Journal of East-West Thought
ISSN: *2168-2259 (online)*
(https://jetjournal.us/)
Volume 15, Issue 1, Jan-March – 2025 Special Issue 2

Impact Factor: 7.665, Peer Reviewed and UGC CARE I

EVALUATION OF TOTAL PHENOL AND TOTAL FLAVONOIDS OF *LEEA MACROPHYLLA* ROXB. IN MELGHAT FOREST AMRAVATI DISTRICT

Mr. Siddharth G. Ikhare, Assistant Professor, Department of Botany, Late Annasaheb R. D. Deore Arts and Science College, Mhasadi
Mr. Ganesh A. Padghan, Assistant Professor, Department of Botany, Vasantrao Naik Arts, Science & Commerce College, Shahada, Dist. Nandurbar

INTRODUCTION

Leea macrophylla Roxb. Known locally as *Hanshin Dabar* or *Hatkhan* in Odisha(India), reportedly has been in use traditionally by many tribes and communities to cure diseases.

Phenolic compounds, such as total phenols and flavonoids, are a varied group of naturally occurring secondary metabolites found in plants. These compounds are well-known for their antioxidant, anti-inflammatory, and antimicrobial properties, which play a crucial role in human health and nutrition. Total phenols are defined by their ability to donate hydrogen atoms or electrons, neutralizing free radicals and safeguarding cells from oxidative stress. This helps reduce the risk of chronic diseases like cardiovascular disease, cancer, and neurodegenerative disorders. Flavonoids, a large subgroup within phenolic compounds, are recognized for their wide range of pharmacological activities, including anti-inflammatory, anticancer, and anti-allergic effects.

METHODOLOGY

Cold Extraction of Plant Extract

Preparation of Sample Plant Material :- Samples of the selected plants materials were collected, washed with tap water to remove impurities and shade-dried for preserving their bioactive components. The dried plant material was then mechanically ground into a fine powder.

Extraction

For extraction, 10 g of the powdered plant material was weighed and crushed using a mortar and pestle in 50 mL of different solvents, including acetone, ethanol, petroleum ether, methanol, and water. The resulting mixture was transferred into 50 mL centrifuge tubes and subjected to centrifugation at 4000 rpm for 15 minutes at 4°C.

Collection and Storage of Extracts :- The supernatant was then carefully collected following centrifugation and allowed to evaporate and get a semi-solid, gummy extract. The extracts were kept at 4°C in a deep freezer for further use in experiments.

Quantification of Total Phenol: Quantifying total phenols in a sample can be done using various methods. One common method is the Folin-Ciocalteau assay. Here's a simplified protocol for it:

Journal of East-West Thought
ISSN: *2168-2259 (online)*
(https://jetjournal.us/)
Volume 15, Issue 1, Jan-March – 2025 Special Issue 2
Impact Factor: 7.665, Peer Reviewed and UGC CARE I

Materials Needed: Sample (e.g., plant extract)**,** Folin-Ciocalteau reagent**,** Sodium carbonate (Na2CO3)**,** Distilled water, Test tubes or micro plates**,** Spectrophotometer.

Protocol:

1. Prepare the sample: Dilute your sample appropriately, depending on its concentration, so that it falls within the linear range of your spectrophotometer (usually 1:10 or 1:100).
2. Prepare the Folin-Ciocalteau reagent: Mix the Folin-Ciocalteau reagent with distilled water (typically 1:10 dilution) to make a working solution.
3. Prepare the sodium carbonate solution: Dissolve sodium carbonate in distilled water to create a 2% (w/v) solution.
4. Reaction mixture: In a test tube or micro plate, add 0.1 mL of your sample, 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 765 nm using a spectrophotometer.
7. Prepare a standard curve: Use a known concentration of a standard phenol solution to create a calibration curve by following steps 4-7.
8. Calculate the total phenol concentration in your sample using the calibration curve. This is typically expressed in milligrams of Gallic acid equivalents (GAE) per gram or milliliter 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 commonly known as the aluminum chloride colorimetric method. It's important to note that the reagents in this method include aluminum chloride, sodium nitrite, and sodium hydroxide, among others. This method is widely used for its simplicity and effectiveness in estimating the total flavonoid content in various samples. Below is a step-by-step protocol for this assay:

