Evaluation of total phenol and flavonoids of wild vegetable *Mililotus alba* Medik from Melghat forest region of Amravati district (MS)

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ABSTRACT

The total phenolic and flavonoid content of *Melilotus alba* Medik using various solvents to evaluate their extraction efficiency. The results demonstrate that acetone, a moderately polar solvent, was the most effective for extracting both phenolic and flavonoid compounds, yielding the highest concentrations (0.656±0.105 mg/g for phenols and 0.571±0.021 mg for flavonoids). Ethanol (0.357±0.063 mg/g for phenols), water (0.303±0.026 mg/g for phenols, 0.064±0.004 mg for flavonoids), and methanol (0.214±0.033 mg/g for phenols) exhibited varying extraction efficiencies, with methanol showing relatively lower results. Petroleum ether showed no measurable content for either compound (0.0±0.0 mg). These findings suggest that solvent polarity plays a significant role in the extraction of bioactive compounds, with acetone emerging as the most efficient solvent for extracting both phenolic and flavonoid compounds. Further investigation into the antioxidant and pharmacological properties of *Melilotus alba* Medik is essential to explore its potential applications in the nutraceutical and medicinal sectors.

Keywords:

Melilotus alba Medik, phenolic, flavonoid, acetone, ethanol, methanol, water, petroleum ether, bioactive compounds, antioxidant.

INTRODUCTION

The Melghat Forest, located in the Amravati district of Maharashtra, is reported to be a biodiversity hotspot with a large number of wild edible plants, which have been serving as a source of food and medicine for many of the local indigenous population. These plants, though of traditional importance, have hardly been studied for their phytochemical composition, particularly

for the presence of phenolic and flavonoids, which are crucial indicators of antioxidant potential.Wild vegetables have a rich content of nutrients and bioactive compounds in traditional diets and medicinal practices. Of these compounds, phenols and flavonoids are more critical for their role in the antioxidant activity; such activity explains various health-related properties, anti-inflammatory, antimicrobial, and anticancer being the most potent.[2,3]

The present study aims to evaluate the total phenol and total flavonoid content in selected wild vegetables from Melghat Forest. By assessing their phytochemical properties, this research seeks to highlight their potential nutritional and medicinal value, thereby promoting their conservation and sustainable utilization.[5]

MATERIAL METHOD

Quantification of Total Phenol: Total phenol content in the sample can be quantified with the help of several methods. One of them is the Folin-Ciocalteau assay. Following is a basic protocol for this:

Materials: Sample (eg, plant extract), Folin-Ciocalteau reagent, Sodium carbonate (Na_2CO_3), Distilled water, Test tubes or micro plates, Spectrophotometer.

1. Sample preparation: Take the sample to be assayed in its diluted form, based on the concentration, for a suitable dilution that falls within the working range of your spectrophotometer. This usually ranges from 1:10 to 1:100.

2. Preparation of Folin-Ciocalteau reagent: Mix and combine Folin-Ciocalteau reagent with distilled water (1:10).

3. Preparation of sodium carbonate solution: Dissolve sodium carbonate in distilled water to give a solution that is 2% (w/v).

4. Reaction mixture:Add 0.1 ml of your sample in a test tube or micro plate, Add 0.5 ml of the Folin-Ciocalteau reagent, Mix well and let it stand for 5-10 minutes in the dark, Add 1.5 ml of the sodium carbonate solution to the mixture. Mix well.

5. Incubate the reaction mixture in the dark at room temperature for 30 minutes.

6. After incubation, measure the absorbance of the reaction mixture at 765nm using a spectrophotometer.

7. Prepare a standard curve: Use a known concentration of a standard phenol solution to prepare a calibration curve by following steps 4-7.

8. Calculate the total phenol concentration in your sample using the calibration curve. This is usually represented in milligrams of Gallic acid equivalents (GAE) per gram or millilitre of sample.

Quantification of Total Flavonoids:-The method you're referring to for the quantification of total flavonoids, which involves sodium hydroxide and sodium nitrite, is oftentimes called the aluminium chloride colorimetric method. Note that the reagents used in this method include aluminium chloride, sodium nitrite, and sodium hydroxide among others. It is a method which largely depends upon the efficiency of its simplicity and ability of estimation of content in various samples. Below is a step-by-step protocol for this assay:

Materials: Sample or extract with flavonoids, Ethanol or methanol for extraction and dilution, Distilled water, Sodium nitrite (NaNO2), Aluminium chloride (AlCl3), Sodium hydroxide (NaOH), Quercetin or any appropriate flavonoid standard for calibration, UV-Visible spectrophotometer, Pipettes and cuvettes.

Sample Preparation: Dissolve 1 mg of extract in an appropriate solvent, centrifuge and keep for further use.

