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Journal of General Medicine and Clinical Practice

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Research Article

Physicochemical, Phytochemical and Antioxidant Study of *Pyrus Malus*

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Received date: February 17, 2025; Accepted date: May 20, 2025; Published date: May 28, 2025

Citation: Rakhi Mishra, Binit Dwivedi, (2025), Physicochemical, Phytochemical and Antioxidant Study of *Pyrus Malus*, *J. General Medicine and Clinical Practice*, 8(5); **DOI:10.31579/2639-4162/256**

Abstract

Background/Aim: *Pyrus malus* (apple), commonly utilized in traditional medicine, is gaining interest for its therapeutic potential. However, standardized information on its mother tincture and raw drug material is limited. This study aimed to establish a comprehensive profile of *Pyrus malus* through physicochemical, phytochemical, and chromatographic analyses to support its pharmaceutical standardization.

Materials and Methods: The mother tincture and raw drug material of *Pyrus malus* leaves were analyzed using standard pharmacopoeial procedures. Physicochemical parameters such as pH, alcohol content, total solids, loss on drying, water and alcohol soluble extractive values were measured. Preliminary Phytochemical screening was performed to detect the presence of various bioactive compounds. High Performance Thin Layer Chromatography (HPTLC) was conducted using chloroform: methanol (9:1, v/v) as the mobile phase, with spot visualization under UV light at 254nm and 366nm and after derivation with anisaldehyde sulphuric acid reagent.

Results: The mother tincture showed a pH of 4.58, alcohol content of 41% v/v, and total solid of 2.05 v/v. The raw drug material exhibited a 4.90% loss on drying and an 18.60% water-soluble extractive value. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, glycosides, and phenolic compounds. HPTLC analysis provided distinct spot patterns, indicating multiple bioactive constituents. The optimized mobile phase demonstrated effective separation, confirming the chemical complexity of the extract.

Conclusions: The study successfully established a physicochemical and phytochemical profile of *Pyrus malus* mother tincture and raw drug material. Chromatographic profile further confirmed the presence of diverse bioactive compounds. These findings support the standardization of *Pyrus malus* for pharmaceutical applications and encourage pharmacological properties and expand its medicinal applications.

Kew Words: HPTLC; UV; physicochemical; *pyrus malus;* homoepathy; antioxidant study

1. Introduction

Pyrus malus also known as apple belongs to the family of Rosaceae[1] and comprises more than 300 species. It is an important remunerative crop grown across the temperature regions of the world. *Pyrus malus* is native to South-West Asia[2]. *Pyrus malus* are rich in vitamins and minerals[3]consuming these fruits aids in weight loss enhances brain health, provides antioxidants, reduces the risk of metabolic syndrome and prevents some forms of cancer[2]. *Pyrus malus* is reported to contain triterpenoids, flavonoids, organic acids and sterols[4]. Pharmacologically *Pyrus malus* is reported to have antioxidants, antiobesity, anticholesterol, anticancer, enzyme inhibitor and antimicrobial activity[5]. *Pyrus malus* is beneficial for general skin health with topical application. *Pyrus malus* strengthens the immune system and prevents diseases. *Pyrus malus* leaves and fruits are rich in vitamins and minerals, trace elements, amino acids and flavonoids[5]. The fruits of *Pyrus malus* contain malic acid, which has tonic, keratolytic and antiseptic properties. The astringent principle of *Pyrus malus* includes tannins, tannin derivatives and colouring materials(flavones)[6]. Due to its rich vitamin and mineral content, it helps in skin degeneration, which causes premature ageing. Its soothing antiseptic and keratolytic agents help loosen dead skin

