

COMPARATIVE AND QUANTITATIVE STUDY OF CARPAINE FROM CARICA PAPAYA COLLECTED DURING THE SEASON AND OFF SEASON GIVEN ACCORDING TO COLLECTION OF DRUG SUBSTANCES IN HOMOEOPATHIC PHARMACOPOEIA OF INDIA

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ABSTRACT

Background:

In the field of homeopathy pharmacy pertains to the timing of drug substance collection, particularly in the context of botanical materials. This gives the significance of collecting components from medicinal plants in its season. Thus be explained within the broader framework of drug standardization.

Methods:

Carica papaya plants are cultivated both during the growing season and the off-season. The collection process includes the procurement and prepares the Mother Tinctures according to HPI and analyzed by High-Performance Thin-Layer Chromatography (HPTLC). The outcomes are graphically presented within the detector, with a specific emphasis on the quantification of alkaloid content.

Results: Extract B i.e., in the peak season shows a slightly higher Carpaine content compared to Extract A i. e in off season, but the difference is minimal.

Conclusion: It is concluded that the collection of drug substance is important to collect in the peak season when the active principles are high concentration.

INTRODUCTION

Carica papaya, scientifically known as *Carica papaya* Linn, is a small evergreen tree belonging to the Caricaceae family. The study looks at collection during seasons to demonstrate the importance of following homoeopathic pharmacopoeia guidelines about collecting the specific plant parts.

In Homoeopathic pharmacopoeia of India (HPI) instructed collecting plant that to collect the fresh fruits must be used soon after picking. The collecting of the drug substance is a crucial phase in the preparation of medicine. It is important to know that every drug has its own peak time of concentration of its chemical constituents in a specific season. The collection of these materials for preparation of medicine during its own peak season will enhance the therapeutic efficacy. The present study examined collecting the *Carica papaya* fruit during two separate seasons, with the goal of demonstrating the critical importance of following the collection guidelines outlined in the homoeopathic pharmacopoeia of India.

The fruits are ready for harvesting when the latex transitions from milky to watery. Warm, dry conditions are favorable for ripening. *Papaya* crops are susceptible to strong winds, frost, and waterlogged conditions. A dry climate with minimal rainfall enhances sweetness.

(Ref. textbook of fruit growing by J.S. Bal). The temperature range from 25^o C- 35^o C is optimum for growth and development of fruit. Lower than the temperature 10^o C restricts growth development of fruit and results less flavor and sweetness of fruits. Carpelloid fruits grow at below 20^o C temperature. (Ref. Textbook of tropical fruit crops by theory to practical by S.N. Ghosh and R. R. Sharma)

Alkaloids are categorized by their structure and origin and it is a class of organic compounds which plays a great role due to their varied and potent activities in the medicine and pharmacology fields. High-performance thin-layer chromatography (HPTLC) is a superior in chromatographic techniques that has power to separates capabilities optimized coating materials, innovative mobile-phase delivery systems, and improved sample application procedures to achieve superior analytical outcomes. The major aspects to focus on the separation of the mixture are include partition coefficients, retention factors (R_f), and capacity factors (k) of individual analytes, selection of the mobile and stationary phase to the solutes, and the plate height that gives the separation efficiency and resolution of each compound in a mixture. The resolution helps to separate components of a mixture depending on the selection of the components in the mobile phase and stationary phase. Mobile phase flow rate depends on particle size and solvent strength that influence capacity factors. (Variyar, P.S., Chatterjee, S., Sharma, A. (2011). Fundamentals and Theory of HPTLC- Based Separation.)

AIM

Comparative and Quantitative Study of Carpaine from *Carica papaya* Collected during the Season and in Off Season given according to Collection of Drug Substances in Homoeopathic Pharmacopoeia of India.

OBJECTIVES

Primary Objective:

To find out the quantity of alkaloid- Carpaine in *Carica papaya* collected during the season and off season.

Secondary Objective:

To find out the importance of collection of drug substance according to given in HPI with the help of above experiment.

