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EVALUATION OF ANTIOXIDANT AND IC50 VALUE OF WILD VEGETABLE MILILOTUS ALBA MEDIK FROM MELGHAT FOREST AMRAVATI REGION (MH).

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Introduction

In recent years, there has been a growing interest in foraging for wild edibles, driven by their potential to enhance nutrition, support sustainable food systems, and promote ecological diversity. The health benefits of wild vegetables are multifaceted, as they have been shown to possess anti-inflammatory, antioxidant, and antimicrobial properties. Often, these natural vegetables take more nutrients from the natural environment than their cultivated counterparts that use chemicals such as pesticides and fertilizers to cultivate. Wild vegetables provide many health benefits, such as an improved digestion system, enhanced immune function, and even chronic disease prevention. To date, however, the knowledge and utilization of wild vegetables in mainstream diets are limited.

Antioxidants protect the human body from oxidative stress, a state of imbalance that occurs between free radicals produced in the body and the ability to neutralize those free radicals. Oxidative stress has been associated with various chronic diseases such as cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders. As a consequence, efforts have been undertaken to identify the presence of natural sources of antioxidants among plant-based foods due to their preventive effect towards oxidative damage in promoting health.

Interestingly, in the last century, such different substances like polyphenol, flavonoids, and carotenoids have been remarked for their free radical scavenging activity and are thus termed antioxidants. Rich sources of many of these substances include fruits and vegetables, herbs, wild plants, and herbs, all of which have been used, albeit unofficially for years to treat almost all diseases caused by oxidative stress. Scientific interest still thrives on their efficacy and mechanisms of action in combating oxidative stress and reducing illness.

Material Method

Medicinally bioactive tissue based drugs separation of plant pairs are important methods of extraction. The separation of components with selective solvents is realized with appropriate extraction technology. The second is termed extraction, which consists of diffusion of solvents into the plant material and solubilizes compounds with similar sign (Green, 2004). The main parameters that can have an impact on the quality of an extract are Solvent to extract and extraction technology; plant material used. The effect of plant material is determined by the nature of plant material; its nature; degree of processing; moisture

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content and particle size, where differences in extraction method are characterized by the extraction type; extraction temperature and time.

Cold method

Preparation of plant extract the plant were cleaned up with tap water and the plant part were extracted. The plant materials were dried in shade and ground to fine inactive powder. About an additional 10 g of material of For each plant, it was crushed in mortar and pestle in 50 mL of respective solvents like Acetone, Platensimycin (20µg) was solubilized in 70% Acetone, 0.5% Ethanol, Petroleum ether, Methanol & Water. The extract was taken into 50 ml centrifuge tubes and subsequently centrifuged at 4000 rpm and at 4°C. Collected the supernatant and evaporated. The semi-solid gummy extract was kept in deep freeze and again administered for further and perform sample stock preparation experiments. Store the extract of all the plants in the respective solvents at 4° C. The working stock of 10 mg/mL concentration was made as stock. The anti-oxidant activity of these compounds can be determined using the colorimetric DPPH assay, which was described by Shimada et al. (Yoshikawa and Naito 2010; Shimada et al. 1992). Were used to assess the radical scavenging activity of the extracts. The ability of the test samples to donate hydrogen is measured in terms of their Deplete of stable organic free radical: DPPH and by subsequent reduction. The absorption of the solution of DPPH deep violet at 517 nm is first measured and then the absorption is reduced to a yellow-white color, if decolorization occurs. This decrease stoichiometric level based on the reduction degree in absorption (Arulpriya et al., 2010)

Antioxidant activity (DPPH Free Radical Scavenging Assay)

Nowadays there is an increase in interest worldwide in identification of pharmacologically potent antioxidant compounds and with no side effects. Such compounds plays an important role as a health-protecting factor and it neutralizes the free radicals, which are unstable molecules and are linked with the development of a number of degenerative diseases and conditions including hepatic disease, immune dysfunction, cataracts and macular degeneration. Scientific reports suggest that antioxidants also reduce the risk for chronic diseases and conditions. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide; hydroperoxide or lipid peroxyl which are thereby involved in reducing the risk of diseases associated with oxidative stress.

The anti-oxidant activity of compounds can be determine by using the colorimetric DPPH assay, as described by Shimada et al., (1992) to determine the radical scavenging activity of the plant extracts.

The hydrogen donating capacity of test samples is quantified in terms of their ability to scavenge the relatively stable, organic free radical DPPH and by consequent reduction. The absorption of the deep violet DPPH solution is measured at 517 nm, after which absorption decreases due to decolorization to a yellow-white color, in the event of reduction.

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This decrease in absorption is stoichiometric according to the degree of reduction (Arulpriya et al., 2010).

