₀

2.3.2 ABTS Radical Cation Scavenging Method

1 mL of distilled DMSO was added to 0.2 mL of various concentrations of the extract or standard, and 0.16 mL of ABTS solution was added to make a final volume of 1.36 mL. Absorbance was measured spectrophotometrically, after 20 min at 734 nm using UV spectrophotometer. Control was maintained without sample. IC₅₀ value obtained is the concentration of the extract required to inhibit 50 % ABTS radical mono cation [22]

2.3.3 Scavenging of Superoxide radical by Alkaline DMSO Method

To the reaction mixture containing 1 mL of alkaline DMSO, 0.3 mL of the drug samples and standard was added in DMSO at various concentrations followed by 0.1 mL of NBT (0.1 mg) to give a final volume of 1.4 mL, and reaction mixture without extract (water in place of extract) used as control. The absorbance was measured at 560 nm [23]

2.3.4 Nitric oxide radical inhibition

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), PBS (pH 7.4, 1 ml) and different concentration of extract or standard solution (1 ml) were incubated at room temperature for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to for min for completion of diazotization. Then, 1ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 540 nm [24].

2.3.5 Phytochemical screening

Methanol extract of *Melaleuca alternifolia* leaves was subjected to phytochemical screening [25].

3.Results and Discussion

3.1 DPPH radical scavenging activity

The values of % inhibition of extract were compared with that of ascorbic acid. However, the % inhibition values in ascorbic acid started at 51.01% at 20 µg/ml and 75.76% at 100 µg/ml while the methanol extract it was 39.56 % at 20 µg/ml and 60.44 % at 100 µg/ml The methanol extract have high IC₅₀ value 67.59 ug/ml when compared to ascorbic acid which is 16.53 ug/ml. These results show that methanol extract of plant have significant DPPH radical scavenging activity (Table 1 and Table 2 and Figure 1 and Figure 2).

Table 1: % Inhibition of DPPH by Ascorbic acid

| Conc. (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC ₅₀ (µg/ml) |
|---------------|----------------------|-----------------------|--------------|--------------------------|
| 20 | 0.291 | 0.594 | 51.01 | 16.53 |
| 40 | 0.253 | | 57.41 | |
| 60 | 0.217 | | 63.47 | |
| 80 | 0.172 | | 71.04 | |
| 100 | 0.144 | | 75.76 | |

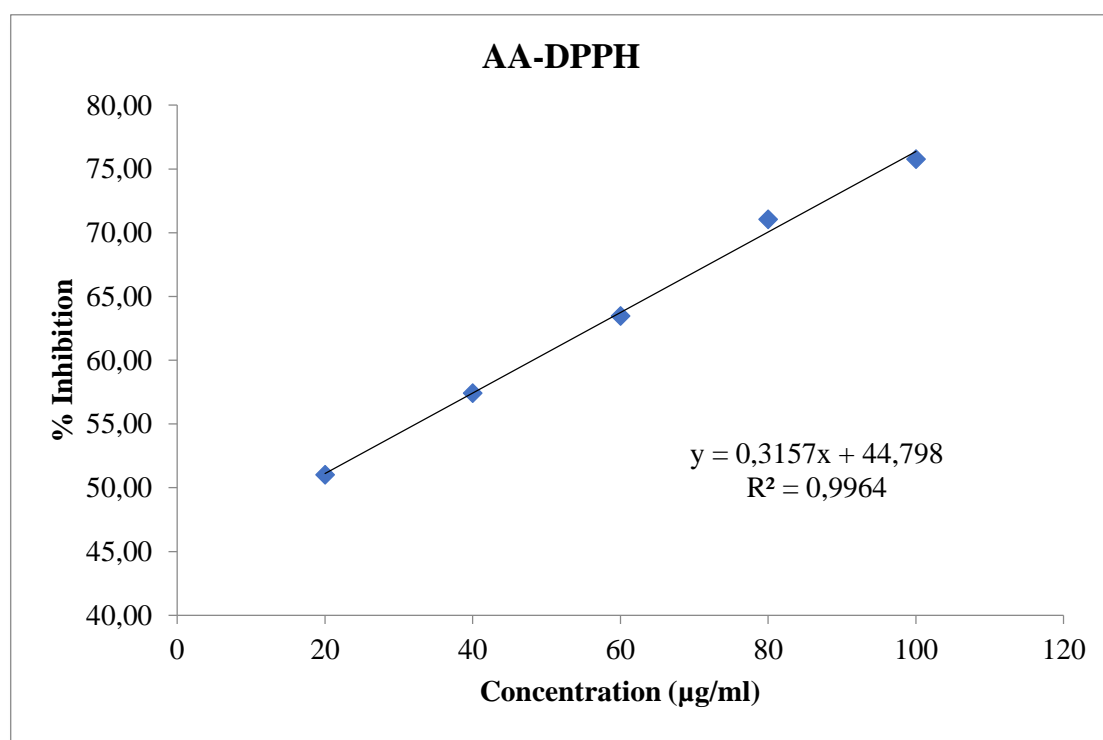


Figure 1: Graph represent regression curve of Ascorbic acid by DPPH assay method

Table 2: % Inhibition of DPPH by *Melaleuca alternifolia* methanol leaves Extracts

