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Comparative Study to Assess Chemo-Profile variation of Maricha (*piper nigrum Linn)* between Non-Grafted and Grafted Variety Sample by RP-UPLC Method

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Abstract: ***In Ayurveda, therapeutic success depends on four essential factors, among which Dravya (medicine) holds prime importance after the physician. Traditionally, medicinal plants were collected directly by Vaidyas; however, current practices largely depend on market-sourced raw materials. Maricha (Piper nigrum Linn.) is an extensively used drug in Ayurvedic formulations, and due to increasing demand, modern cultivation techniques such as grafting are being adopted over conventional methods. Grafting is known to enhance yield, improve disease resistance, and reduce harvesting time.***

The present study aims to compare the pharmacognostic, physicochemical, phytochemical, and chemical profiles of non-grafted and grafted Maricha. Fresh seeds of Piper nigrum cultivated through non-grafted and grafted methods were collected and shade-dried following Good Cultivation Practices (GCP). Both samples were evaluated through pharmacognostic, physicochemical, and phytochemical analyses, along with chromatographic profiling using the RP-UPLC method.

The results revealed that grafted plants exhibited superior morphological characteristics, including thicker stems, larger leaves, and bigger fruits. Phytochemical screening showed the presence of similar constituents such as saponins, tannins, alkaloids, volatile oils, and starch in both samples, while physicochemical parameters remained within standard limits. RP-UPLC analysis indicated a higher piperine content in grafted Maricha (4.57%) compared to non-grafted samples (3.57%).

The study concludes that grafting positively influences the morphological and chemical attributes of Maricha, supporting its use for improved quality and efficacy of Ayurvedic formulations and emphasizing the need for further research on genetic and environmental influences.

Keywords: Chemo-profile variation; Grafting; harvest period; Good Cultivation Practice (GCP); RP-UPLC

I. INTRODUCTION

Dravya is one of the four essential components of treatment described under Chikitsa Chatushpada [(1)], playing a crucial role next to the physician (*Bhishak*). When administered as Aushadha, dravyas restore doshic equilibrium and alleviate disease. Classical Ayurvedic texts emphasize that the efficacy of a drug depends not only on its inherent properties but also on proper cultivation, collection, storage, and processing. Acharya Charaka states that even potent or toxic substances can be therapeutically beneficial when processed appropriately. Traditionally, physicians themselves collected medicinal plants; however, in modern practice, raw materials are largely procured from commercial sources, raising concerns regarding quality and authenticity.

Cultivation practices significantly influence the quality, potency, and therapeutic efficacy of medicinal plants used in Ayurvedic formulations. Scientific and sustainable cultivation ensures a consistent supply of high-quality raw materials, minimizes overexploitation of wild resources, and maintains phytochemical integrity. With the increasing global demand for Ayurvedic medicines, standardized cultivation practices have become essential for ensuring safety, efficacy, and sustainability.

In Ayurveda, several medicinal plants serve multiple roles as food, spices, and therapeutic agents. Maricha (Piper nigrum Linn.), commonly known as black pepper, is one such plant widely used since ancient times.

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It is an important ingredient in several classical formulations such as Trikatu [2], Chaturushana[3], and Shadushana[4], and is extensively used in both classical and proprietary medicines. Due to its high demand, cultivation practices of Maricha have evolved from conventional propagation methods to advanced techniques such as grafting.

Grafting is an innovative propagation technique increasingly adopted in black pepper cultivation to enhance disease resistance, yield, and plant longevity. In this method, a desirable scion is grafted onto a disease-tolerant rootstock, commonly Piper colubrinum, to manage foot rot disease caused by *Phytophthora capsica* [5]. While grafting offers agronomic advantages such as improved productivity and early harvesting, its impact on the chemical composition and medicinal properties of Maricha remains inadequately explored.

At present, grafted Maricha is widely used in medicinal practice without sufficient evidence regarding potential variations in its phytochemical and therapeutic profile compared to non-grafted varieties. This necessitates a systematic physicochemical and chromatographic evaluation to assess any chemoprofile variations arising due to different cultivation methods.

II. AIMS AND OBJECTIVES

A. *Aim of the Study*

Compare the Chemo-profile variation between Non-grafted and Grafted variety of Maricha (*Piper nigrum* Linn*)*

B. *Objectives of the Study*

1) Collection of *Piper nigrum* Linn grown from Non-grafted and Grafted methods of cultivation.
2) Comparative Pharmacognostic, Physicochemical and Phyto-chemical analytical study of collected Maricha (*Piper nigrum* Linn).
3) Comparative quantitative evaluation of PIPERINE phytoconstituents of Maricha (*Piper nigrum* Linn) samples collected from Non-grafted and Grafted methods of cultivation through RP-UPLC method of analysis.

III. MATERIALS AND METHODS

The study was designed under the following phases:

1) Collection, authentication and preapration of drug maricha.
2) Pharmacognostical evaluation of the trial drug. Macroscopic evaluation and microscopic evaluation of both samples were carried out following standard methods.
3) Physicochemical Evaluation Foreign Matter, Moisture Content/Loss on drying, Total Ash, Acid Insoluble Ash, Water-Soluble Extract Value, Alcohol Soluble Extract Value and pH was carried out following standard methods.
4) Phytochemical Evaluation Organic Constituents, Inorganic Constituents, TLC analysis and RP-UPLC analysis carried out following standard methods.

A. *Collection Of Trial Drug*

The trial drug MARICHA was collected At the Locality of Honnavar Taluk, Uttara Kannada District, Karnataka, 581334.