MATERIALS AND METHODS

1. Selection of Tool: HPTLC

2. Site of Study: Medisynth Chemicals PVT. Ltd, Turbhe and Bharati Vidyapeeth Poona college of Pharmacy, Pune, Maharashtra, India
3. Equipment used: HPTLC
4. Investigation Products:
Carica papaya mother tinctures prepared from the drug substance collected in the peak season and in the off season.
5. Preparation: Unripe green fruit of *Carica papaya* Linn were collected from Pune in the month of February 2023 (in off season) and September 2023 (in the peak season) and *C. papaya* L., authenticated by Botanist, Bharati Vidyapeeth Deemed to be College, Pune, Maharashtra, India. The collection process involves the procurement of unripe green fruit, excluding seeds, and including the moist mixture composed of 100 grams of solid material and 400 milliliters of moisture. This mixture is subsequently combined with 500 milliliters of strong alcohol to yield a total volume of one thousand milliliters, thus giving rise to the Mother Tincture (according to HPI) and analyzed with the HPTLC to know the quantitative variation of major alkaloid Carpaine from *Carica papaya* mother tinctures of homoeopathic medicines Extract A (Off-season) and Extract B (in peak season).
6. Analysis method: The analysis method used in the present study is HPTLC. The stationary phase prepared with HPTLC Silica gel 60 F₂₅₄ plates (Merck) measured 100 x 100 mm prewashed with Methanol, followed by heating at 105^o C. The Instrument used as Linomat 5 (S/N: 150721) with Band type application locating position Y: 8.0 mm, length: 8.0 mm, width: 0 mm and track position: First position X: 21.5 mm, distance between tracks: 11.4 mm using 2.0 µL sample per application with 50 nL/s dosage speed and water as a solvent. Chromatography development chamber measured TTC 10x10 tank, Mobile phase prepared Methanol: Ethyl acetate : Water : Formic acid (4.0 : 4.0 : 1.5 : 0.2 v/v/v/v) with saturation time 20 min and Whatman No. 1 Filter paper as saturation pad with development distance 70 mm drying 5 min at room temperature.

In the present study, the HPTLC method used with different solvents and instruments, such as TLC Visualizer 2(S/N: 290326) With modes for White light wavelength UV 254 nm, and UV 366 nm and TLC Scanner 3(S/N: 151015) with wave lengths 254 nm with Deuterium lamp, 366 nm with Mercury lamp. The procedures involved developing the plate, scanning the plate, and derivatizing the plate using Dragendorff's reagent. Post-derivatization analysis with Tungsten lamp Scanning at 520 nm and Deuterium & Tungsten lamps spectrum scan under 200-800 nm, additionally at 289 nm scanned. The data analyzed with the software visionCATS (version 3.2.23095.1) with integration parameters of Savitzky- Golay smoothing (window 7), lowest slope baseline correction and peak detection Gauss (legacy) with sensitivity 0.1, separation 1, and threshold 0.1. The main compound of focused was identified as Carpaine, with an average R_f value of 0.684 ± 0.086.

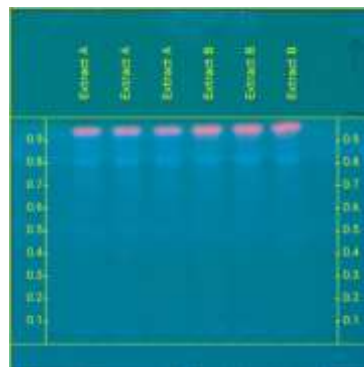
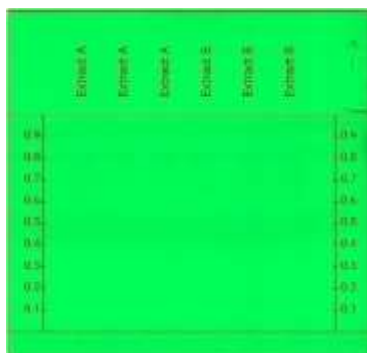
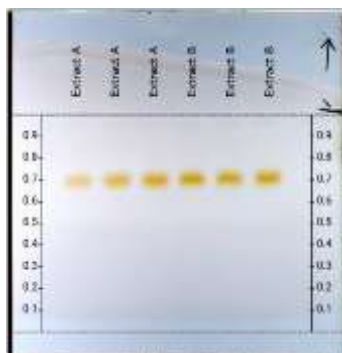
RESULTS:

The results of the evaluation include the RF values of the substance Carpaine, which are 0.684 +/- 0.086, 0.691 +/- 0.093, and 0.698. The calibration results show

an area calibration for substance Carpaine at 520 nm with a linear-2 range deviation of 5.00%.