Preparation of Sample

The free-radical scavenging activity was estimated by DPPH assay. The reaction mixture contained 10 µl of test sample and positive control ascorbic acid with 1 mg concentration and 190 µl of methanolic solution of 0.1 mM DPPH radical. The mixture was then shaken vigorously and incubated at 37° C for 5 min. The absorbance was measured at 517 nm on ELISA plate reader indicated higher free radical scavenging activity, which was calculated using the following equation:

$$(\%)\text{Free radical scavenging effect} = \frac{[\text{Absorbance of control (Ac)} - \text{Absorbance of sample (As)}]}{\text{Absorbance of control (Ac)}} \times 100$$

Antioxidant Potential of synthetic compounds

The antioxidant activities was successfully performed of the both selected plant extract were analyzed for their antioxidant potential by DPPH (2, 2 diphenyl-1-picryl hydrazyl) free radical scavenging assay. The results are shown in the table, graph and picture below;

Fig. Mililotus alba Photo

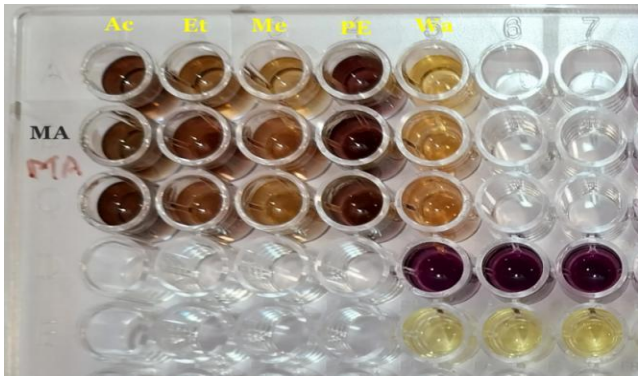

Fig. Antioxidant Activity Photographs

Result and Conclution

Table: % Antioxidant Potential Using DPPH Assay Method (Conc. 1 mg)

No.	Name of Sample	Antioxidant Potential (Mean±SD)
1	MA-A	4.856±0.225
2	MA-E	6.455±2.035
3	MA-M	12.138±2.546
4	MA-P	0
5	MA-W	28.410±1.323

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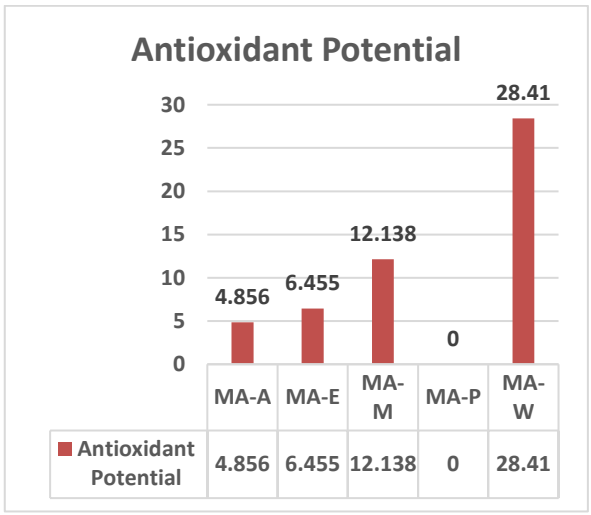
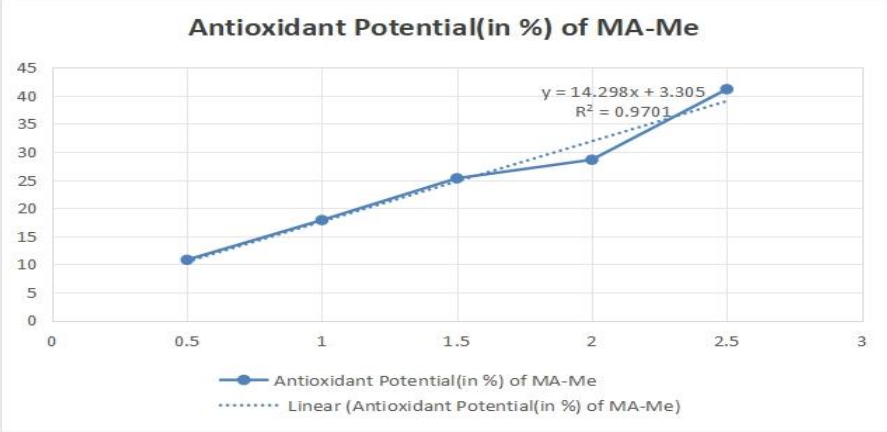


*All the data statistically analyzed with mean±SD (n=3)	Graph : Free Radical Scavenging Potential (Antioxidant Potential) of

Name of Sample	Concentration (in mg)	% Antioxidant Activity	Y-equation	R^2 value	IC_{50} value
MA-Me	0.5	10.795±1.251	14.298x + 3.305	0.9701	3.265
	1	17.871±1.363			
	1.5	25.309±2.005			
	2	28.615±2.454			
	2.5	41.167±1.115			

Table: Antioxidant potential of Mililotus alba -Methanol extract at different concentration & determination of IC_{50}



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Graph: Antioxidant potential of Mililotus alba -Methanol extract at different concentration.

Conclution

Mililotus alba Medik antioxidant activity of Mililotus alba Medik extracts was measured using various solvents: acetone, ethanol, methanol, petroleum ether, and water. The maximum antioxidant activity was recorded for the water extract (28.410 ± 1.323), followed by methanol extract (12.138 ± 2.546). Ethanol extract also exhibited high antioxidant activity (6.455 ± 2.035), whereas acetone extract exhibited moderate activity (4.856 ± 0.225). No antioxidant activity was observed in the petroleum ether extract. The IC50 value of methanol extract of Mililotus alba (Mililotus alba –Methanol) is given in the table. The IC50 value is 3.265 mg, which is the concentration at which the extract exhibits 50% antioxidant activity. The low IC50 value suggests relatively strong antioxidant potential of the Mililotus alba methanol extract because lower IC50 values reflect higher antioxidant activity. The R2 value of 0.9701 also suggests good fit of the data to the linear regression model represented by the Y-equation (14.298x + 3.305), suggesting strong correlation between concentration and antioxidant activity.

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