EVALUATION OF ANTIOXIDANT ACTION OF MELALEUCA BRACTEATA F. MUELL. LEAF EXTRACT

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ABSTRACT

The leaves of the plant grow alternate, are sessile and spirally arranged. The leaves are narrow-ovate or elliptically lanceolate, 1-2 cm long and glabrous with 5-11 veins. The apex of the leaf is acute to acuminate. The extraction yield of the leaf using different solvents is presented in Figure 5.3. The extraction abilities of different solvents were found in the order: ethanol (46.3%)>water (16.7)>chloroform (10.5)>n-hexane (6.3). The findings of the phytochemical analysis suggest the presence of saponin glycosides, phenolics, terpenoids, protein, and flavonoids in the leaves. The total phenolic content of n-hexane, chloroform, ethanolic and aqueous extract of M. bracteata were 7.39±0.91, 48.31±0.26, 74.27±0.59 and 51.83±0.44 GAE mg/g, GAE mg/g, respectively. All the extract of the plant was subjected to in vitro determination of antioxidant potential. The IC50 value of the DPPH scavenging potential for n-hexane extract was found to be more than 250µg/mL whereas for chloroform, ethanol and aqueous extracts it was found to be 152.17, 89.28 and 166.46 µg/mL respectively. The reducing potential was found to be dose dependent and the followed the order ethanolic>aqueous>chloroform>n-hexane. The IC50 value of the percent HRSA for the n-hexane, chloroform, ethanol and aqueous extracts was found to be 250.0, 226.13, 164.31 and 134.4µg/mL respectively. The IC50 value of the percent PRP for the chloroform, methanol and aqueous extracts was found to be 243.24, 169.90, and 210.28µg/mL respectively while the IC50 for n-hexane extract was more than 250µg/mL.

Keywords: - *Melaleuca Bracteata F. Muell.; Antioxidant activity; In – Vitro; Free Radicals.*

1. INTRODUCTION

A medicinal plant, according to the World Health Organization, is any plant, which in one or more of its organs contains substances that can be used for the therapeutic purposes or which, may be precursors for the synthesis of new drugs [1]. The medicinal properties of plants could be largely based on the antioxidant phytochemicals present in them. Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Nimse and Pal, 2015). Antioxidants are responsible for the defense mechanisms of the organism against the pathologies associated to the attack of free radicals. Various antioxidants show substantially varying antioxidative effectiveness in different food systems due to different molecular structure. The antioxidants can be classified as primary or secondary (Hurrell, 2003) else they can also be classified as enzymatic or non-enzymatic (Ratnam et al., 2006) [2]. Free radicals are the types of Reactive Oxygen Species (ROS) that include all highly reactive, oxygen-containing molecules. Different types of ROS include the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical and various lipid peroxides. All these radicals are capable of reacting

with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage [3]. Antioxidants have the capability of stabilizing or deactivating free radicals even before they attack cells. An antioxidant is a substance that can efficiently reduce a pro-oxidant with simultaneous formation of products having no or low toxicity [4].

2. PLANT PROFILE

Melaleuca bracteata cultivar 'Revolution Gold' is a plant belonging to the Myrtaceae family native to Australia. It is a fast-growing evergreen perennial that is widely grown for its attractive golden foliage. The genus of Melaleuca is known to contain around 230 species worldwide predominantly in Australia, Indonesia, tropical America and South Asia. Golden Bottle Bush or Golden Melaleca is a very delicate looking beautiful shrub/tree with delicate branches that wave out in the wind like a flag. It grows to about 8-10 m tall and 1-2 m wide with narrow leaves, which are lance-shaped to linear, 8–28 mm long and 1–3 mm wide with usually no stalk, or a very short stalk. The leaves are arranged spirally around the stem and crowded together. The upper surface of the leaf is hairy, especially when young, with many oil dots [5].

3. MATERIALS AND METHODS [6]

3.1. Collection and Identification of Plant Material

The leaves of M. bracteata were collected from the plant obtained from a local plant nursery of Bhopal, Madhya Pradesh in the month of February and the leaves were submitted for authentication at the Taxonomical department of Saifia Science College, Bhopal.

3.2. Pharmacognostic Study

The pharmacognostic study of the leaves was performed by macroscopic and microscopic examination of the leaves. The cross section of the green leaf was investigated and compared to the reported literature. The leaf type, shape and other macroscopic features were observed and reported.

3.3. Extraction of leaves

The leaves were washed with distilled water, dried under shade and powdered using a blender at low speed. The powdered leaves were sieved to remove any unwanted debris. 100 g of powder was evenly packed in the extractor of the Soxhlet apparatus and subjected to successive solvent extractions using solvent of increasing polarity (n-hexane, chloroform, and ethanol by hot continuous extraction process for about 32 h. The aqueous extraction was done by cold maceration process after completion of the solvent extraction process. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation to reduce the volume to one-tenth. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture. The dried extracts were stored in desiccators for further processing [7].