fragrant. *Pyrus malus* has low antigen content which is useful in managing immune-mediated diseases[7]. *Pyrus malus* is believed to be a good source of antioxidants[8]. Previous research reported that *Pyrus malus* fruit contains epicatechin, procyanidins, gallic acid, protocatechuic, chlorogenic, ferulic and p-coumaric, quercetin, and myricetin[9]. *Pyrus malus* plant has high levels of antioxidants and anti-inflammatory activities, which help to reduce age-associated diseases such as cardiovascular disease, hypertension, diabetes, CVD associated with obesity, and stroke and improve bone health[10] Homoeopathy, *Pyrus malus* leaves are used. The major characteristics of a plant are affected by its genetic background and the environment where it is grown. *Pyrus malus*, commonly known as the apple tree, has long been a subject of interest in homoeopathy and phytochemistry due to its medicinal properties. While there's limited specific research on the homoeopathic mother tincture of *Pyrus malus* regarding its phytochemical composition, antioxidant activity, and HPTLC profiling, addressing these factors is crucial for authenticating and enhancing its medicinal potential. Homoeopathic mother tinctures are primarily alcoholic extracts, retaining a variety of secondary metabolites such as flavonoids, phenolics, tannins, and alkaloids. These compounds are often responsible for antioxidant and therapeutic properties. Comprehensive phytochemical screening involves determining the presence of bioactive constituents commonly found in *Pyrus malus* leaves homoeopathic mother tincture. The antioxidant potential of *Pyrus malus* homoeopathic mother tinctures can be evaluated using assays like DPPH[11], ABTS[12], or FRAP[13]. These studies help quantify the free radical scavenging capacity of the tincture, which is a significant indicator of its therapeutic efficacy. High-Performance Thin Layer Chromatography (HPTLC)[14] is a standard method for identifying and quantifying specific phytochemicals in herbal tinctures. HPTLC can be employed to fingerprint phenolic acids and flavonoids, ensuring the consistency and authenticity of the homoeopathic preparation. The quality and composition of phytochemicals [15] in *Pyrus malus* mother tincture depend significantly on climatic conditions, soil type, altitude, and agricultural practices. Plants grown in temperate regions with optimal sunlight and humidity are likely to yield higher levels of bioactive compounds. Documenting and standardising raw material sources is crucial for ensuring batch-to-batch consistency in the tincture. Homoeopathic mother tinctures should be prepared following strict pharmacopoeia guidelines, provided the raw drug is fresh, authentic, and contamination-free. The origin of the raw drug must be authenticated through botanical identification and traceability to reputable sources. Studies focusing on standardization, bioactivity, and clinical efficacy of *Pyrus malus* leaves homoeopathic mother tincture are needed to establish its scientific basis. Comparative studies using different *Pyrus malus* leaves mother tinctures might reveal variations in effectiveness and bioactivity, emphasizing the importance of genetic and environmental factors. Standardization parameters were determined by the guidelines given by the World Health Organization (WHO)[15]. Parameters including extractive values, ash values and loss on drying were determined. Preliminary phytochemical test, antioxidant stud and chromatographic profiling were performed for the identification and standardization of *Pyrus malus* leaves. *Pyrus malus* leaves are a rich source of antioxidant compounds Antioxidant components are micro constituents that inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions and are involved in scavenging free radicals[16]. In view of that, we designed the study to evaluate the antioxidant potential of *Pyrus malus* by various assay methods. The present study is helpful for the determination and quantification of antioxidant compounds which are useful for producing *Pyrus malus* safer drugs for the treatment of various ailments of human beings.

Figure 1: Image of *Pyrus malus* leaf

2. Materials and Methods

The leaves of *Pyrus malus* were collected from the flora of the Nilgiri District, Tamil Nadu by Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu, India and was authenticated by the staff of the Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Ooty. The voucher specimen has been deposited in the herbarium and the laboratory of DDPR Central Research Institute for Homoeopathy, Noida, Uttar Pradesh, India, for future reference. Authentic plant material was used to prepare the alcoholic extract. The solvents ethanol, HPLC water, methanol and chloroform were of analytical grade purity (Merck Ltd., India).

2.1. Physicochemical studies of raw drug standardization

2.1.1. Determination of Loss on Drying

Loss on drying method used for determination of moisture content as per methods recommended in Homoeopathic Pharmacopeia of India [17]. Percentage loss on drying was calculated.

2.1.2. Determination of Foreign matter

For foreign matter determination, 100 g of plant raw material has been taken and outspread in the form of a thin-layer. The sample was examined by a 6x lens or with an unaided eye, and the foreign organic matter was picked

manually. The ratio of total foreign matter weighed, and the weight of the drug taken gave the % of foreign matter.