- Samples- M120: Grafted Maricha samples
  M121: Non-grafted Maricha samples
- Place of collection: Both M120 and M121 samples were collected from the same field grown adjacent to each other with support of Areca plant (*Areca catechu*) to avoid differences in factors such as climate, water, soil, temperature, sunlight and pollination.
- Collection method- Handpicked
- Collection period- End of January [6]
- Post Harvest procedures- As per G.C.P and Wealth of India [7] textbook is followed.

B. *Authentication*

The collected raw drug MARICHA (*Piper nigrum* Linn) seeds were verified under the guidance of experts from the Dept. Of Dravyaguna Taranath Government Ayurvedic Medical College and Hospital Ballari.

C. Preparation Of Trial Drug

3 kgs of Maricha drug (Phala) were collected from the different method of cultivation (Non-Grafted and Grafted) of Karnataka, Fruit was dried for 15 days till it completely dried as Per GCP. After drying 2.5 kg of drug was obtained, which was later made into coarse powder by using pulverize and fine powder using mixer grinder. Finally, 1.3 kg of fine powder and 1.2 kg of coarse powder was obtained. The ethanolic extract was carried out by General method, The Standard Operating procedure was followed.

Pharmacognostic evaluation of the trial drug

Macroscopic evaluation: The macroscopic character of the trial drug *Piper nigrum* was observed for the following sensory features:

Microscopic evaluation: Powder microscopy was carried out as per standard operating procedure.

D. Physicochemical Evaluation

The standard for the trial drug is available in API book /Quality Standards of Indian Medicinal Plants book, and the tests were carried out as per the standard operating procedures mentioned in the above books. Determination of Foreign matter, Loss on drying, Total Ash, Acid Insoluble ash, Alcohol soluble Extractive value, Water soluble Extractive value and pH were carried out.

Phytochemical evaluation:

The Phyto- chemical analysis for detection of organic chemical constituents was carried out for choornas ethanolic extracts. Test for Primary metabolites, Secondary metabolites, test for Detection of Inorganic constituents were carried out.

E. TLC Analysis Of Maricha

Carried out thin-layer chromatography on a TLC plate using Piperine as reference standards.

Extract: Methanol

Mobile Phase (Solvent system): Toluene: ethyl acetate: formic acid: water (20:60:15:5)

Stationary phase: marked TLC plate.

F. Reversed Phase - Ultra Performance Liquid Chromatography (RP-UPLC) Analysis

Estimation of Piperine.

Ethanolic Extract of Maricha.

Reversed Phase Ultra Performance Liquid Chromatography conditions:

Column: RP-UPLC C-18 column

Size: 4.6mm×250mm

Instrument used: Shimadzu SPD-10A.

Chromatographic Conditions

Table no [32] showing Chromatographic Condition for Piperine.

Mobile Phase	ACN: Water: acetic acid (60 :39.5:0.5 %v/v)
Stationary Phase	Symmetry C18 (4.6 x 250mm, 5µ particle size)
Wavelength	345nm
Run time	10 Mins
Flow Rate	1ml/Min
Injection Volume	100 µL
Temperature	Ambient Isocratic elution
Mode of Operation	Isocratic

G. Preparation of Standard Stock Solution

An accurately weighed quantity of piperine(10mg) was transferred to a 10mL volumetric flask, dissolved and diluted to the mark with Methanol to obtain standard stock solution of 1000ppm.

Preparation of Calibration Curve

Aliquots of 1, 2, 3, 4 and 5mL standard stock solution (1000µg/mL) was transferred to 10mL of volumetric flasks and made up to the mark with Methanol to get concentration of 100, 200, 300, 400 and 500µg/ml.

H. Sample Preparation

10mg of different Maricha powder was taken in 10ml of volumetric flask added Methanol and sonicated for 30minutes then taken filter by using 0.2 µ filter membrane and subjected to analysis.

Procedure:

- The mobile phase was forced through the packed column with flow rate of 1ml/min and under pressure gradient elusion 345nm.
- The chromatograph with the concentration 1mg/ml is done by injecting 100µl of standard Piperine solution.
- The chromatograph of Maricha choorna solution with concentration 10ml was done.
- The computer recorded the peaks of Absorbance of the substance.
- The report contains the retention time (RT).
- The amount of Piperine present in all the sample was estimated using the formula:

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard amount}}{\text{Dilution of Standard}} \times \frac{\text{Dilution}}{\text{Sample amount}} \times \text{Mean weight}$$

IV. RESULT

Comparative macroscopic characteristics between non-grafted maricha plant and grafted maricha plant

Table no [33] Showing Comparative Macroscopic characteristics between Non-grafted Maricha plant and Grafted Maricha plant

Characteristics	Non-grafted Maricha plant	Grafted Maricha plant
Stem width	2.4 cm	3.6 cm
Internodal length	7.4 cm	6.1 cm
Leaf color	Slight light Green	Green
Petiole length	2.6 cm	2.7 cm
Veins number	7	7
Leaf dimensions	17.6 x 12.6 cm	19.4 x 15.8 cm
Inflorescence size	Larger	smaller
Fruit/Drupe size	12.1 cm and dense	13.9 cm and sparse



Figure [16] Comparison between Non-grafted and Grafted variety of Maricha Stem width



Figure [17] Comparison between Non-grafted and Grafted variety of Maricha Internodal Length



Figure [18] Comparison between Non-grafted and Grafted variety of Maricha Leaf width



Figure [19] Comparison between Non-grafted and Grafted variety of Maricha Leaf length



Figure [20] Comparison between Non-grafted and Grafted variety of Maricha Leaf petiole