| Conc (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC ₅₀ (µg/ml) |
|--------------|----------------------|-----------------------|--------------|--------------------------|
| 20 | 0.359 | 0.594 | 39.56 | 67.69 |
| 40 | 0.336 | | 43.43 | |
| 60 | 0.315 | | 46.97 | |
| 80 | 0.295 | | 50.34 | |
| 100 | 0.235 | | 60.44 | |

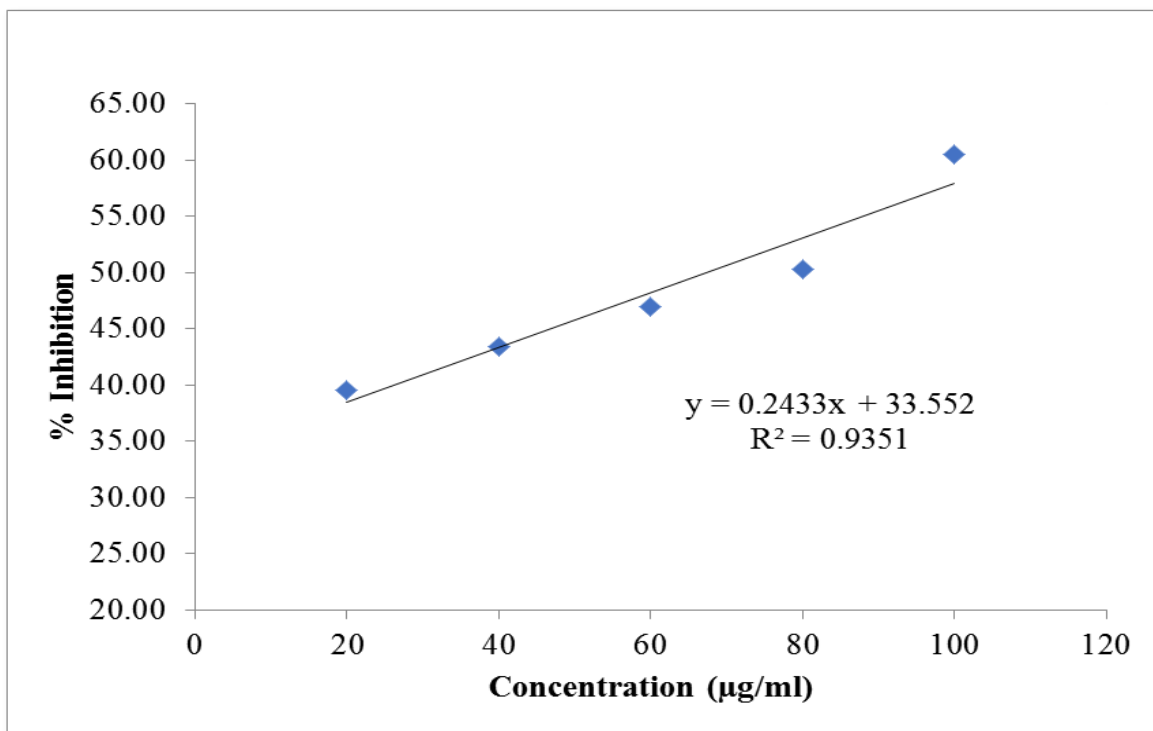


Figure 2: Graph represent regression curve of DPPH by *Melaleuca alternifolia* methanol leaves Extracts

3.2 ABTS Assay

The methanol extract of shows significant ABTS radical scavenging method. The % inhibition of ascorbic acid started at 10ug/ml is 50.89 and for 50 ug/ml is 73.94 whereas in methanol extract the % inhibition started at 10ug/ml is 25.50 and for 50 ug/ml is 50.11. The IC₅₀ value of methanol extract is 51.70 which is higher as compared to ascorbic acid. (Table 3 and Table 4; Figure 3 and Figure 4)

Table 3: ABTS assay % inhibition by Ascorbic acid

| Conc (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC ₅₀ (µg/ml) |
|--------------|----------------------|-----------------------|--------------|--------------------------|
| 10 | 0.441 | 0.898 | 50.89 | 8.64 |
| 20 | 0.396 | | 55.90 | |
| 30 | 0.328 | | 63.47 | |
| 40 | 0.285 | | 68.26 | |
| 50 | 0.234 | | 73.94 | |