Determination of Flavonoid Content:- Preparation of reaction mixture in a test tube, mix a known volume of the extract, e.g., 1 ml. Add 1 ml of 2% $NaNO_2$ solution. After 5 minutes, add 1 ml of 10% $AlCl_3$ solution. Wait for another 6 minutes, then add 2 ml of 1 M NaOH. Finally, add enough distilled water to bring the total volume to 10 ml. Incubation: Shake contents well after incubation allow the mixture at room temperature to stand for 15 minutes. Measurement of Absorbance: Measure the reaction mixture's absorbance at 510 nm, using a UV-Visible spectrophotometer. Prepare the blank sample of all reagents except the extract. Preparation of standard solution: The standard curve prepare using known solutions of concentration by a flavonoid standard Quercetin like.

Repeat the protocol followed for the test samples.

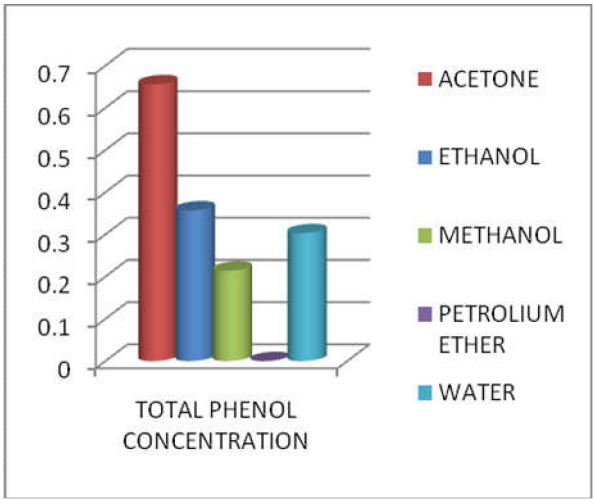
Calculation: Calculate the total flavonoid content using the standard curve. Express the results as mg of Quercetin equivalents (QE) per g or ml of the sample.[7,8,9]

RESULT AND DISCUSSION

Quantification of total phenol in mg in *Mililotus alba*plants.

Name of Solvent	*Mililotus alba*
Acetone	0.656±0.105
Ethanol	0.357±0.063
Methanol	0.214±0.033
Petroleum Ether	0.0±0.0
Water	0.303±0.026

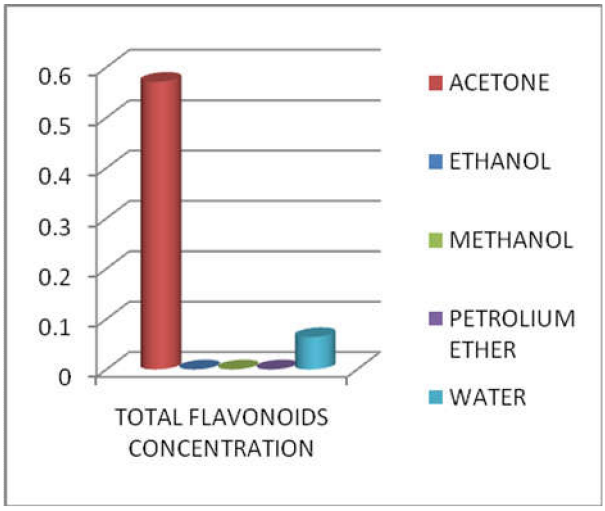
Table : Quantification of total phenol in mg in *Mililotus alba*plants



Graph: - Quantification of total phenol in mg in *Mililotus alba*plants.

Name of Solvent	*Mililotus alba*
Acetone	0.571±0.021
Ethanol	0.000
Methanol	0.000
Petroleum Ether	0.000
Water	0.064±0.004

Table:- Quantification of total flavonoid in mg in *Mililotus alba* plants.



Graph :- Quantification of total flavonoid in mg in *Mililotus alba* plants.

The present study evaluated the total phenol and flavonoid content in *Melilotus alba* Medik using different solvents. The results indicate that acetone was the most effective solvent for extracting phenolic and flavonoid compounds, yielding the highest concentration (0.656±0.105), followed by ethanol (0.357±0.063) and water (0.303±0.026). Methanol showed relatively lower

extraction efficiency (0.214±0.033), while petroleum ether did not extract any measurable phenolic or flavonoid content (0.0±0.0). These findings suggest that solvent polarity significantly influences the extraction efficiency of bioactive compounds from *Melilotus alba* Medik Acetone, being a moderately polar solvent, appears to be the most suitable for obtaining maximum phenolic and flavonoid content. Further research on antioxidant and pharmacological activities can help explore the potential applications of *Melilotus alba* Medik in nutraceutical and medicinal fields.

The quantification of total flavonoid content in *Melilotus alba* Medik using different solvents revealed significant variations in extraction efficiency. Among the tested solvents, acetone exhibited the highest flavonoid yield (0.571±0.021 mg), indicating its effectiveness in extracting flavonoid compounds. In contrast, ethanol, methanol, and petroleum ether did not extract any measurable flavonoids (0.000 mg). Water showed a minimal flavonoid content (0.064±0.004 mg), suggesting limited solubility of these compounds in aqueous solutions. These findings highlight the importance of solvent selection in flavonoid extraction, with acetone being the most effective for *Melilotus alba* Medik Further studies on antioxidant and pharmacological activities are recommended to explore the potential health benefits and applications of these bioactive compounds.

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