Figure [21] Comparison between Non-grafted and Grafted variety of Maricha Leaf colour front and back view

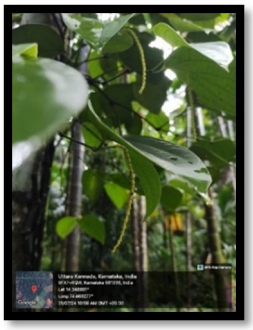


Figure [22] Image of Non-grafted Maricha Inflorescence



Figure [23] Im of Grafted Mar Inflorescenc

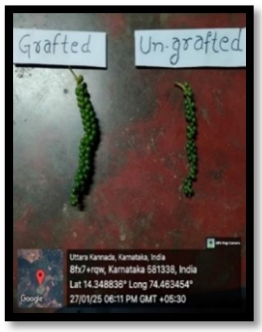


Figure [24] Comparison between Non-grafted and Grafted variety of Maricha



Figure [25] Comparison between Non-grafted and Grafted variety of Maricha spike length

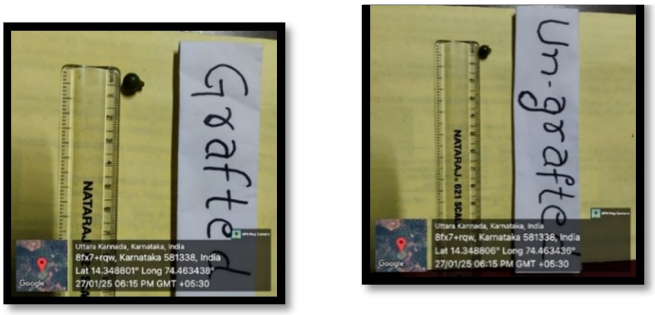


Figure [26] Comparison between Non-grafted and Grafted variety of Maricha seeds diameter

Comparative organoleptic characteristics between non-grafted maricha sample and grafted maricha sample

Table no [34] Showing Comparative Organoleptic characteristics between Non-grafted Maricha sample and Grafted Maricha sample

characteristics	Non-grafted Maricha sample	Grafted Maricha sample
Color	Green	Green
Odor	Intense Pungent	Pungent
Taste	Intense Spicy	Spicy
Texture	Smooth	Smooth
Size	0.5 x 0.6 cm	0.8 x 0.9 cm

Comparative powder microscopic characteristics between non-grafted maricha sample and grafted maricha sample

Table no [35] Showing Comparative Powder Microscopic characteristics between Non-grafted Maricha sample and Grafted Maricha sample

Characteristics	Standard	Non-grafted Maricha sample	Grafted Maricha sample
Stone cells	Beaker shaped isodiametric or slightly elongated	Beaker shaped isodiametric or slightly elongated	Beaker shaped isodiametric or slightly elongated
Starch grains	Single oval to round	Single oval to round	Single oval to round
Aleurone grains	Few	Few	Few
Oil globules	Few	Few	Few

Images of powder microscopy of grafted and non-grafted maricha samples

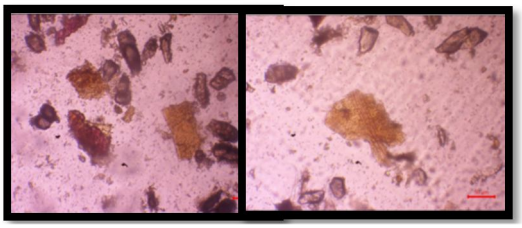


Figure [41] Comparison between Epicarp cells in surface view and stone cells of Grafted and Non-grafted Maricha samples

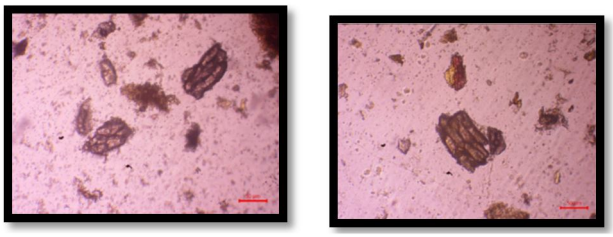


Figure [42] Comparison between Agglutinated masses of Starch grain from Perisperm of Grafted and Non-grafted Maricha samples

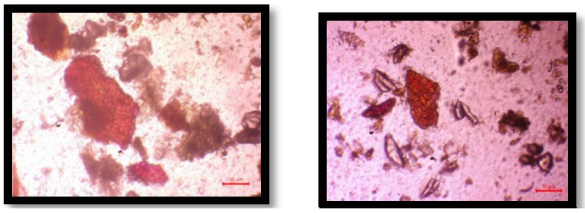


Figure [43] Comparison between Group of Stone cells of Grafted and Non-grafted Maricha samples

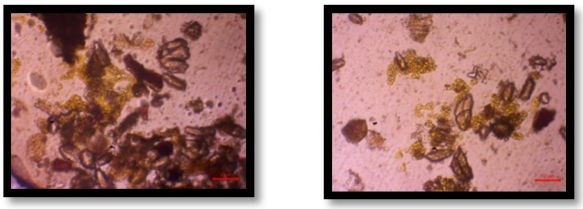


Figure [44] Comparison between Parenchymatous cells filled with Oil Globules of Grafted and Non-grafted Maricha samples

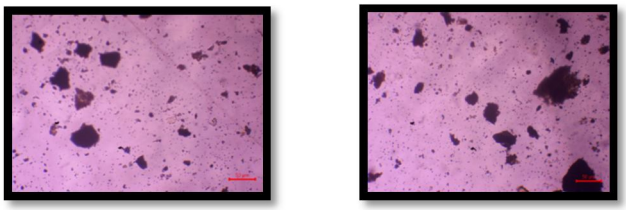


Figure [45] Comparison between Starch grains of Grafted and Non-grafted Maricha samples