Table 4: % ABTS assay % inhibition by *Melaleuca alternifolia* methanol leaves Extracts

| Conc (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC ₅₀ (µg/ml) |
|--------------|----------------------|-----------------------|--------------|--------------------------|
| 10 | 0.669 | 0.898 | 25.50 | 51.70 |
| 20 | 0.619 | | 31.07 | |
| 30 | 0.564 | | 37.19 | |
| 40 | 0.526 | | 41.43 | |
| 50 | 0.448 | | 50.11 | |

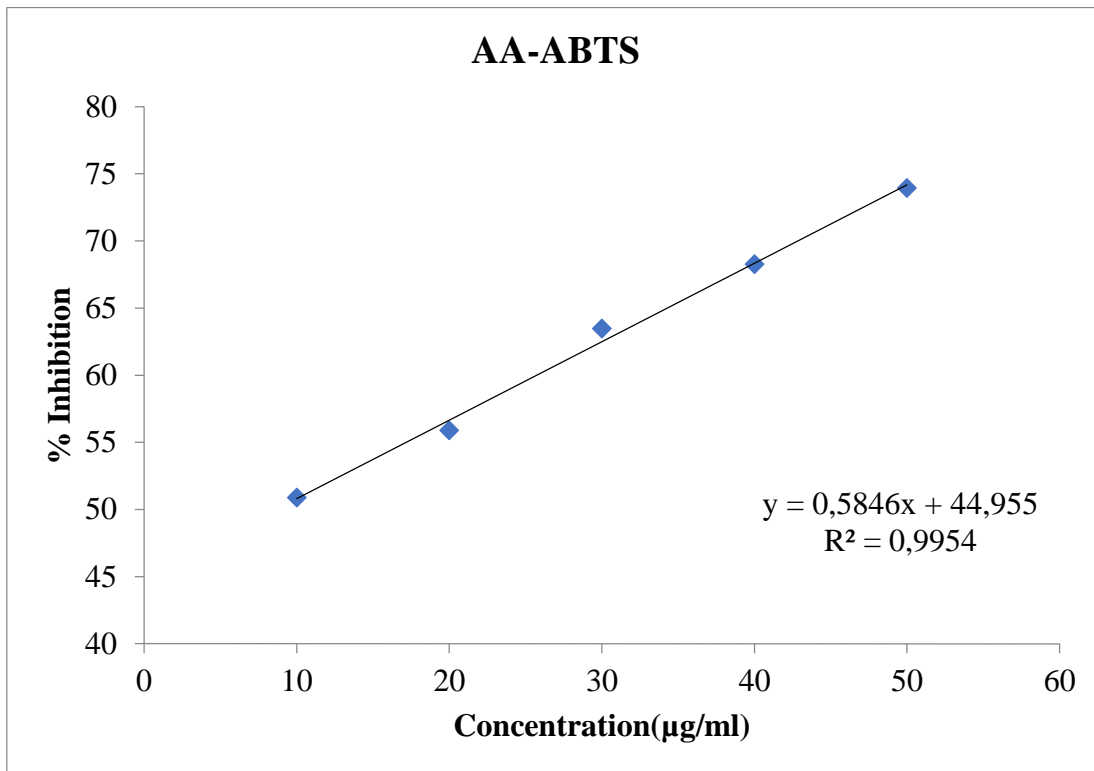


Figure 3: Graph represent regression curve of Ascorbic acid ABTS assay method

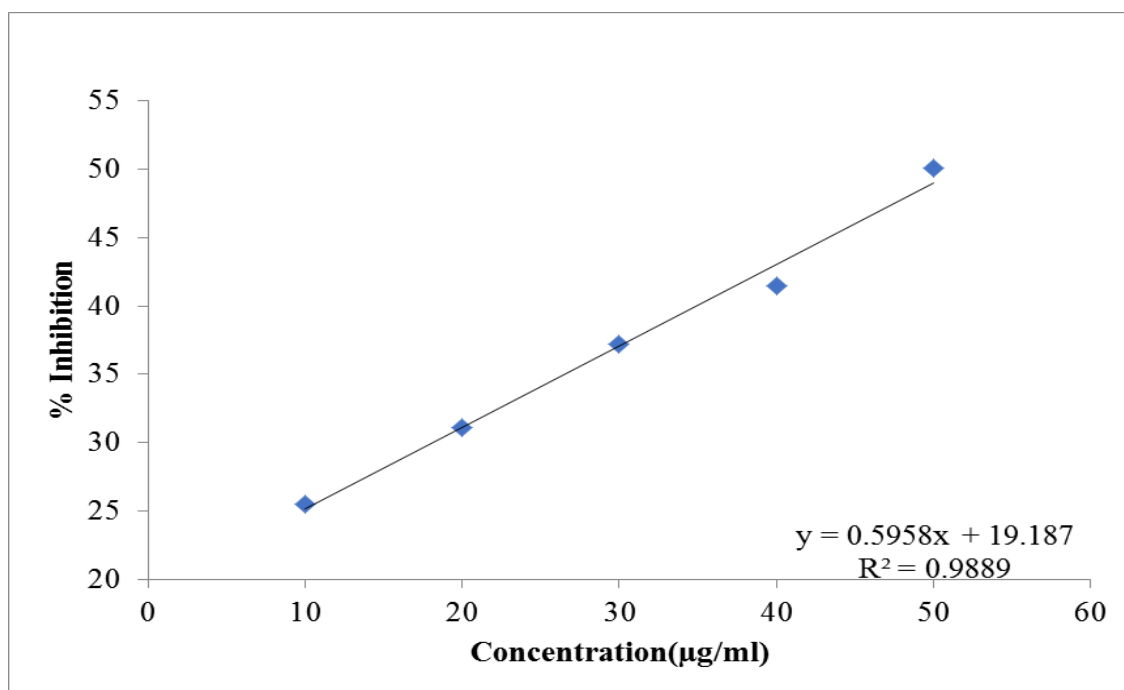


Figure 4: Graph represent regression curve of ABTS assay by *Melaleuca alternifolia* methanol leaves Extracts

3.3 Scavenging of Superoxide radical by Alkaline DMSO Method

Super oxide assay was performed where ascorbic acid was taken as standard. Values of test and sample were compared by measuring % inhibition from concentration 20 µg/ml to 100 µg/ml and then the values were compared to evaluate antioxidant activity of the sample. The IC₅₀ value of methanol extract of plant is 89.73 which is higher as compare to ascorbic acid. (Table 5 and Table 6; Figure 5 and Figure 6).

Table 5: % Inhibition of Superoxide assay by Ascorbic acid

| Conc (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC ₅₀ (µg/ml) |
|--------------|----------------------|-----------------------|--------------|--------------------------|
| 20 | 0.688 | 1.307 | 27.52 | 27.50 |
| 40 | 0.599 | | 33.93 | |
| 60 | 0.572 | | 39.98 | |
| 80 | 0.526 | | 44.88 | |
| 100 | 0.473 | | 48.71 | |