Materials: Plant extract or sample containing flavonoids, Ethanol or methanol (for extraction and dilution),Distilled water, Sodium nitrite (NaNO2),Aluminum chloride (AlCl3),Sodium hydroxide (NaOH), Quercetin or other suitable flavonoid standard for calibration, UV-Visible spectrophotometer, Pipettes and cuvettes.

Procedure: Sample Preparation: Dissolve 1 mg extract in the suitable solvent, centrifuge it and store for use.

Flavonoid Content Determination: 1. Preparation of Reaction Mixture: In a test tube, mix a known volume of the extract (e.g., 1 mL), Add 1 mL of 2% NaNO2 solution. After 5 minutes, add 1 mL of 10% AlCl3 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. **2. Incubation:** Mix the contents well. Allow the mixture to stand for 15 minutes at room temperature. **3. Measurement**

Journal of East-West Thought
ISSN: *2168-2259 (online)*
(https://jetjournal.us/)
Volume 15, Issue 1, Jan-March – 2025 Special Issue 2
Impact Factor: 7.665, Peer Reviewed and UGC CARE I

of Absorbance: Measure the absorbance of the reaction mixture at 510 nm using a UV-Visible spectrophotometer. Use a blank sample containing all reagents except the extract.**4. Preparation of Standard Solution:** Prepare a standard curve using solutions of known concentrations of a flavonoid standard like Quercetin.

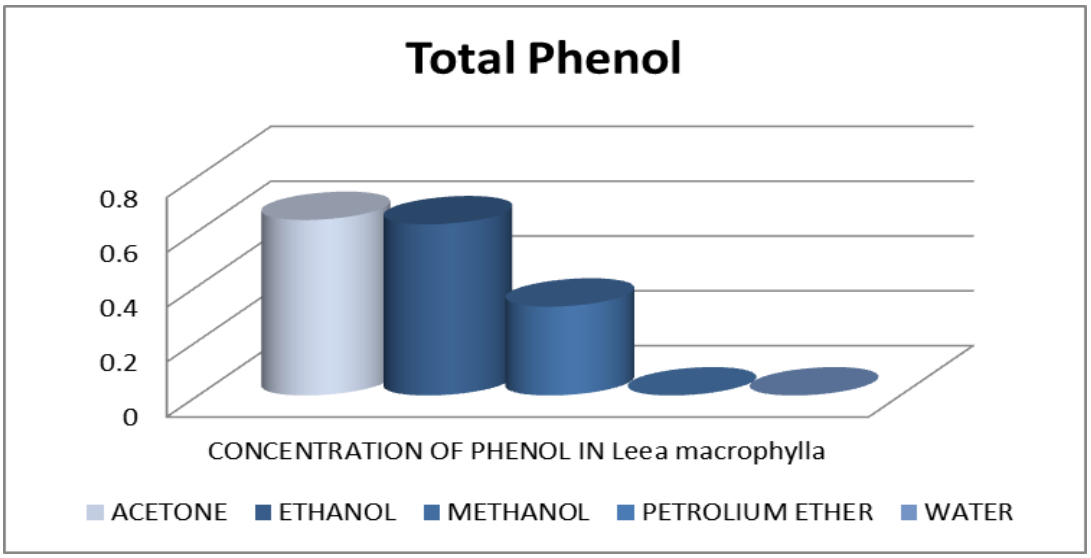
Follow the same procedure as for the test samples.

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.