2.1.3. Determination of Total Ash value

In drug, the impurity present in the form of organic matter was determined with the help of total Ash value. For its determination, 2 g of the dried raw drug was weighed in powdered form in a pre-weighed silica crucible[18]. The sample was incinerated in silica crucible by gradually increasing the temperature up to 450°C for 4 hours or until it became carbon free. The crucible was cooled and weighed until constant weight was obtained. Percent of total ash value was then calculated by taking the ratio of loss in weight to weight of sample taken.

2.1.4. Determination of Acid Insoluble Ash value

After total cash value determination, 25 mL of 5 M hydrochloric acid was added to the dried ash and boiled in a water bath for 10 minutes. The solution was concentrated till its colour changed to yellow. Acid insoluble matter was filtered using ashless Whatman paper number 1 followed by washing with distilled water. The paper was again ignited in a crucible at a temperature not more than 450°C for 4 hours[18], after which the crucible was kept in a desiccator, cooled and weighed. With reference to the originally taken air-dried powdered drug, % of acid insoluble ash value was calculated.

2.1.5. Determination of Water-soluble extractive value

The extractive value determines the number of active constituents in a given amount of plant raw drug material when extracted with a solvent. These values indicate the extent of polar and non-polar components present in the plant material[19]. For determination of water extractive value, 2 g of sample was accurately weighed, air dried powdered drug was put in a conical flask with 100 mL water added in it. The solution was allowed to stand for 24 hours with intermittent shaking of the flask after every 4 hours. The water-soluble extractive was filtered using Whatman filter paper. 25 mL of this filtrate was completely dried on a pre-weighed petri plate at 105°C[20]. The increase in weight of the petri dish was noted to calculate the water-soluble extractive value determination. With reference to the originally taken air-dried powdered drug, the % of water-soluble extractive value was calculated.

2.1.6. Determination of Alcohol-soluble extractive value

For determination of alcohol soluble extractive value accurately weighed 2 g air dried powdered drug was put in a conical flask and 100 mL absolute alcohol added to it. The whole solution was left for 24 hours for complete extraction at room temperature. The solution was shaken vigorously for a few minutes after every 6 hours. The extract was filtered with the help of Whatman filter paper taking precautions to avoid evaporation and loss of alcohol from the extract. Weighed the empty flat-bottomed petri dish[20]. The petri dish with 25 mL of filtrate was heated at 105°C[21] in an electric oven then cooled in a desiccator and weighed. With reference to the originally taken air-dried powdered % of alcohol-soluble extractive value calculated and recorded in Table 2.

2.1.7. Preparation of crude extract/Inhouse mother tincture

100 g of coarsely dried powdered *Pyrus malus* leaves were taken in which 600 ml distilled water and 430 ml strong alcohol (95%) was added to make one thousand millilitres of the mother tincture using the percolation method (as per Homoeopathic Pharmacopoeia of India)[17]. This tincture was transferred to a tightly packed amber glass container and stored for further study.

2.1.8. Qualitative Phytochemical screening

Phytochemical tests were performed on crude extract for qualitative estimation of phytochemicals present in in-house mother tincture of *Pyrus malus* with all respective testing procedures as described in the textbook of Harborne JB (eds.)[22] (Table 3).

2.1.9. Standardization of mother tincture

Standardization of mother tincture was conducted to identify the organoleptic and physicochemical properties of mother tincture [23]. Organoleptic properties measurement was done for colour, odour and clarity of solution. The samples were tested for various physicochemical properties like sediments, pH, total solids, Wt/mL and total alcohol content.

2.2. Preparation of chloroform extract

25 mL of Mother Tincture was taken in a 50 mL beaker. To remove the ethanol, the solution was evaporated in a water bath and extracted three times with 20 mL chloroform. Combined and concentrated chloroform extract up to 2 mL volume. Carried out TLC[24] of chloroform extract of mother tincture on silica gel 60 F254 pre-coated plate [25].