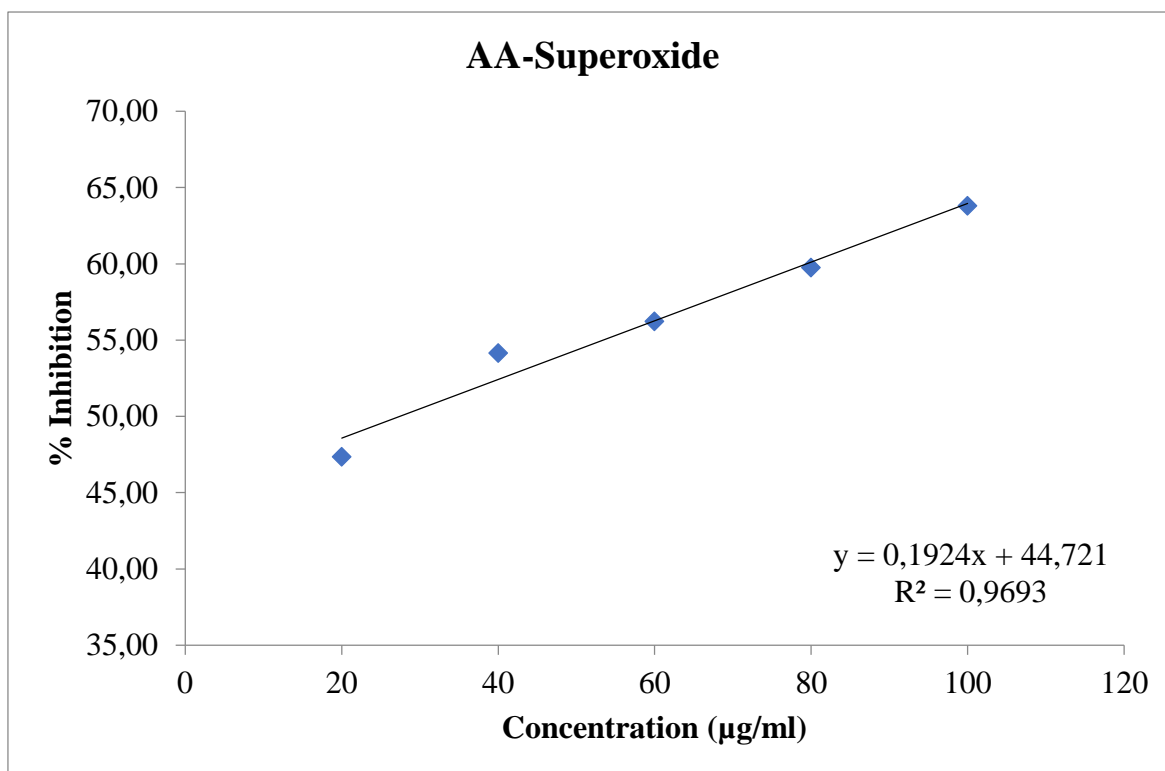


Figure 6: Graph represent regression curve of Ascorbic acid by superoxide assay method

Table 6: % Superoxide assay % inhi by *Melaleuca alternifolia* methanol leaves Extracts

| Conc (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC ₅₀ (µg/ml) |
|--------------|----------------------|-----------------------|--------------|--------------------------|
| 20 | 0.911 | 1.307 | 30.30 | 89.73 |
| 40 | 0.853 | | 34.74 | |
| 60 | 0.783 | | 40.09 | |
| 80 | 0.694 | | 46.90 | |
| 100 | 0.602 | | 53.94 | |

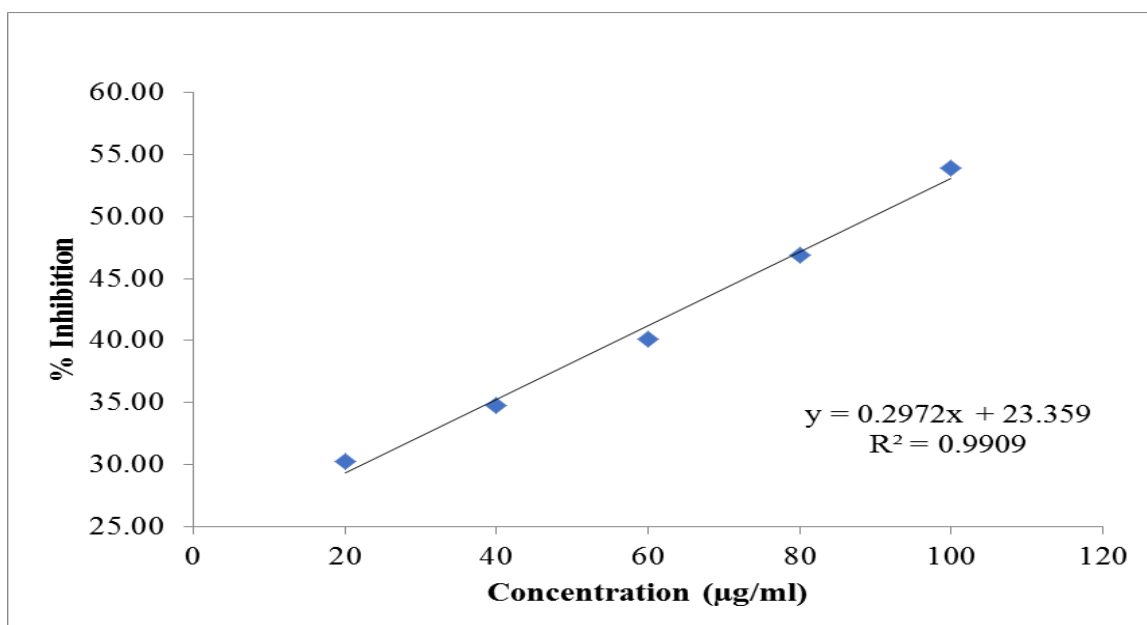


Figure 7: Graph represent regression curve of *Melaleuca alternifolia* methanol leaves extracts by Superoxide assay method.

3.4 Nitric oxide assay

In this assay the % inhibition of ascorbic acid started at 10ug/ml is 48.99 and for 50 ug/ml is 68.32 whereas in methanol extract the % inhibition started at 10ug/ml is 25.60 and for 50 ug/ml is 56.04. The IC_{50} value of methanol extract is 56.11 which is higher as compared to ascorbic acid which is 10.60 which shows significant nitric oxide antioxidant activity (Table 7 and Table 8; Figure 7 and Figure 8).