OBSERVATION AND RESULT

Name of Solvent	Total Phenol concentration(mg GAE/g extract)
Acetone	0.640±0.014
Ethanol	0.624±0.077
Methanol	0.324±0.085
Petroleum Ether	0.0±0.0
Water	0.0±0.0

Table 1. Total Phenol of Leea macrophylla (mg GAE/g extract)



Graph 1. Total Phenol of Leea macrophylla

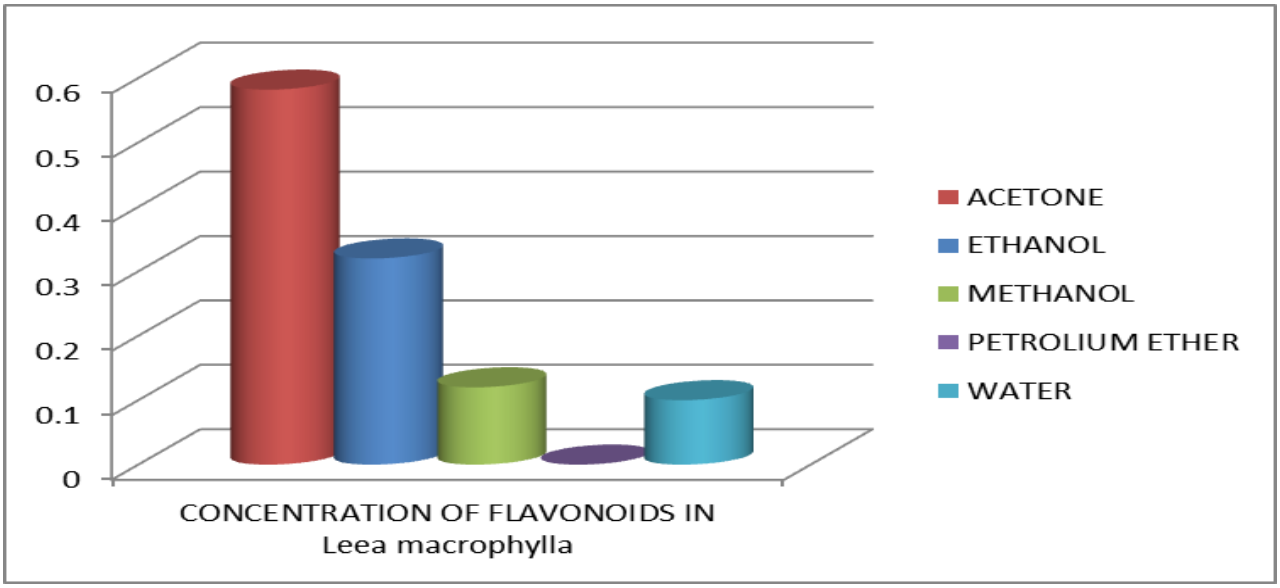
RESULT

The results indicate that acetone exhibited the highest total phenol concentration, followed closely by ethanol. Methanol extracted a lower phenolic content, while petroleum ether and water showed no detectable phenolic compounds.

Journal of East-West Thought
ISSN: *2168-2259 (online)*
(https://jetjournal.us/)
Volume 15, Issue 1, Jan-March – 2025 Special Issue 2
Impact Factor: 7.665, Peer Reviewed and UGC CARE I

Name of Solvent	Total Flavonoids Concentration(mg QE/g extract)
Acetone	0.582±0.037
Ethanol	0.320±0.014
Methanol	0.120±0.013
Petroleum Ether	0000
Water	0.100±0.002

Table 2. Total Phenol of Leea macrophylla (mg QE/g extract)



Graph 2. Total Phenol Of Leea macrophylla

RESULT: The results indicate that acetone was the most effective solvent for flavonoid extraction, yielding the highest concentration. Ethanol also demonstrated a moderate extraction efficiency, while methanol and water resulted in significantly lower flavonoid concentrations. Petroleum ether did not extract detectable flavonoids.