2.3. Preliminary phytochemical screening [8]

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them.

3.4.1. Test for Alkaloids

3.4.1.1. Mayer's Test: To a few ml of plant sample extract, two drops of Mayer's reagent was added along the sides of test tube.

3.4.1.2. Wagner's test: A few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube.

3.4.1.3. Hager's test: A few drops of Hager's reagent were added to few ml of plant extract along the sides of test tube.

3.4.1.4. Dragendroff's Test: A few drops of Dragendroff's reagent were added to 1 ml of each extract.

3.4.2. Test for Glycosides

3.4.2.1. Saponin glycosides

3.4.2.1.1. Froth test: 1 ml solution of the extract in water was placed in a test tube and shaken vigorously.

3.4.2.2. Anthraquinone glycosides

3.4.2.2.1. Borntrager's test: The extract was boiled with 1.0 ml of dilute sulphuric acid in a test tube for 5 minutes filtered while hot. The filtrate was cooled and shaken with an equal volume of dichloromethane and the lower layer (dichloromethane) was separated and shaken with half its volume of dilute ammonia.

3.4.3. Test for Tannins and phenolic compounds

3.4.3.1. Gelatin test: To the extract was added 1% gelatin solution containing 10% sodium chloride.

3.4.3.2. Ferric chloride test: To the extract was added a freshly prepared solution of ferric chloride.

3.4.3.3. Vanillin hydrochloride test: Test solution of the extract was treated with few drops of vanillin hydrochloride reagent.

3.4.3.4. Alkaline reagent test: Test solution of the extract was treated with sodium hydroxide solution.

3.4.4. Test for Flavonoids

3.4.4.1. Shinoda test: To the test solution of the extract, few fragments of magnesium ribbon were added and conc. hydrochloric acid was mixed drop wise to it.

3.4.4.2. Zinc hydrochloride reduction test: To the test solution a mixture of zinc dust and conc. hydrochloric acid was added.

3.4.5. Test for Proteins and amino acids

3.4.5.1. Millons test: Test solution of the extract was allowed to react with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).

3.4.5.2. Ninhydrin test: The solution of extract was boiled with 0.2% solution of ninhydrin.

3.4.6. Test for Sterols and triterpenoids

3.4.6.1. Libermann Burchard test: Extract was treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added from the sides of the test tube.

3.4.6.2. Salkowski test: The extract was dissolved in chloroform and a few drops of conc. sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time.

4. TOTAL PHENOLIC CONTENT (Shabir et al., 2011)

The extraction of phenolic compounds was based on a modified method by Hsu et al. Briefly 5 g dried powder of leaves was mixed with 80 mL of methanol and kept overnight. The suspension was filtered through a

qualitative cellulose filter paper and the filtrate was diluted to 100 mL with methanol. The solution was stored at 4°C in amber bottles and served as the stock solution (50 mg/mL) for subsequent analyses. For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% Na2CO3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample [9].

5. ANTIOXIDANT ACTIVITY OF M. BRACTEATA EXTRACTS [10]

5.1. DPPH radical scavenging assay (Mishra et al., 2017)

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Separately, 1mM solution of DPPH and extract solution (50-250 µg/mL) were prepared in ethanol. 1.5ml of the extract solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates.

5.2. Reducing power assay (Shabir et al., 2011)

Different concentrations of the extracts (50-250 µg/mL to the final concentration) in methanol (1.0 mL) were diluted with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and mixed with 2.5 mL 1% potassium ferricyanide. After incubation at 50°C for 20 minutes, 2.5 mL of 10% TCA were added to the mixture. 2.5 mL of the reaction mixture was diluted with an equal amount of distilled water and absorbance was measured at 700 nm after treatment with 0.5 mL of 0.1% FeCl3. Increased absorbance of the reaction mixture indicates an increase in reduction capability.

5.3. Phosphomolybdenum assay

10mg of plant extract was dissolved in 1 mL of DMSO. 100µl of the sample was taken and 1 mL of the reagent solution was added to it. The mixture was incubated in a boiling water bath at 95°C for 90 min. After 90 min, the absorbance of the solution was read at 695 nm. Ascorbic acid (10 mg/mLin DMSO) was used as standard. The Phosphomolybdenum reduction potential (PRP) of the studied extracts were reported in percentage using the formula

% of inhibition = (control OD - sample OD/ Control OD) x 100.

6. Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one-way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result was considered statistically significant when P- value less than 0.001 ($P<0.001$) vs control.

7. RESULTS AND DISCUSSIONS

The present work focused on preparing successive solvent extracts of Melaleuca bracteata F. Muell. and evaluating its antioxidant potential using in vitro models. The results obtained from the investigation are presented:

7.1. Extraction Yields: The extraction yield of the leaf using different solvents i.e. ethanol>water>chloroform>nhexane is 46.3>16.7>10.5>6.3, respectively.