2.3. HPTLC fingerprinting profile study

HPTLC fingerprinting study [26] was carried out by following the methodology of High-Performance Thin Layer Chromatography for the analysis of Medicinal plant. A densitometric HPTLC Camag Linomat 5 instrument was used for the study. As a sample applicator Camag Linomat 5 was used for spotting TLC plate. Spots were made on silica gel 60 F254 pre-coated plate (Merck) 10 × 10 cm plate with an aid of sampling machine and solvent front was run up to 70mm height [27]. For development of mobile phase, a saturating chamber Camag Twin Trough glass chamber was used. Camag TLC Scanner and software vision CATS were used for scanning purpose [28]. HPLC grade solvents were used for all the extracts solution. Volume applied for sample 2 µL. For detection of active constituents' various mobile phase was used. TLC spots were visualized after illumination at 254 nm, 366 nm and after derivatization.

2.4. Study of Antioxidant Potential

2.4.1. Determination of Total Phenolic content

The total phenolic content of the extracts was determined by Folin-Ciocalteu's reagent procedure reported by Singleton [29]. The total phenol content was estimated in *Pyrus malus* mother tincture. Ascorbic acid was used as reference standard. Different concentration (0.2661-8.517 mM) of ascorbic acid were prepared and analysed at 736 nm and calibration curve was plotted as absorbance versus concentration. Total Phenolic content was estimated by using Ascorbic acid as standard approximately 50 µl of the mother tincture was mixed with 5 mL 10 % Folin-Ciocalteu's [30] (phenol reagent) and 4 mL of sodium carbonate. The mixture was allowed to stand for one hour in dark. After one hour the colour changed from yellow to blue. The absorbance of the solutions was measured at λmax 736 nm using a UV-VIS spectrophotometer. The total phenolic content was calculated from the calibration curve and in results total phenolic content of *Pyrus malus* mother tincture was calculated as the ascorbic acid equivalents using standard ascorbic acid (Y= 0.0753x +0.0256, R2 0.9995) curve standardised in the lab for the calculation of ascorbic acid equivalent. Total Phenolic content was expressed in mM concentration of ascorbic acid equivalent[31].

2.4.2. Determination of DPPH Radical Scavenging Assay

The free radical scavenging activity of *Pyrus malus* sample in-house mother tincture(A) and market sample (B, C and D) were measured by 2,2-diphenyl-1-picryl-ydraxyl (DPPH) radical scavenging assay[32]. The standard solution of DPPH was prepared by dissolving 0.025 g in 25 mL methanol and different concentration of standards/mother tincture sample (100µl) was mixed with 4 mL methanol and 1 mL of DPPH standard. The mixture was allowed to stand for one hour in dark, after which the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The percentage inhibition was determined by comparing the test result and the control (methanol used as solvent blank). Percentage degradation was calculated by the formula:

$$\text{DPPH radical Scavenging}(\%) = [(\text{B} - \text{A}) \times 100] \div \text{B}$$

Where,

A= absorbance of sample

B= absorbance of control

The inhibiting effects of mother tincture showed varied levels of DPPH radical scavenging activity, expressed as percentage degradation [29].

2.4.3. Determination of Total Flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric assay method. Total flavonoid content using rutin hydrate as standard. An aliquot of 1 mL of the mother tincture mixed with 4 mL distilled water add 300 µl (5%) sodium nitrite in it. After five minutes add 300 µl (10%) aluminium chloride then after five minutes add 2 mL methanol and 2 mL (1M) sodium hydroxide then add 2.4 mL distilled water to make up the volume up to 10 mL. The mixture was shaken vigorously and left to stand in dark at room temperature. The resulting mixture colour change yellow to pink. The absorbance of the reaction mixture was measured at λ max 510 nm with a spectrophotometer. A standard calibration curve was constructed using rutin hydrate standard solutions of (31.25- 500 µg/mL) of each standard was treated in the same manner as the samples above to generate calibration curve. The total flavonoid content of mother tincture was determined from the curve and the results recalculated and expressed as the rutin hydrate equivalents.

2.4.5. Determination of ABTS Assay

Free radical scavenging activity of *Pyrus malus* mother tincture was determined by ABTS (2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) [33] radical cation decolourization assay. ABTS. + cation radical was produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulfate (1:1) stored in the dark at room temperature for 16 hours [34] before use. ABTS. + solution was then diluted with methanol to obtain an absorbance of 0.700 at 746 nm. After the addition of 10 µl of mother tincture/standard in 2 mL of diluted ABTS. + solution, the absorbance was measured at 5 min after the initial mixing. An appropriate solvent blank (methanol) was run in each assay.

