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EXTRACTION AND ISOLATION OF CARPAINE FROM CARICA PAPAYA LEAF

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ABSTRACT

There have been numerous studies conducted on humans and animal models to confirm the effectiveness of papaya leaves extract for the treatment of thrombocytopenia. In the present study, the alkaloid, carpaine extracted from papaya leaves was found to have platelet stimulating activity. Carpaine is isolated from the papaya leaf extract by using the Soxhlet extraction method. For extraction 64 g of leaves powder was extracted with a suitable mixture of solvents like Methanol, Ethanol, Glacial acetic acid, HCL, and Water in Soxhlet apparatus for 48 h at 70°. Then the Carpaine should be isolated from the papaya leaf extract by treating the extract with the solvent like petroleum ether, ammonia, chloroform, n-hexane. Carpaine present in papaya leaf shows Anti-thrombocytopenic activity. The isolated Carpaine has been evaluated for the various evaluation parameters such as Phytochemical, TLC, HPTLC, FTIR, and DSC analysis. The result of preliminary phytochemical test screening ofextract of carica papaya leaf powder revealed that presence of alkaloids. Thin layer chromatography studies of this compound were optimized with the solvent system which showed a spot at RF = 0.74. In HPTLC the RF value of Carpaine was found to be 0.94. DSC the thermogram obtained having two peaks at 133.22 and 242.56°C respectively. In FTIR the presence of functional groups was confirmed and matched with standard range and observed value. From above result, It is concluded Carpaine was Extracted and Isolated from papaya leaf.

KEYWORDS: Carica Papaya Leaf, Anti-thrombocytopenia, Carpaine, Extraction, Isolation, HPTLC, DSC, FTIR.

INTRODUCTION

Carica papaya linn: Belonging to family Caricaceae has been used to treat the ailments like malaria, dengue and jaundice. Carica papaya leaf is used as food or as medication in folk medicine. Traditionally, the leaf extract was used as a tonic for the heart, analgesia, and treatment for stomach ache. Leaf of Carica papaya plant contains carpaine, carpinine, pseudocarpine, vitamin C, vitamin E, choline and carposide.[1] Its young leaves are rich in flavonoids, alkaloids, phenolic compounds, cynogenetic compounds and carotenoids.[2] Leaves extracts from Carica papaya is generally used for patients with dengue fever. The extract is available in the form of capsules, tablets and syrup.

Carpaine (fig. 1) belongs to the class of macrolide analogues. It is one of the major alkaloids of papaya leaves which have been studied for its cardiovascular effects. Carpaine is isolated and identified from Carica papaya leaves with the content of 0.93 g/kg.

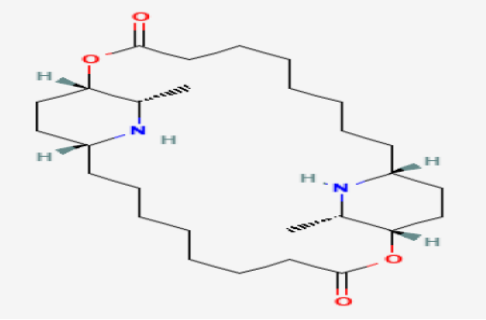


Fig. 1: Structure of carpaine.

It is also reported to increase platelet count in rats. Carpaine (C28H50N2O4) from papaya leaves is responsible for the anti-thrombocytopenic activity, e.g., raising patient's platelet count, during treatment of dengue fever. There are some marketed formulations that contain the Carpaine in their formulation like Caripill-1100mg, Platigain-1100mg, Platfast-1100mg etc.[3]

Therapeutic Application of Carpaine slows the heart rate in humans and thus reduces blood pressure, anthelmintic action, anti-plasmodial and anticancer activity. It is also reported to increase platelet count in rats.[4]

MATERIAL AND METHOD

All solvents (analytical grade) Methanol, Glacial acetic acid, H C L, Petroleum ether, N-hexane, Chloroform, Ammonia, Toluene and Ethyl acetate were purchased from S.D fine Chemical limited Mumbai (India).

List of Equipments and Instruments are Ultra-Sonicator, Hot air oven, Electrical Water-bath, Lyo-philizer, Digital weighing balance, HPTLC, DSC, FTIR.

➢ Solvent extraction method for alkaloid[5]

First step: The powdered defatted drug is mixed with an alkaline aqueous solution which displaces the alkaloids from their combinations as salts; the free bases are then extracted with an organic solvent (Dichloromethane, chloroform, ethyl acetate or diethyl ether).