Physichochemical parameters: non-grafted maricha sample and grafted maricha sample

Table no [36] Showing physicochemical characteristics of Non-grafted Maricha sample and Grafted Maricha sample

Sl.No	Test	Non grafted Maricha samples	Grafted Maricha samples	Standard value
1.	Foreign matter	0.8%	0.9%	< 2%
2.	Loss on drying	9.5%	10.8%	-
3.	Ash value	4.5%	4.5%	< 5%
4.	Acid insoluble ash	1%	1%	> 0.5%
5.	Water soluble extract value	8.8%	7.6%	> 6%
6.	Alcohol soluble extract value	7.6%	9.6%	> 6%
7.	pH	10.34	10.71	-

Phytochemical parameters: non-grafted maricha sample and grafted maricha sample

Table no [37] Showing Phytochemical characteristics of Non-grafted Maricha sample

Sl.No	Test	Non-grafted Maricha sample	Grafted Maricha sample
1	Carbohydrate	Absent	Absent
2	Protein	Absent	Absent
3	Saponin	Present	Present
4	Tannin	Present	Present
5	Steroid	Absent	Absent
6	Alkaloids	Present	Present
7	Flavonoids	Absent	Absent
8	Glycoside	Absent	Absent
9	Reducing Sugar	Absent	Absent
10	Volatile oil	Present	Present
11	Oil and Fats	Present	Present
12	Starch	present	present

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TLC analysis: non-grafted maricha sample

Table no [38] Showing TLC analysis of Non-grafted Maricha sample

Sl.No	wavelength	Rf value result
1	White light/Visible rays	0.12 0.41
2	Short UV Wave (254 nm)	0.06 0.12 0.35 0.38 0.45 0.55 0.59 0.76
3	Long UV Wave (365 nm)	0.06 0.12 0.35 0.39 0.45 0.55 0.65 0.76 0.88

TLC analysis: grafted maricha sample

Table no [39] Showing TLC analysis of Grafted Maricha sample

Sl.No	wavelength	Rf value result
1	White light/Visible rays	0.12 0.38
2	Short UV Wave (254 nm)	0.09 0.15 0.32 0.35 0.38 0.45 0.49
3	Long UV Wave (365 nm)	0.09 0.15 0.21 0.38 0.41 0.45 0.59 0.65 0.76 0.82 0.94

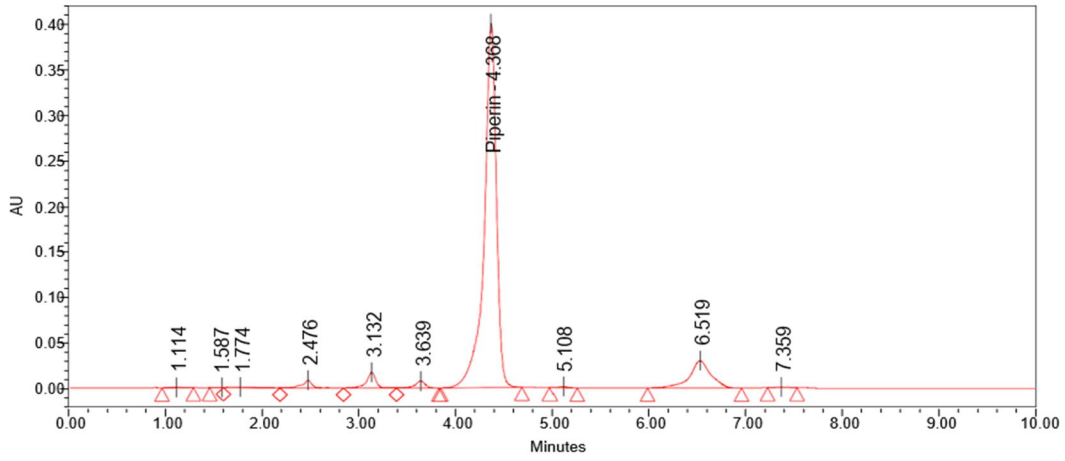
RP-UPLC analysis of the samples

Non-grafted maricha sample

Table no [55] Showing information of Non-grafted Maricha sample

	SAMPLE	INFORMATION	
Sample Name:	M121	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	Maricha non-grafted samples
Vial:	1: A,3	Acq. Method Set:	Piperine20_05_23
Injection #:	1	Processing Method:	M121
Injection Volume:	10.00 ul	Channel Name:	345.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 345.0 nm (PDA Spectrum)
Date Acquired:	8/8/2025 12:35:35 PMIST		
Date Processed:	8/9/2025 10:31:26 AMIST		

Graph No [06] Showing Piperine conc in Non-grafted Maricha sample RP-UPLC values



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Table no [56] Showing Peak results of Non-grafted Maricha samples

Sl.No	Name	RT	Area	Height	Amount	Units
1		1.104	6743	681		
2		1.712	1956	412		
3		2.477	14446	857		
4		3.138	55729	8197		
5		3.646	110999	17060		
6		3.639	54569	7704		
7	Piperine	4.375	3469704	399664		µg/mL
8		5.109	8837	1184		
9		6.538	455154	29927		
10		7.380	5107	587		