Percent inhibition of absorbance at 746 nm was calculated using the formula:

$$\text{ABTS ion scavenging effect (\%)} = [(\text{AB-AA}) \times 100] \div \text{AB}$$

Where,

AB is absorbance of ABTS radical+ methanol

AA is absorbance of ABTS radical + sample/standard

Trolox was used as standard substance [34]..

3. Results

3.1. Physiochemical and Phytochemical studies

The physicochemical properties of the *Pyrus malus* mother tincture for parameters like sediments, pH, total solids, alcohol content and weight per mL were analysed and tabulated in Table 1. The results obtained for various physiochemical studies of *Pyrus malus* raw drug are tabulated in Table 2. Phytochemical tests performed on the crude extract of whole plant of *Pyrus malus* showed positive results for various tests as mentioned in Table 3. Organoleptic observations of the prepared in-house mother tincture indicated formation of a clear green solution with characteristic tincture odors.

S. No.	Parameter	In-house sample
1	Sediments	Nil
2	pH	4.58
3	Total solid	2.05% w/v
4	Wt/mL	0.94g
5	Alcohol content	41.0% v/v

Table 1: Result of physicochemical properties of In-house sample of *Pyrus malus* mother tinctures.

S. No.	Name of test	Results %
1	Foreign matter	2.00
2	Loss on drying	4.90
3	Total ash value	3.69
4	Acid Insoluble ash value	1.20
5	Water-soluble extractive value	18.60
6	Alcohol-soluble extractive value	5.65

Table 2: Result of physicochemical properties of *Pyrus malus* raw drug material.

S. No.	Name of Phytochemical test	Results
1	Tannins (lead acetate test)	Positive
2	Flavonoids (alkaline reagent test)	Positive
3	Alkaloids	Positive
	Mayer's test	
	Hager's test	
	Wagner's test	
	Dragendorff's test	
4	Phenolic compounds (ferric chloride test	Positive
5	Glycoside (Sodium hydroxide reagent test)	Positive

Table 3: Result of Phytochemical tests for screening various phytochemicals present in the mother tincture of *Pyrus malus*.

3.2. Results of HPTLC study

Pyrus malus chloroform extract was prepared in the laboratory. Based on extensive literature reviews, various combinations of solvent systems were studied to have an appropriate mobile phase composition for the best and most efficient HPTLC chromatographic separation of active constituents in Pyrus malus chloroform extract. Among all the mobile phase combinations studied, chloroform: methanol (9:1, v/v) was finalized as the ideal. Thus, the most appropriate mobile phase composition for the entire HPTLC method development study was finalized. The profile of Chromatographic separation was scanned at UV 254nm and 366nm wavelength. At white light nine spots appear at $R_{f.}$ 0.26(yellow), 0.33(yellow), 0.40(green), 0.47(green), 0.55(green) and 0.79(light green) Figure 2. At UV 254nm six brown spots appear at $R_{f.}$ 0.26, 0.32, 0.40, 0.46, 0.55 and 0.82 Figure 3. At UV 366nm eight spots appear at $R_{f.}$ 0.24(green), 0.33(red), 0.38(red), 0.46(red), 0.55(red), 0.67(red),0.79 (red) and 0.84(red) Figure 4.

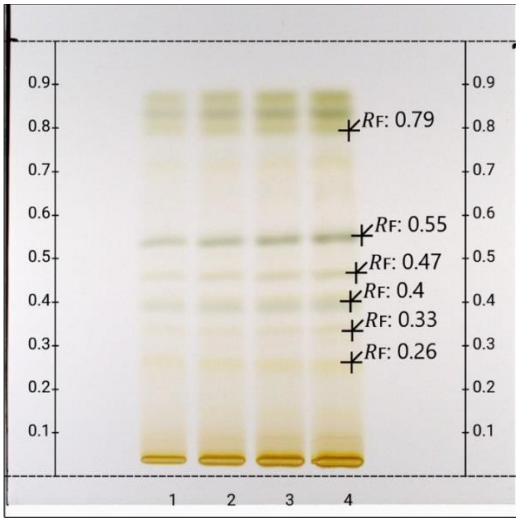

Figure 2: High-performance thin-layer chromatography fingerprints of Pyrus malus at white light. Track 1-4 Pyrus malus mother tincture.