Second step: The organic solvent containing the alkaloids as bases is separated from the residue and if necessary, partially concentrated by distillation under reduced pressure. The solvent is then stirred with an acidic aqueous solution: the alkaloids go into solution in the aqueous phase as salts, whereas the neutral impurities remain in the organic phase. The operation is repeated as many times as necessary until the organic phase The operation is repeated as many times as necessary until the organic phase no longer contains any alkaloids. Many acids are used (hydrochloric, sulfuric, citric, tartaric), but always in very dilute solutions.

Third step: The aqueous solutions of the alkaloid salts, combined, and if necessary, "washed" with a polar solvent (hexane, diethyl ether) are alkalinized with a base in the presence of an organic solvent not miscible with water. The alkaloids as bases precipitate and dissolve in the organic phase. The extraction of the aqueous phase continues until the totality of the alkaloids has gone into the organic phase (which is easy to verify as Mayer's reaction on the aqueous phase becomes negative).

Finally, the organic solvent containing the alkaloids as bases is decanted, freed from possible traces of water by drying over an anhydrous salt (For example sodium sulphate), and evaporated under reduced pressure. A dry residue is left the total basic alkaloid.

➢ Isolation of carpaine from alkaloid[6]

The extract obtained by Soxhlet extraction was further washed with petroleum ether ((PE), 160 ml) until PE layer was colourless. After removal of chlorophyll, the extract was basified with concentrated ammonia (28 %, 8 ml) followed by partitioning the extract in distilled water (80 ml) and PE (200 ml). The mixture was sonicated for 10 min and then was kept overnight without disturbance. On next day, the PE layer was collected and evaporated for removal of PE. The residue obtained was alkaloid fraction. Obtained alkaloid fraction was partitioned in n-hexane (80 ml), sonicated for 5 min, and kept aside for 1 h. Hexane layer contained Carpaine and its derivatives. Isolated fraction from hexane was separated using preparative thin layer chromatography (TLC) by using mobile phase toluene: ethyl acetate: glacial acetic acid (5:3.5:1.5, v/v/v) to get pure Carpaine.

Fig. 2: Soxhlet extraction.

Fig. 3: Dried papaya leaf.

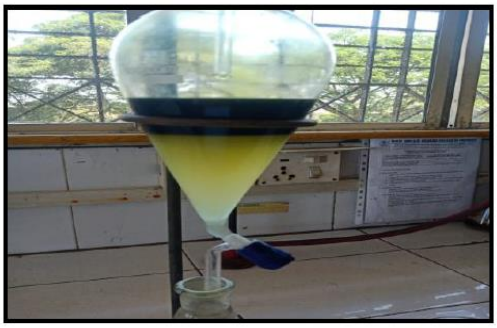


Fig. 4: Extract washed with petroleum ether.

Fig. 5: Solvent evaporation.

Fig. 6: After evaporation of solvent.

Fig.7: Lyophilization of Sample.

➢ **Phytochemical analysis**

The analysis for alkaloid were carried out according to standard methods, Dragendroff's Mayer and Wagner's test.

➢ **Determining the Thin Layer Chromatography (TLC) Profile[7]**

The thin-layer chromatography method used silica gel 60F 254 as the stationary phase and toluene: ethyl-acetate: glacial acetic acid (5:3.5:1ug/ml) as a mobile phase for the identification of alkaloid was performed using the dragendroff's reagent.

➢ **HPTLC Profiling[8]**

Sample preparation

An amount of 0.25g of isolated Carpaine was dissolved in 10 mL methanol and further diluted to 10-fold to prepare 0.1 g/mL working solutions.

➢ **Structure analysis**

Structure and functional group were analysis by Fourier Transform Infrared Spectroscopy (FT-IR) for carpaine alkaloid form papaya leaf extract.

➢ **Thermogram analysis**

Thermogram Peak were determined the sample of carpaine alkaloid from papya leaf extract 4.180mg by using the Differential scanning calorimetry Analysis (DSC).

RESULT AND DISCUSSION

Alkaloids were identified in the dried papaya leaves based on a phytochemical investigation (Table 1). Alkaloid content, which demonstrates its numerous medicinal uses. It has been observed that the papaya leaf extract, which is used to treat cancer, also raises the platelet count in rats. When treating dengue fever, carpaine (C28H50N2O4) derived from papaya leaves is what has the anti-thrombocytopenic effect, such as increasing the patient's platelet count. The presence of the carpaine alkaloid, which is extracted from Carica papaya leaves, was clearly demonstrated by the observed value in (Table 2), where the FTIR result verified and matched the presence of functional groups with the standard range.