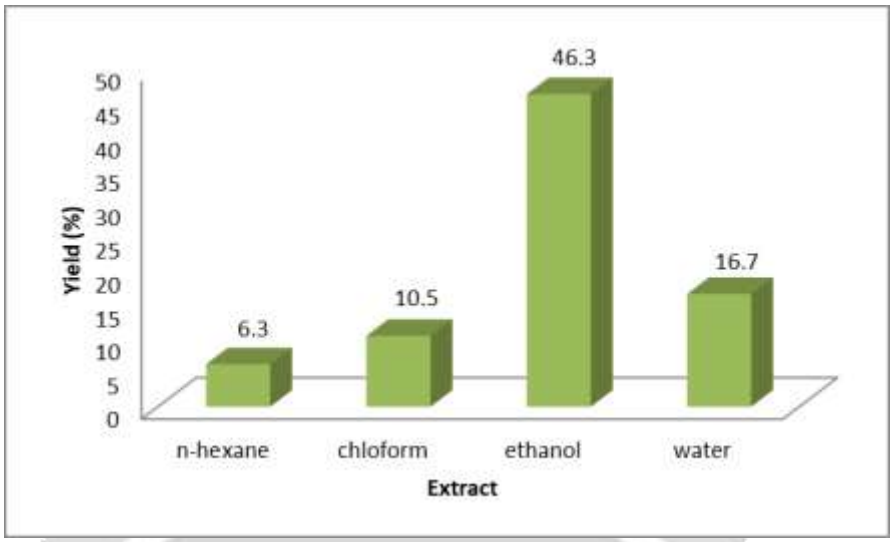

Chart 1. Extraction yields of different solvents

7.2. Evaluation of Antioxidant Activity

The IC50 value of the percent PRP for the chloroform, methanol and aqueous extracts was found to be 243.24, 169.90, and 210.28µg/mL respectively while the IC50 for n-hexane extract was more than 250µg/mL. It was found that the ethanolic fraction was best able to inhibit the phosphomolybdenum complex. A comparative analysis of the results of % PRP for the different extracts is presented in chart 2.

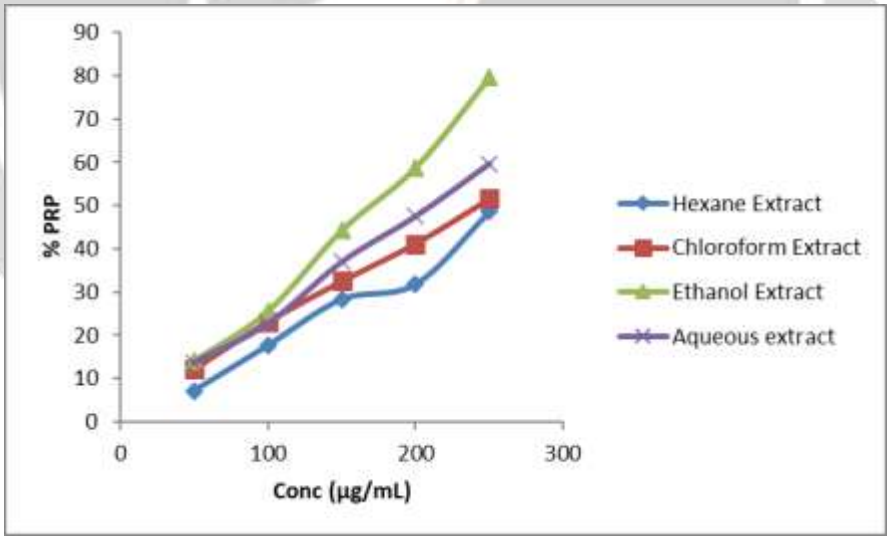

Chart 2. % PRP of various extracts of *M. bracteata*

It was witnessed from the results that all the extracts of plant M. bracteata had potential antioxidant activity which may be attributed to the presence of flavonoids and terpenoids in the extracts. The antioxidant potential of the extracts against various mechanisms of oxidation suggests that the plant may act through inhibiting various mechanisms working for oxidation inhuman body. Although the n-hexane extract was not able to inhibit oxidation mechanism in optimal doses, yet the antioxidant potential of the plant may be considered as a good source of natural antioxidants.

8. CONCLUSION

The objective of the present study was to assess the antioxidant potential of different leaf extracts of Melaleuca bracteata using the in vitro models. The results obtained led to the conclusion that Melaleuca bracteata leaves are a rich source of potential antioxidants. Despite being extracted at high temperature, the inhibition of the oxidation species suggests the presence of phytochemical that are heat stable and possess high antioxidant potential. The availability and adaptability of the plant make it a good source of natural antioxidant that may be converted as nutraceutical supplements.

9. REFERENCES

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