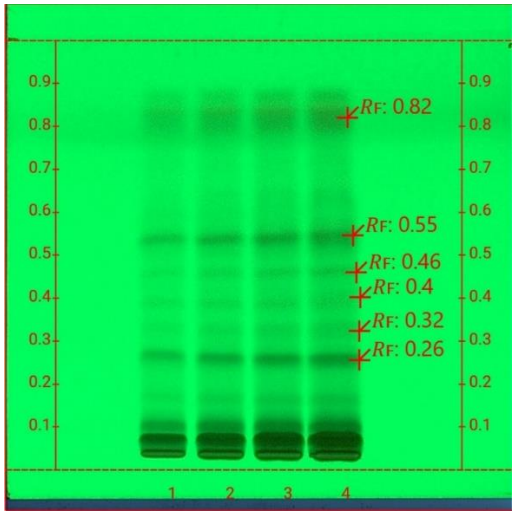

Figure 3: High-performance thin-layer chromatography fingerprints of Pyrus malus at UV 254nm. Track 1-4 Pyrus malus mother tincture.

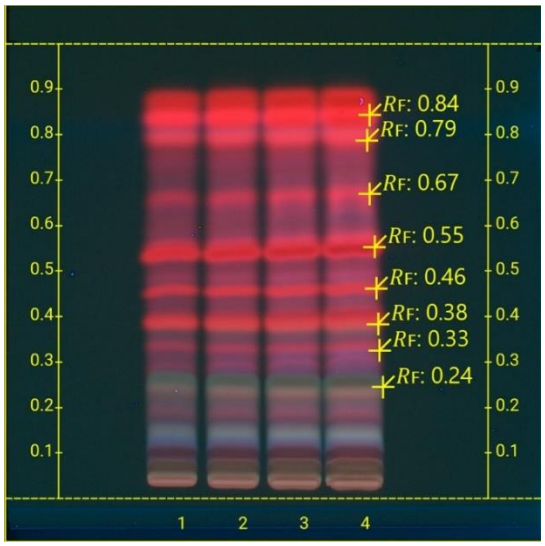

Figure 4: High-performance thin-layer chromatography fingerprints of Pyrus malus at UV 366nm. Track 1-4 Pyrus malus mother tincture.

3.3. Results of Antioxidant study

3.3.1. Result of Total phenol content

In the present study the total phenolic content of Pyrus malus in-house mother tincture was determined by Folin–Ciocalteu method and reported as ascorbic acid equivalents. Study reveals total phenolic content found in Pyrus malus in-house sample (75µL) is 17.47 mM AAE (Ascorbic acid equivalents) Table 4.

Sample.	**Concentration in (mM) of AAE**	**Absorbance**
Pyrus malus mother tincture	17.47	2.3633
Control	0.10	0.0151

Table 4: Clinical Studies on Sugar Intake and Cognitive Decline.

3.3.2. Result of DPPH Assay

In the present study the DPPH assay of Pyrus malus in-house mother tincture was determined by DPPH radical scavenging assay method and reported as ascorbic acid equivalents (AAE). Study reveals Pyrus malus in-house sample was able to decolorize DPPH free radical, the DPPH scavenging increased with the concentration of the extract. The percentage of inhibition found in 100 µL volume of Pyrus malus in-house mother tincture was 86.18% Table 5.

Sample	**Concentration in (mM) of AAE**	**Absorbance**	% Degradation
Pyrus malus mother tincture	5.56	0.0005	86.18%
Control	0.27	0.0363	

Table 5: Result of DPPH Assay in Pyrus malus mother tincture.

In DPPH assay a significant correlation coefficient (R, 0.9944) was found between the antioxidant activity of alcoholic extracts (mother tinctures) of Pyrus malus in-house sample. The hydrogen radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPH by antioxidant compounds present in the mother tincture. Our study indicates that the Pyrus malus mother tincture exhibits a high content of Phenolic compound i.e.,17.47 mM which was significantly correlated with DPPH radical scavenging activity % i.e., 86.18% Table 4 & 5.