Table 1: Phytochemical Screening of papaya leaves extract.

Name of test	Methadology	Interference	Result
Dragendroff's test	3ml of extract was taken in tets tube and few drop of Dragendroff's reagent	Orange brown precipitated was observed, indicates presence of alkaloids	+

Mayer's test	3ml of extract was taken in a test tube and few drop of Mayer's reagent was added	A yellowish or white ppt was formed, indicate presence of alkaloids	+
Wagner's test	3ml of extract was taken in a test tube and few drop of Wagner's reagent was added	Reddish brown precipitate was observed indicates presence of alkaloids.	+

Fig. 8: Phytochemical test for alkaloid.

The result of preliminary phytochemical test screening of extract of Carica papaya leaf powder revealed that presence of alkaloids.

Determining the Thin Layer Chromatography (TLC) Profile

TLC Profile

$$\text{Rf value} = \frac{\text{Distance Travelled by Solute}}{\text{Distance travelled by Solvent}}$$

= 4.3/5.8
= 0.74cm

Detailed Thin layer chromatography studies of this compound were optimized with the solvent system toluene: ethyl acetate: glacial acetic acid (5:3.5:2v/v/v) which showed a spot at **RF = 0.74**

HPTLC Profiling

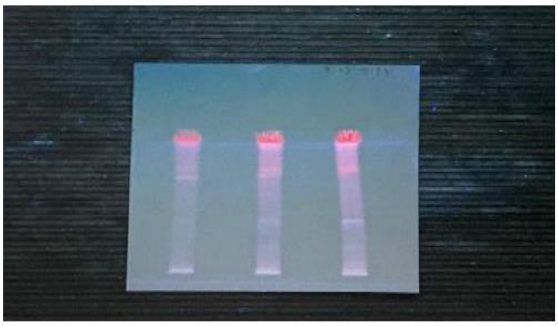
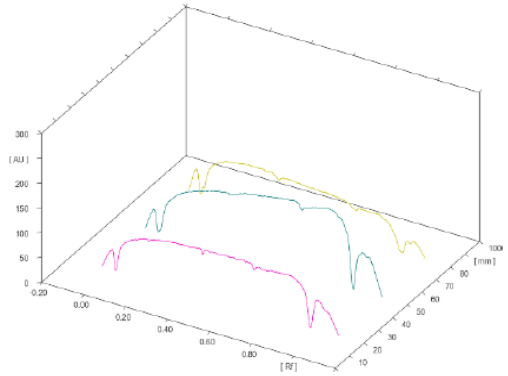
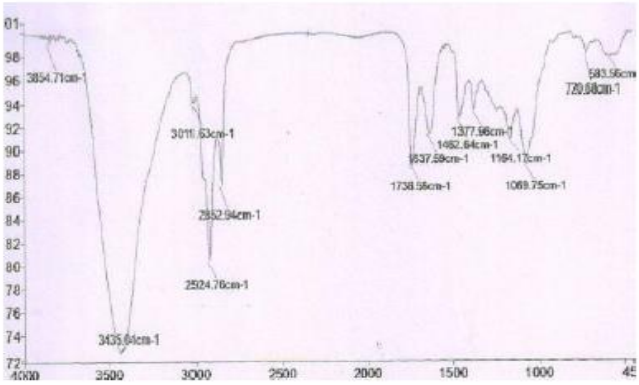


Fig. 9: HPTLC Photo Documented of Carpaine.