Table 7: NO assay % Inhibition by Ascorbic acid

| Conc. (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC_{50} (µg/ml) |
|---------------|----------------------|-----------------------|--------------|-------------------|
| 10 | 0.277 | 0.543 | 48.99 | 10.60 |
| 20 | 0.242 | | 55.43 | |
| 30 | 0.223 | | 58.93 | |
| 40 | 0.193 | | 64.46 | |
| 50 | 0.172 | | 68.32 | |

Table 8: NO assay % Inhibition by *Melaleuca alternifolia* methanol leaves extracts

| Conc (µg/ml) | Absorbance of Sample | Absorbance of Control | % Inhibition | IC_{50} (µg/ml) |
|--------------|----------------------|-----------------------|--------------|-------------------|
| 10 | 0.404 | 0.543 | 25.60 | 56.11 |
| 20 | 0.371 | | 31.68 | |
| 30 | 0.335 | | 38.31 | |
| 40 | 0.316 | | 41.80 | |
| 50 | 0.293 | | 46.04 | |

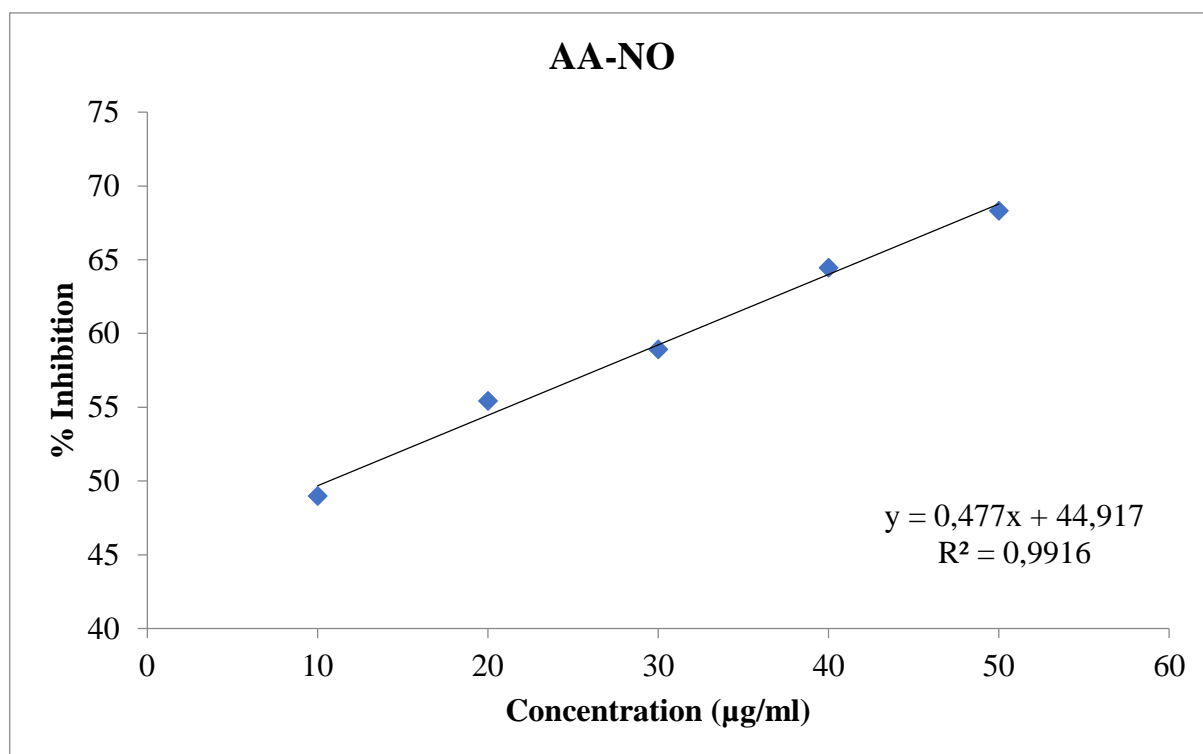


Figure 7: Graph represent regression curve of Ascorbic acid NO assay method

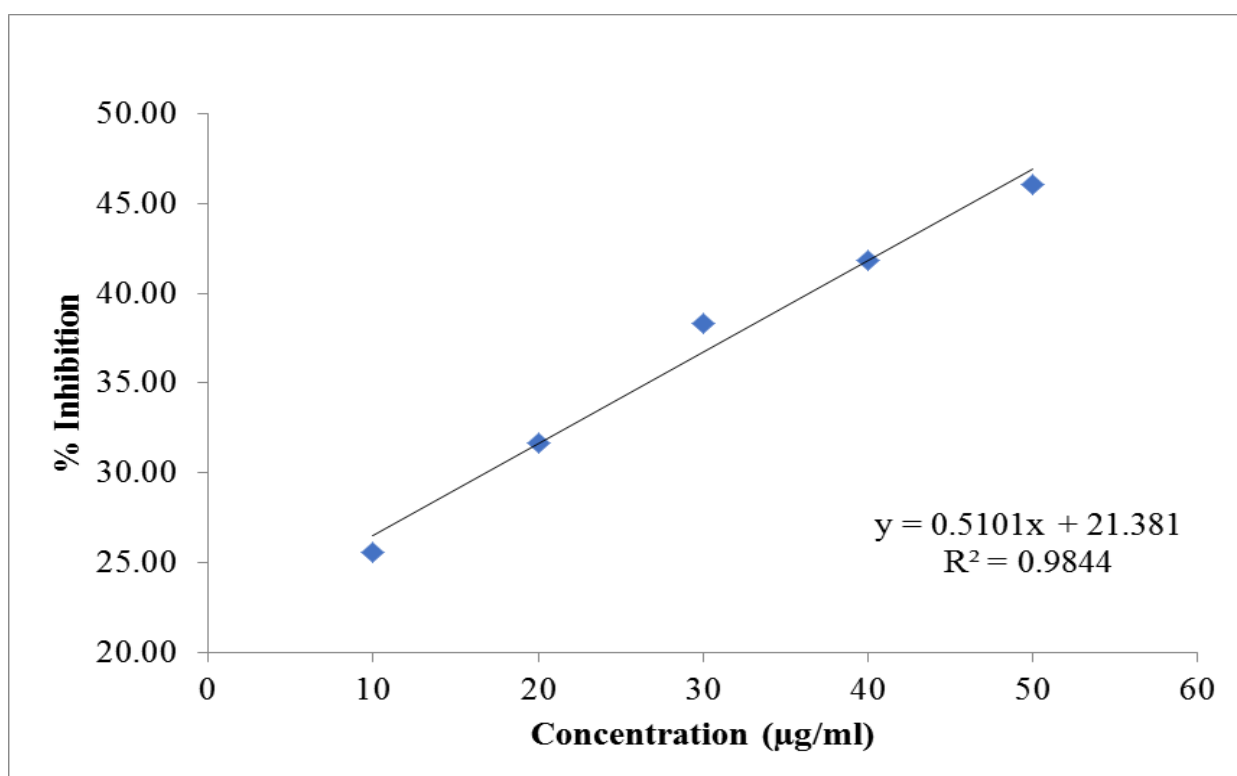


Figure 8: Graph represent regression curve of *Melaleuca alternifolia* methanol leaves extracts by NO assay method

3.5 Phytochemical evaluation

Methanol extract containing leaves shows existence of various phytoconstituent such as terpenoids, phenolic compounds, flavonoids and steroids (Table 9).

Table 9: Preliminary phytochemical analysis for methanolic extract of *Melaleuca alternifolia* leaves extract

| S. No. | Test | Crude Extract |
|--------|---|---------------|
| 1 | Carbohydrates Molisch's test | - |
| 2 | Glycosides Keller-Killiani test | - |
| 3 | Saponins Foam test | + |
| 4 | Alkaloids A.Mayer's test B.Dragendroff's test | - - |
| 5 | Flavonoids A. Alkaline reagent test | + |
| 6 | Phenolics and Tannins A. Ferric chloride test B. Test for Tannins | + + |