Table no [57] Showing PDA result of Non-grafted Maricha sample

Sl.No	Name	RT	Purity 1 Angle	Purity 1 Threshold	Match 1 Spect Name	Match 1 Angle	Match 1 Threshold
1		1.104					
2		1.712					
3		2.477					
4		3.138					
5		3.646					
6		3.639					
7	Piperine	4.375	0.035	0.050	Piperine Standard	0.012	0.020
8		5.109					
9		6.538					
10		7.380					

GRAFTED MARICHA SAMPLE

Table no [58] Showing information of Grafted Maricha sample

	SAMPLE	INFORMATION	
Sample Name:	M120	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	Maricha grafted samples
Vial:	1: A,2	Acq. Method Set:	Piperine20_05_23
Injection #:	1	Processing Method:	M120
Injection Volume:	10.00 ul	Channel Name:	345.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 345.0 nm (PDA Spectrum)
Date Acquired:	8/8/2025 12:46:43 PMIST		
Date Processed:	8/9/2025 10:29:50 AMIST		

Graph No [07] Showing Piperine concentration in Grafted Maricha sample RP-UPLC values

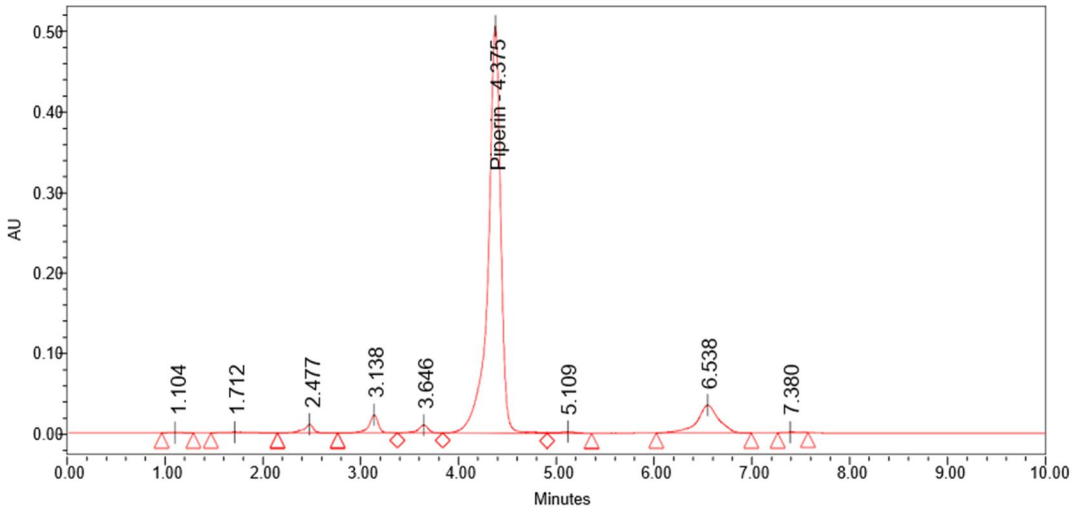


Table no [59] Showing Peak results of Grafted Maricha samples

Sl.No	Name	RT	Area	Height	Amount	Units
1		1.104	6449	649		
2		1.712	19709	949		
3		2.477	73587	10713		
4		3.138	148177	22382		
5		3.646	72846	9868		
6	Piperine	4.375	4460962	506134		µg/mL
7		5.109	22341	1891		
8		6.538	525678	34499		
9		7.380	5368	641		

Table no [60] Showing PDA results of Piperine in Grafted Maricha samples

Sl.No	Name	RT	Purity 1 Angle	Purity 1 Threshold	Match 1 Spect Name	Match 1 Angle	Match 1 Threshold
1		1.104					
2		1.712					
3		2.477					
4		3.138					
5		3.646					
6	Piperine	4.375	0.05	0.25	Piperine standard	0.05	0.25
7		5.109					
8		6.538					
9		7.380					

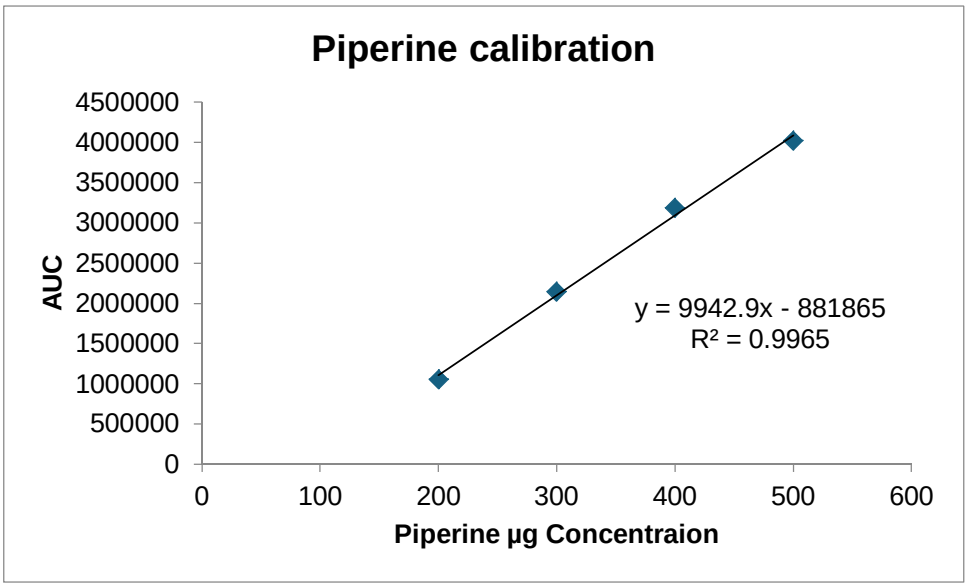
Piperine quantification

Table no [61] Showing concentration of Piperine and Area under curve

Concentration	Area under curve
100	1044049
200	1051277
300	2140282
400	3183040
500	4017988

Table no [62] Showing Piperine content in both samples

Drug	AUC	Piperine content in microgram	mg	%
M120	4460962	457.568	0.457	4.57
M121	3469704	357.8646	0.357	3.57



Graph No [08] Showing Piperine calibration

V. DISCUSSION

Discussion, in any research context, acts as the intellectual arena where data interpretation, hypothesis re-examination, and critical thinking converge to pave the way for valid conclusions. It is a vital component in the research process because any hypothesis must undergo rigorous scrutiny from multiple perspectives before it can be accepted as an established principle. This scrutiny involves analysing the hypothesis considering existing theories, data gathered, methodological strengths and limitations, and broader implications.