3.3.3. Result of Total flavonoid content

In the present study the total flavonoid content of Pyrus malus mother tincture was determined by $AlCl_3$ method and reported as rutin hydrate equivalents. Study reveals Pyrus malus mother tinctures (1ml) contain 3293.75 µg/ml amount of flavonoid compound Table 6.

Sample	**Concentration in ((µg/ml))**	**Absorbance**
Pyrus malus mother tincture	3293.75	3.0479
Control	0.15	0.0157

Table 6: Result of Total Flavonoid content in *Pyrus malus* mother tincture.

3.3.4. Result of ABTS assay

In the present study the ABTS assay of *Pyrus malus* in-house sample was determined by ABTS assay method and reported in terms of Trolox equivalents. A significant correlation coefficient (R, 0.9901) was found between the antioxidant activity of alcoholic extracts (mother tinctures) of *Pyrus malus* in-house sample. Study reveals *Pyrus malus* in-house mother tincture was able to decolorize ABTS+ free radical, the ABTS radical cation scavenging activity increased with the concentration of the extract. The result showed the percentage of inhibition found in 10 µL volume of *Pyrus malus* in-house mother tincture was 99.44% Table 7

Sample	Concentration in (µg/ml))	Absorbance	% Degradation
***Pyrus malus* mother tincture**	266.87	0.0016	99.44%
Control	0.41	0.0420	

Table 7: Result of ABTS radical cation scavenging activity against *Pyrus malus* mother tincture.

4. Discussion

The present study provides a comprehensive analysis of the antioxidant potential of Pyrus malus in-house mother tincture through the quantification of total phenolic and flavonoid contents, and its evaluation via DPPH and ABTS radical scavenging assays. The findings strongly support the hypothesis that Pyrus malus contains bioactive compounds capable of exerting significant antioxidant effects. The total phenolic content was found to be 17.47 mM AAE at 75 µL concentration, indicating a high level of polyphenolic compounds. Phenolic compounds are well-known for their capacity to donate hydrogen atoms or electrons, thereby neutralizing free radicals and contributing to the plant's antioxidant defense mechanism. Similarly, the flavonoid content, measured as 3293.75 µg/mL of rutin hydrate equivalents, further confirms the richness of Pyrus malus in phytochemicals with antioxidant properties. Flavonoids act as potent scavengers of reactive oxygen species (ROS) and are associated with various health benefits, including anti-inflammatory and cardioprotective effects.The strong radical scavenging activity observed in both DPPH and ABTS assays provides functional evidence of antioxidant capability. The DPPH assay demonstrated 86.18% inhibition at 100 µL concentration, while the ABTS assay showed even higher scavenging activity with 99.44% inhibition at only 10 µL. These values are significant, especially considering the small volumes used, and suggest a high potency of the mother tincture. Importantly, strong correlation coefficients were found between phytochemical content and antioxidant activity (R = 0.9944 for DPPH and R = 0.9901 for ABTS), supporting a direct association between phenolic/flavonoid presence and free radical neutralization. These findings align with previous studies highlighting the antioxidant potential of apple-derived products and extracts, and further suggest that the preparation method of the mother tincture retains or possibly concentrates these bioactive compounds. The results not only validate the traditional use of Pyrus malus in herbal medicine but also open new avenues for its application in pharmaceutical formulations aimed at oxidative stress-related disorders.

Conclusion

This study demonstrated that the alcoholic extract (mother tincture) of Pyrus malus leaves possesses favorable physicochemical properties and significant antioxidant potential. HPTLC fingerprinting confirmed the presence of bioactive compounds in the chloroform extract, supporting the chemical richness of the formulation. The antioxidant activity, evaluated through total phenol content (17.47 mM AAE), total flavonoid content (3293.75 µg/mL), DPPH assay (86.18% inhibition), and ABTS assay (99.44% inhibition), showed a strong correlation between polyphenol concentration and radical scavenging capacity. These findings suggest that the potent antioxidant effects of Pyrus malus may contribute to its therapeutic efficacy in homoeopathic applications. The study establishes a foundational framework for the standardization and scientific validation of Pyrus malus mother tincture in alternative medicine. Future research should aim at the quantitative estimation and pharmacological evaluation of additional phytoconstituents to further elucidate the mechanisms behind its medicinal properties and broaden its clinical applications.

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DOI:10.31579/2639-4162/256