| 7 | Phytosterols and Triterpenoids A. Leiberman-Bucharat test B. Salkowaski test | + + |
| 8 | Test for fixed oils and fats A. Oily spot test | - |

Conclusion

The various results shows that *Melaleuca alternifolia* leaves have antioxidant activity due to presence of terpenoids, phenolic compounds, flavonoids and steroids. In DPPH, ABTS, NO, and SO assay the methanol extract of leaves shows various antioxidant activity because the IC₅₀ value of methanol extract is high when compared to ascorbic acid. We can say that that the plant might useful for free radical disorder like, aging, carcinoma etc. Hence further studies are required for plant extracts for identification of phytoconstituents which is responsible for pharmacological activity.

Acknowledgement

The authors thanks to Principal, Khalsa College of pharmacy, Amritsar for providing research and facilities.

References

1. Y. Haidan, M. Qianqian, Y.E. Li, P. Guangchun; The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*. 21(559)(2016) 1-18.
2. O.O. Johnson & G.A. Ayoola,; Antioxidant activity among selected medicinal plants combinations (multicomponent herbal preparation). *Int. J. Res. Health Sci*. 3(3) (2015) 526–532.
3. W.R. Sawadogo, A. Meda, C. Lamien, M. Kiendrebeogo, I.P.Gissou, O.G Nacoulma; Phenolic content and antioxidant activity of six Acanthaceae from Burkina Faso. *Journal of Biological Sciences*. 6 (2006) 249–252.
4. S. Maqsood, S.Benjakul, F.Shahidi,; Emerging role of phenolic compounds as natural food additives in fish and fish products. *Critical Reviews in Food Science and Nutrition*.53(2013) 162–179.
5. J. Gruz, F.A. Ayaz, H.Torun, M. Strnad; Phenolic acid content and radical scavenging activity of extracts from medlar (*Mespilus germanica*, L.) fruit at different stages of ripening. *Food Chem*. 124 (2011) 271-277.