The phrase, “A hypothesis gets established as a principle if the reasoning is given satisfactorily; otherwise, it remains as it is,” underscores the importance of providing thorough logical reasoning and empirical evidence to support claims. Without this rigor, findings risk remaining preliminary observations rather than contributions to the scientific comity.

In the present study on Maricha (*Piper nigrum* Linn), the hypothesis centred on whether the cultivation method (non-grafted vs grafted) influences the phytochemical content, especially Piperine concentration. This discussion aims to revisit this hypothesis by analysing findings through scientific, agronomical, and Ayurvedic lenses, ensuring the evidence is contextualized holistically.

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The results of the current study discussed under the following headings:

1) Discussion on selection of Topic.
2) Discussion on Review of Literature.
3) Discussion on Analytical study.

A. Discussion on Selection of Topic

The selection of Maricha (*Piper nigrum* Linn) for this research is rooted in both its classical medicinal importance and the modern necessity for standardization and quality control in herbal medicine. Black pepper, known as Maricha in Ayurveda, has been described in classical Ayurvedic literature as an indispensable herb with wide-ranging therapeutic applications. It is frequently used in formulations aimed at enhancing bioavailability of drugs.

Choosing this topic was motivated by several key factors:

- Medicinal Significance: Piperine, the chief alkaloid in black pepper, is responsible for many of these pharmacological effects. It enhances absorption of various drugs and nutrients and possesses anti-inflammatory, antioxidant, and anticancer properties as documented in multiple modern studies.
- Agricultural Innovation: Grafting is an advanced horticultural technique known to improve crop yield, disease resistance, and plant Vigor. However, its impact on phytochemical production remains underexplored in many medicinal plants. This gap presents a unique opportunity to assess how grafting may affect not only agronomic traits but also medicinal quality.
- Quality Assurance: The herbal industry faces challenges regarding the variability of active compounds due to differences in cultivation, harvesting, and processing. Understanding how cultivation influences phytoconstituent profiles directly addresses the need for reliable quality control.
- Bridging Traditional and Modern Knowledge: Integrating traditional Ayurvedic wisdom with modern analytical tools represents the future direction of herbal drug research. The topic embodies this integration by exploring how a classical herb's efficacy can be scientifically validated.

The topic's selection thus aligns with current scientific priorities and traditional medicine values, offering the potential to impact agricultural practices, pharmacognosy, and ultimately patient care.

B. Discussion on Review of Literature

A thorough literature review is foundational to research, providing insights into what is known, unknown, and debated within the scientific and traditional domains.

Ayurvedic Classical Literature holds a prominent place for Maricha as multifaceted potent drug useful in many ailments, enhancing Bioavailability of other Drugs. It is capable of action on different Dhatus, Shrotas, and Rogamarga.

These classical references illustrate the herb's significance and establish the framework within which its therapeutic virtues are understood. However, these texts describe herbal drugs primarily through sensory and experiential criteria, lacking quantifiable parameters.

Modern Scientific Literature

Modern research has identified Piperine as the principal active compound responsible for many of black pepper's medicinal effects:

- Pharmacological Properties [8]: Antioxidant, antimicrobial, anti-inflammatory, anticancer, and bioavailability enhancing.
- Analytical Methods: Several studies have employed chromatographic methods, including HPLC and UPLC, to quantify Piperine, although comparative studies of cultivation methods are rare.
- Agricultural Impact: Limited data exist on how grafting influences phytochemical synthesis in medicinal plants, especially Maricha.

Thus, the literature highlights the research gap—a lack of integrated studies evaluating how agricultural practices affect the medicinal quality of herbs.

C. Discussion on Analytical Study

1) Pharmacognostic Analysis

Pharmacognostic evaluation forms the basis for identifying crude drugs and establishing their authenticity. In this study, both non-grafted and grafted varieties of Maricha were subjected to organoleptic and microscopic examination.

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The organoleptic characteristics such as size, shape, colour, odour, and taste were comparable between the two varieties, though minor differences in colour intensity and surface texture were observed, possibly due to altered secondary metabolite profiles influenced by grafting with the *Piper colubrinum*. The taste and Odour of Non-grafted Maricha was more compared to Grafted samples suggesting possible higher Volatile and aromatic components.

Microscopic examination of both the non-grafted and grafted Maricha (*Piper nigrum* Linn) samples with Transverse section and Powder microscopy method did not reveal any significant differences in their structural features. Key identifying characteristics such as the presence of stone cells, oil cells, starch grains, and pericarp tissues were observed in both varieties and appeared similar in arrangement and density. This suggests that grafting did not cause major changes in the internal anatomy of the fruit. Overall, the microscopic analysis indicates that the grafting process did not significantly alter the cellular structure of Maricha (*Piper nigrum* Linn).