CONLUSION:- Total phenol - This research finding shows that the choice of solvent has a great influence on the extraction of phenolic compounds. Among all the tested solvents, acetone showed the highest efficiency for extracting total phenols, closely followed by ethanol. Methanol exhibited a lesser extraction capacity. The petroleum ether and water showed an inability to extract phenolic compounds. The results imply that polar organic solvents, especially acetone and ethanol, are better for the extraction of phenolic compounds. Such knowledge is very essential in the selection of an optimum solvent in subsequent studies related to the extraction of bioactive compounds from plant material.

Journal of East-West Thought
ISSN: *2168-2259 (online)*
(https://jetjournal.us/)
Volume 15, Issue 1, Jan-March – 2025 Special Issue 2
Impact Factor: 7.665, Peer Reviewed and UGC CARE I

Total flavonoids :- The findings of this research show that the type of solvent has a strong influence on flavonoid extraction. Among the solvents evaluated, acetone was the best in terms of flavonoid extraction efficiency followed by ethanol. Methanol and water had very low extraction capabilities, while petroleum ether was not able to extract flavonoids. These results indicate that polar solvents such as acetone and ethanol are more appropriate for flavonoid extraction. This knowledge can be helpful in optimizing solvent selection for the extraction of bioactive compounds in future research and industrial applications.

REFERENCES

Aberoumand, A., & Deokule, S. S. (2009). Studies on nutritional values of some wild edible plants from Iran and India. Pakistan Journal of Nutrition, 8(1), 26-31.

Antonieta R., Hermosin-Gutierrez, I., Mardones, C., Vergara, C., Herlitz, E., Vega, M., ... & von Baer, D. (2010). Polyphenols and antioxidant activity of calafate (Berberis microphylla) fruits and other native berries from Southern Chile. Journal of Agricultural and Food Chemistry, 58(10), 6081-6089.

Aruoma, O.I. (1993). Free radicals and food. Chemistry in Britain. pp. 210-214.

Behl C. (1999). Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. Progress in Neurobiology. 57(3): 301-23

Bhogaonkar Prabha Y.1, Vishal R. Marathe2 and Prachi P. Kshirsagar (2010). Documentation of Wild Edible Plants of Melghat Forest, Dist. Amravati, Maharashtra State, India. Ethnobotanical Leaflets 14: 751-58.

Bonoli M, Verardo V, Marconi E and Caboni MF. (2004). Antioxidant phenols in barley (Hordeum vulgare L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic acids. J Agric Food Chem. ; 52: 5195-5200.

Brand-Williams W, Cuvelier M, Berset C. (1995). Use of free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 28(1): 25-30.

Chatha SAS, Anwar F, Manzoor M and Bajwa JR. (2006) Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasas Aceites Sevilla.; 57: 328-335.

Choudhury, R., Choudhury, M. D., De, B., & Paul, S. B. (2010). Importance of certain tribal edible plants of Tripura

Danesi, F. (2009). Biological effects of bioactive components and extracts derived from edible plants commonly used in human nutrition.

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. Japi, 52(794804), 4.

Fernández-López, J. A., Almela, L., Obón, J. M., & Castellar, R. (2010). Determination of antioxidant constituents in cactus pear fruits. Plant Foods for Human Nutrition, 65, 253-259.

Journal of East-West Thought
ISSN: *2168-2259 (online)*
(https://jetjournal.us/)
Volume 15, Issue 1, Jan-March – 2025 Special Issue 2
Impact Factor: 7.665, Peer Reviewed and UGC CARE I

Pal, R. S., Kumar, R. A., Agrawal, P. K., & Bhatt, J. C. (2013). Antioxidant capacity and related phytochemicals analysis of methanolic extract of two wild edible fruits from north western Indian Himalaya. Int J Pharm Bio Sci, 4(2), 113-123.

Saklani, S., Chandra, S., & Mishra, A. P. (2011). Evaluation of antioxidant activity, quantitative estimation of phenols, anthocynins and flavonoids of wild edible fruits of Garhwal Himalaya. Journal of Pharmacy Research, 4(11), 4083-4086.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of agricultural and food chemistry, 40(6), 945-948.