In HPTLC the RF value of Carpaine was found to be 0.94

FTIR Analysis

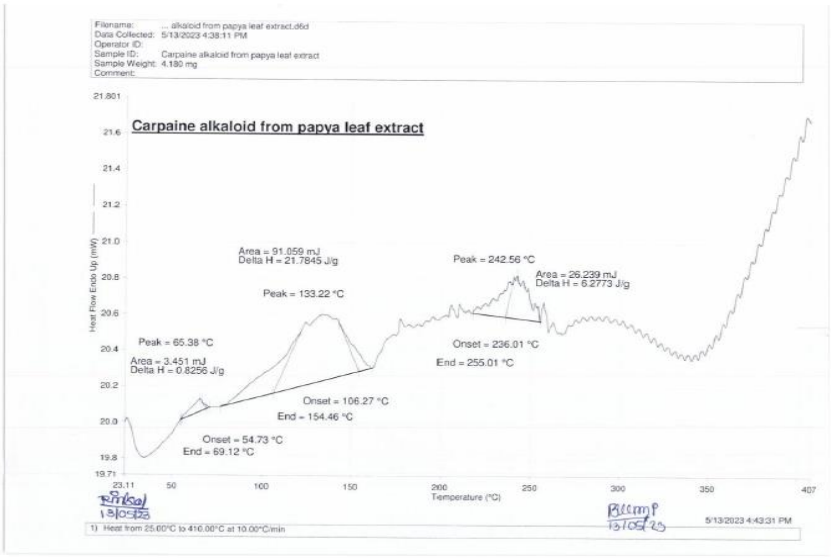


Peak of FTIR
Table 2: FTIR result.

Functional group	Standard (cm^{-1})	Observed(cm^{-1})
N-H	3500-3100	3435.04
C-H	3000-2850	2924.76
C=O	1725-1705	1738.58
C-O	1300-1000	1069.75

From the above table of FTIR result reveled that the presence of functional groups was confirmed and matched with standard range and observed value.

Differential scanning calorimetry Analysis (DSC)



Thermogram peak of Carpaine

Based on the result obtained from the above given thermogram the peaks were found to be 133.22°C and 242.56°C indicating the presence of Carpaine alkaloid from papaya leaf extract.

CONCLUSION

The phytochemical test for identification of alkaloids were performed such as Dragendorff, Wagner and Mayer show a positive result and confirmed the presence of alkaloid.

From TLC we obtain the RF value 0.74cm in TLC by using mobile phase toluene: ethyl acetate: glacial acetic acid (5:3.5:1.5, v/v/v) to get pure Carpaine. In HPTLC Carpaine sample was detected by CAMAG TLC SCANNER mobile phase 8:2 ml chloroform: methanol at wavelength 510.

From DSC the thermogram obtained having two peaks at 133.22 and 242.56°C respectively indicating the presence of Carpaine alkaloid from papya leaf extract.

In FTIR the presence of functional groups was confirmed and matched with standard range and observed value.

Standard range of N-H is 3500-3100 cm¯1 and observed range was found to be 3435.04 cm¯1 and C-H standard range is 3000-2850 cm¯1 and observed range is 2924.76 cm¯1.

From above result, it is concluded Carpain was extracted and isolated form papaya leaf.

REFERENCES

1. Thakur S. Formulation and characterization of herbal tablets for the management of dengue. *EPRA International Journal of Research and Development (IJRD)*, 2021; 6, 105: 4.
2. Yogiraj V, Goyal PK, Chauhan CS, Goyal A, Vyas B. Carica papaya Linn: an overview. Int J Herb Med, 2014; 2(5): 1-8.
3. "National Center for Biotechnology Information.," PubChem Compound Summary for CID 442630, Carpaine., [Online]. Available: https://pubchem. ncbi. nlm. nih. gov/compound/Carpaine. . [Accessed 14 May 2023].
4. Munir S, Liu ZW, Tariq T, Rabail R, Kowalczewski PŁ, Lewandowicz J, Blecharczyk A, Abid M, Inam-Ur-Raheem M, Aadil RM. Delving into the Therapeutic Potential of Carica papaya Leaf against Thrombocytopenia. Molecules, 2022; 25, 27(9): 2760.
5. C.K. Kokate, A.P. Purohit, S.B. Gokhale, Pharmacognosy, Nirali Prakshan, Nov, 2018.
6. Zunjar V, Dash RP, Jivrajani M, Trivedi B, Nivsarkar M. Antithrombocytopenic activity of carpaine anHead WF, Lauter WM. Phytochemical Examination of the Leaves of Carica papaya L. Economic Botany, 1956; 10: 258-260.
7. alkaloidal extract of Carica papaya Linn. leaves in busulfan induced thrombocytopenic Wistar rats. Journal of ethnopharmacology, 2016; 2, 181: 20-25.
8. Devi S, Ropiqa M, Murti YB, Nugroho AK. Screening of Extraction Process and The Estimation of Total Alkaloids in Carica papaya Linn. Leaf. Majalah Obat Tradisional, 2020; 25(2): 90-95.
9. Haldar S, Mohapatra S, Singh R, Katiyar CK. Isolation and quantification of bioactive Carpaine from Carica papaya L. and its commercial formulation by HPTLC densitometry. Journal of Liquid Chromatography & Related Technologies, 2020; 20, 43(11-12): 388-393.