2) Physicochemical Analysis

Physicochemical parameters such as foreign matter, loss on drying, ash values (total ash, acid-insoluble ash), extractive values (alcohol and water soluble), and pH are vital indicators of genuinity.

The analysis revealed presence of foreign matter within the pharmacopeial limits as per standards [9] of API.

The loss on drying was slightly higher in the grafted variety, possibly due to enhanced vascular connectivity or altered transpiration dynamics in grafted plants. However, this could increase the risk of microbial growth and may reduce shelf life if not properly stored. Total ash and acid-insoluble ash values were within pharmacopeial limits in both samples, indicating minimal inorganic adulteration. The water and alcohol-soluble extractive values were notably higher in the grafted variety, suggesting a greater concentration of water- and alcohol-soluble constituents such as alkaloids, glycosides, and tannins.

The pH of the grafted Maricha sample was found to be slightly higher compared to the non-grafted sample, indicating that the grafted variety is a bit more alkaline.

Although the difference is not large, it shows that grafting can cause subtle changes in the plant's internal chemistry.

3) Phytochemical Analysis

Preliminary phytochemical screening revealed the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and phenols in both samples.

4) TLC analysis

The TLC (Thin Layer Chromatography) analysis of grafted and non-grafted *Maricha* (*Piper nigrum* Linn) samples under different wavelengths revealed variations in their chemical profiles. Under white light, both samples showed two prominent spots, with similar Rf values around 0.12 and a second spot slightly differing (0.38 in grafted vs. 0.41 in non-grafted), suggesting comparable major compounds. However, under short UV (254 nm) and long UV (365 nm), the non-grafted sample exhibited more distinct spots at multiple Rf values, particularly at 0.06, 0.55, and 0.76, indicating a richer diversity of UV-active compounds. The grafted sample also showed several spots but had additional peaks at Rf values like 0.09, 0.15, and 0.94, which were absent in the non-grafted sample. This suggests that grafting may lead to the formation or suppression of certain phytochemicals. The presence of unique spots in each sample under UV light reflects differences in their secondary metabolite composition. Overall, the TLC results indicate that grafting causes measurable changes in the phytochemical profile of *Maricha*, which could influence its therapeutic properties.

5) RP-UPLC analysis

To assess the quantitative and qualitative variation of bioactive compounds, Reverse Phase Ultra Performance Liquid Chromatography (RP-UPLC) was employed. The RP-UPLC profile revealed significant variation in the chemo-profile, particularly in the concentration of piperine, which is the primary bioactive alkaloid responsible for the pungency and many pharmacological effects of Maricha (*Piper nigrum* Linn). The RP-UPLC analysis showed that the grafted *Maricha* (*Piper nigrum* Linn) sample had a higher concentration of the targeted compound, with a content of 4.57%, compared to 3.57% in the non-grafted sample. This indicates that grafting may have a positive influence on the biosynthesis or accumulation of certain phytoconstituents, possibly due to improved nutrient uptake or stress-induced metabolic activity at the graft union. The increase in the percentage of the active compound suggests that grafted plants may offer enhanced potency for specific therapeutic applications.

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6) Results Interpretation

The analysis revealed that grafted Maricha samples contain a significantly higher percentage of Piperine than non-grafted samples. This finding implies:

- Influence of Grafting on Secondary Metabolism: Grafting may induce physiological changes that enhance secondary metabolite production.
- Agronomic and Pharmacological Benefits: Grafted plants could be preferred for cultivation when medicinal potency is the priority.
- Ayurvedic Validation: The concept of *Desha* (environment) and *Upakrama* (method) affecting drug potency is empirically supported.

The comparative analysis indicates that grafting influences the pharmacognostic features, physicochemical properties, and phytochemical composition of Maricha (*Piper nigrum* Linn) While grafted plants may offer advantages in terms of agricultural productivity, appears superior in terms of phytochemical richness, particularly with higher levels of piperine and other medicinally relevant compounds.

Thus, for pharmaceutical and Ayurvedic applications where quality and efficacy are of prime importance, the grafted variety may be preferred.

Further metabolomic studies and bioactivity assays are recommended to understand the full impact of grafting on therapeutic potential and to explore whether certain graft combinations can balance yield with quality.

7) Integration with Ayurvedic Principles

Acharya Sushruta's statement, *"Satatadhyayanam vadah paratantravlokanam,"* [(10)] meaning continuous study and examination of other disciplines, underlines the necessity of combining modern analytical tools with classical knowledge. This research embodies that philosophy by:

- Using modern RP-UPLC technology to validate traditional claims.
- Emphasizing the dynamic nature of herbal drug efficacy, influenced by cultivation practices.

8) Broader Implications

This study's findings have several broader implications:

- For Farmers and Cultivators: Adoption of grafting can lead to enhanced product value and market competitiveness.
- For Pharmaceutical Industry: Ensuring the quality of raw materials by selecting cultivars with higher active content improves formulation efficacy.
- For Ayurvedic Practice: Validates traditional concepts with empirical data, promoting evidence-based practice.
- For Research and Development: Provides a framework to further explore cultivation influences on other medicinal plants.

9) Limitations of the study

- Geographical Scope: Sampling was limited; wider geographical variation needs exploration.
- Other Phytoconstituents: Only Piperine was quantified; other active components should be analysed.
- Clinical Correlation: Direct pharmacological or clinical studies correlating Piperine content with therapeutic outcomes are necessary.

In summation, the discussion thoroughly examines the hypothesis, supporting the conclusion that grafting significantly influences the phytochemical profile of Maricha (*Piper nigrum* Linn) This supports a more scientific understanding of how agricultural practices intersect with medicinal plant quality, offering a promising avenue to improve herbal drug efficacy.