6. T.P. Devasagayam, J.C Tilak, K.K Boloor, K.S Sane, S.S Ghaskadbi, R.D Lele; Free radicals and antioxidants in human health. *current status and future prospects*.52(2004) 794-804.
7. A.M. Adly Amira; Oxidative stress and disease: an updated review.*Research journal of immunology*.3(2) (2010), 129-145.
8. V.A. Kangralkar, S.D Patil & R.M. Bandivadekar; Oxidative stress and diabetes a review. *International Journal of Pharmaceutical Applications*. 1(1) (2010) 38-45.
9. M. Valko, D. Leibfritz, J.Moncola, M.T.D Cronin, M.Mazura, J.Telser; Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*. 39 (2007) 44–84.
10. G.S. Jensen, W.U. Xianli, K.M. Patterson, J. Barnes, S.G. Carter, L. Scherwitz, R. Beaman, J.R. Endres, A.G. Schauss; In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-Blinded, placebo-controlled, crossover study. *Journal of Agricultural and Food Chemistry*.56(2008) 8326-8333.
11. W. Droge; Free radicals in the physiological control of cell function. *Physiological Reviews*. Vol. 82(2002) 47-95.
12. I. Aruoma Okezie; Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists Society*. 75(2)(1998) 199-212.
13. S.E Atawodi; Antioxidant potential of African medicinal plants. *African Journal of Biotechnology*. 4 (2) (2005) 128-133.
14. Y. Christen; Oxidative stress and Alzheimer disease. *The American Journal of Clinical Nutrition*, 71(Suppl) (62) (2000) 1S-9S.
15. E. Souri, G.Amin, H.Farsam, M. Barazandeh Tehrani; Screening of antioxidant activity and phenolic content of 24 medicinal plants extracts. *DARU Journal of Pharmaceutical Sciences*. 16(2) (2008) 83-87.
16. L. Halcon, K. Milkus; *Staphylococcus aureus* and wounds: A review of tea tree oil as promising antimicrobial. *Am J Infect control*.32(7) (2004) 402-408.
17. D.S Buck, D.M. Nidorf, J.G. Addino; Comparison of two topical preparations for the treatment of onychomycosis: *Melaleuca alternifolia* (tea tree) oil and clotrimazole. *J Farm Practice*.38(6) (1994) 601-605.
18. J.B. Bassett, DL. Pannowitz, RS. Barnetson; A comparative study of Tea tree oil versus benzyl peroxide in the treatment of acne. *Med J Aust*. 153(8)(1990) 455-458.
19. J.J. Brophy, NW. Davies, IA Southwell, IA.Stiff, LR Williams; Gas chromatographic quality control for oil of Melaleucaterpinen-4-ol type (Australian tea tree). *J. Agric. Food Chem*. 37(5)(1989) 1330-1335.
20. R.V Tatiana, CA Luiz, RA Célia, FP Vanderlúcia, AN Evandro; Chemical constituents from *Melaleuca alternifolia* (Myrtaceae). *Chemical New*.27(4) (2004) 1-6.
21. I. Gulçin, R. Elias, A. Gepdiremen L Boyer L; Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). *Eur Food Res Technol*.(223) (2006) 759-767.
22. R. Jain and S.K Jain; Total Phenolic Contents and Antioxidant Activities of Some Selected Anticancer Medicinal Plants from Chhattisgarh State, India. *Pharmacologyonline* 2 (2011) 755-762.
23. P. Muthukumar, P. Shanmuganathan, C. Malathi; In Vitro Antioxidant Evaluation of *Mimosa pudica*. *Asian J. Pharm. Res*.1(2) (2011) 44- 46.
24. P. Murali, V.Ashok, V. Madhavan; In Vitro Antioxidant Activity And HPTLC Studies; On The Roots And Rhizomes Of *Smilax zeylanica* L. (Smilacaceae). *Int J Pharm Sci*. 3(1) (2012) 192-195.
25. J.B Harborne; *Phytochemical Screening Guide to modern techniques of plant analysis* 2nd ed. NewYork. Chapman and Hall. (1991). p.653

(2019) ; <http://www.jmaterenvironsci.com>