With the help of the HPTLC analysis method, the peak for Extract B (In the peak season) is slightly taller and larger area compared to Extract A (in the off season) range from 0.1036 AU-0.0994 AU, 0.00623 AU-0.00607 AU respectively. The slight difference in Rf values indicates that the Carpaine

in both extracts is likely the same compound and is interacting with the stationary and mobile phases in the same manner. Whereas in Extract B before derivization with Dragendorff's reagent, there are bands other than Carpaine which indicates the other phytoconstituents of the Carica papaya of the respective plant in the peak season.



After Dragendorff's reagent (fig.1)

Before Dragendorff's reagent 254nm (fig.2)

Before Dragendorff's reagent 366nm (fig.3)

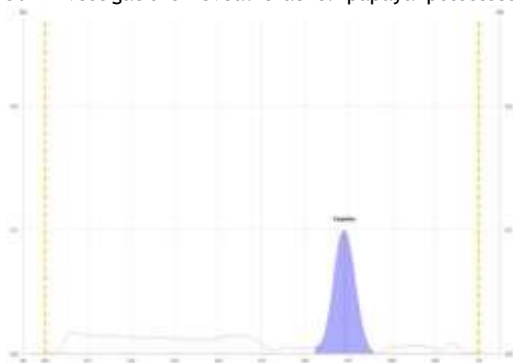
CONCLUSION

The results presented in this study reveal that HPTLC is an effective method to determine the total alkaloid content in the mother tincture extract (A & B) from Carica papaya fruit. Based on the above experiment Extract A and Extract B have slight differences in peak height and area suggested that Extract B contain a marginally higher concentration of Carpaine. The nearly identical Rf values indicate that the Carpaine in both extracts is likely the same compound and is interacting with the stationary and mobile phases in the same manner. The evaluation has the lack of a standard reference application available for the substance Carpaine but it is to be noted that the peaks were correctly detected and assigned for this substance.

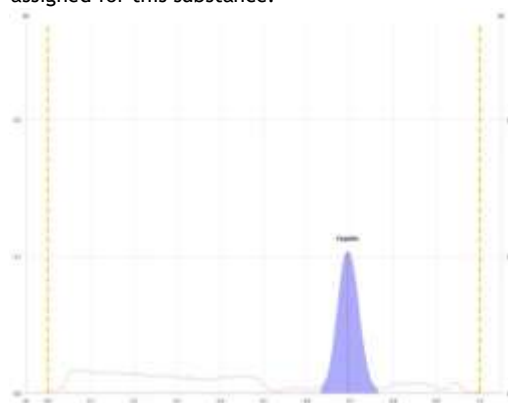
DISCUSSION

This study aims to conduct a comprehensive quantity assessment of C. papaya mother tincture, focusing on the quantitative analysis of its metabolite profile using analytical approaches. Our investigations reveal that C. papaya possesses a complex

phytochemical composition, with a notable presence of alkaloids. Among these, Carpaine has been identified as a major alkaloid constituent in the Carica papaya mother tincture. Analysis with the HPTLC method to determining the total alkaloid content in the mother tincture extract from Carica papaya fruit with the above experiment the Extract A (in the off season) and Extract B (in the peak season) have slight differences in peak height and area suggested that Extract B contain a marginally higher concentration of Carpaine. The nearly identical Rf values indicate that the Carpaine in both extracts is likely the same compound 0.684 +/- 0.086, 0.691 +/- 0.093, and 0.698. The evaluation has the lack of a standard reference application available for the substance Carpaine, but it is to be noted that the peaks were correctly detected and assigned for this substance.



Extract A (In the off season. fig.4)



Extract B (In the peak season. fig.5)

The implementation of advanced analytical techniques enables the generation of more robust and precise scientific data pertaining to the plant's chemical constituents. This enhanced understanding of the importance of collection of homoeopathic drug substances. The study is needed for further research to fully elucidate the bioactive compounds in C. papaya mother tincture.

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