VI. CONCLUSION

1) Macroscopic study reveals better growth of the Grafted Maricha variety compared to Non-grafted Maricha variety in size, shape and plant growth.

2) Organoleptic study shows presence of higher concentrations of aromatic compounds in Non-grafted Maricha sample compared to Grafted Maricha sample.

3) Microscopic study of both non-grafted and grafted samples confirmed that grafting didn't alter internal anatomy of Maricha.

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4) Physicochemical and Phytochemical studies showed the results as per API standards in both the samples thus confirming genuinity.
5) TLC results confirmed additional bioactive components in the grafted variety of Maricha sample.
6) RP-UPLC study carried out for quantification of Piperine in both the samples confirmed the genuinity and higher Chemo-profile was observed in Grafted variety of Maricha.
7) Grafting was effective not only for increasing yield, Reduction of Harvest period and Foot rot infestation in cultivation of Maricha but also have additional chemo-profile variation compared to conventional method of cultivation with quantitative increase in important bioactive compounds such as Piperine.
8) Thus, Grafting may be beneficial in additional medicinal properties compared to conventional method of cultivation.

A. *Scope for Further Study*

1) Wider Geographical Sampling – to understand how environmental factors such as soil composition, altitude, and climate impact phytochemical concentrations.
2) Longitudinal Studies – to assess seasonal variations and long-term stability of Piperine in both grafted and non-grafted plants.
3) Genetic Profiling – to explore the influence of genetic markers on Piperine biosynthesis and accumulation in grafted vs. non-grafted plants.
4) Pharmacological Correlation Studies – to validate whether the increased Piperine content in grafted samples leads to improved therapeutic efficacy in clinical settings.

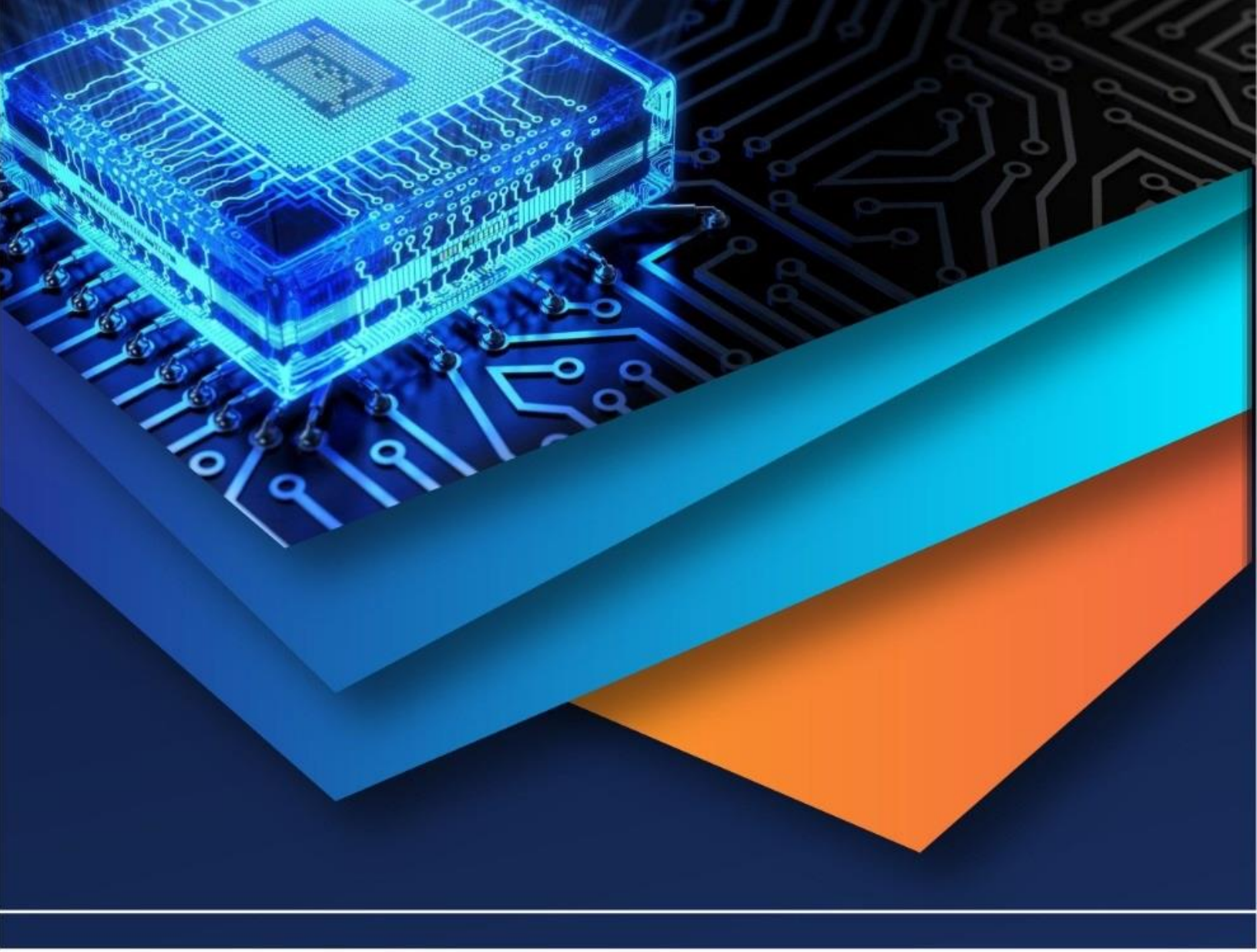
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