Atomic Spectroscopy

Article

Ftir, Nmr, And Phytochemical Analysis Of Isolates From Methanolic Extract Of Neurocalynx Calcinus

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ABSTRACT: Paccha chedi is the vernacular name of Neurocalynx Calcinus, Using the soxlet extraction method, several solvents of variable polarity (chloroform, ethyl acetate, methanol, and water) were used to extract the plant Neurocalynx Calcinus. According to analysis, these extracts contained alkaloids, saponins, proteins, amino acids, terpenoids, and flavonoids. It identified specific chemical groups, including -OH, -COOH, -CH2, and C=O, through their absorption bands at 3456 cm-1 and 2939 cm-1, with FTIR spectroscopy analyzing with reference to a standard reference chart. Methanolic extract was used for accurate results Additionally, Nuclear Magnetic Resonance (H1 NMR) was employed to investigate the electronic states and characteristics of protons within these compounds. Further research is required to examine the possible therapeutic applications of these active chemicals in the creation of certain compounds very active in nature which can be further used to explore new drug development based on their therapeutic lineage.

Keywords: Neurocalynx Calcinus, Phytochemicals, Alkaloids, Fourier Transform Infrared (FTIR) spectroscopy, chemical constituents, and Nuclear Magnetic Resonance (NMR) analysis.

INTRODUCTION

CONTEXT: Wound healing involves the intricate process of repairing damaged tissue, by anabolic progression as a result of enhanced cellular function, matrix signalling, and various physiological processes. Wounds are essentially physical injuries caused to living tissues due to discontinuation, disruption of cellular function, anatomical rupture, and functional issues in the concerned parts. However, an acceptable treatment is still impractical for fully healing a wound. A natural wound healing product that is readily available, reduces pain and costs, promotes tissue integration, leaves minimal scarring, and promotes rapid healing is urgently needed. For thorough research on Wound healing studies conducted within a laboratory setting *(IN-VITRO)* and within living organisms *(IN-VIVO)* examination, Neurocalynx Calcinus (Rubiaceae) was chosen based on ethnopharmacological investigations. This shrub, known as "Pacha chedi," is indigenous to the jungles of Kerala and the southern India. The plant is said to have anti-oxidant, analgesic, and anti-inflammatory qualities. It also demonstrated considerable burn healing, immune-enhancing, and wound healing properties. By using IR and NMR analysis on isolation, several compounds, including flavonoids, polyphenols were found in the NCME extract. Additionally, the anti-inflammatory, antibacterial, and acute dermal healing effects of solutions with different NCME crude concentrations created using this extract will be further evaluated, along with their safety, in a rat excision, incision wound models.[1,2]

COLLECTION AND PREPARATION: *Neurocalynx Calcinus* plant was collected from the Nilambur forest area Palakkad and was authenticated by Venkateshwara college of Botanical college, Tirupathi, using solvents of differing polarities such as chloroform, ethyl acetate, methanol, and water,the procedure involved immersing a mixture containing 1g of the sample and 10ml of the chosen solvent for a duration of 8 hours.

The best rate of wound contraction will be co-related in the histopathological findings. Compound identified in NCME as a wound healer, were basically quercetin which are flavanols catechins which are flavonoids similar to glucoside and kaempferol, which is a flavonoid lipid molecule. The outcome also demonstrated that NCME extract had high antioxidant, anti-inflammatory, and antibacterial action. Based on the phytochemical background, it is likely to be the next target for a novel medicine and can significantly speed up wound healing, supporting traditional use.[3,4]

METHODS

Plant material collection and various extract preparation: NC plant was collected from the Nilambur forest area Palakkad and was authenticated by Venkateshwara College of Botanical college, Tirupathi. About 200gms of shade-dried powder was successively extracted by Chloroform, Ethyl acetate, methanol and Water at room temperature by Soxhlet extraction method, the extraction indicated completion on change of initial colour of percolate from dark green to brown colour very light colour like straw colour, each of the solvent extracts was concentrated in the rotatory evaporator under reduced pressure and the methanolic extract was freeze-dried. The methanolic extracts of NC called NCME were prepared by 100g of powdered plant extract for 30 min using 500 ml of methanol. The extract was reconstituted for the required concentration of 5mg/kg and 10mg/kg in sterile methanol.

The phytochemical components of medicinal plants are combinations that more clearly or indirectly show how to prevent or treat various ailments. The Ayurvedic medicinal tradition commonly employs the Soxhlet extraction method, a traditional extraction approach, for addressing diverse health conditions according to specific criteria. Samples are immersed in distinct solvents and left to interact for a 24-hour period. Phytochemicals are categorized as essential or non-essential constituents based on their effectiveness in aiding plant digestion. Isolation procedures involve techniques such as TLC and HPLC. The functional groups within the sample's compounds are detected through FTIR analysis, while the count of protons in the compounds is determined using H1 NMR. This is also utilized to ascertain functional groups within the sample's compounds and to ascertain the number of protons within the compounds.[5]

PHYTOCHEMICAL SCREENING:

To identify the phytoconstituents, all of the extracts were subjected to phytochemical screening employing a traditional technique, as described by Tiwari et al[6]. Numerous tests were conducted, including those on alkaloids, carbohydrates, phenols, glycosides, saponins, proteins, phenol, terpenoids, flavonoids, and tannins. [7]

Table 1: Screening of Phytochemicals of *NEUROCALYNX CALCINUS*
Plant utilizing extracts of different solvents

TESTS	CHLOROFORM	ETHYL-ACETATE	METHANOL	AQUEOUS
Alkaloid	ABSENT	PRESENT	PRESENT	ABSENT
Carbohydrates	ABSENT	ABSENT	ABSENT	ABSENT
Glycosides	ABSENT	PRESENT	PRESENT	ABSENT
Saponins	PRESENT	ABSENT	PRESENT	PRESENT
Proteins &	ABSENT	ABSENT	ABSENT	ABSENT
Amino acids	ABSENT	ABSENT	PRESENT	ABSENT
Phenol	ABSENT	ABSENT	PRESENT	ABSENT
Terpenoids	PRESENT	ABSENT	ABSENT	PRESENT
Flavonoids	ABSENT	PRESENT	PRESENT	PRESENT
Tanins	ABSENT	PRESENT	PRESENT	PRESENT
Steroids	ABSENT	ABSENT	PRESENT	ABSENT
Cardiac	ABSENT	ABSENT	ABSENT	ABSENT
glycosides	ABSENT	ABSENT	ABSENT	ABSENT

FT-IR (Fourier Transform Infrared Spectroscopy)

An FT-IR spectrometer (Varian Instruments, Shimadzu), KBr bar splitter, and Attenuated Total Reflection (ATR) accessories were used to analyse the material. The Dura Sample IR single-pass jewel-covered internal reflection surface, together with an electronic load display and regulated contact pressure through a hardened steel bar, were used in the ATR inspection equipment. This made sure that the fibre testing completely Placed a cover over the ATR device's 2 mm diameter window. With co-added outputs of 4 cm and 16 cm, five measurements were conducted for each individual fibre test in the 4000-600 cm-1 range. There was no link between any of the spectra's absorbance units and the ATR standard calibration. [8,9,10]

Thermo Fisher Scientific's Grams/AI (Version 9.1) was used to integrate the spectra into the GRAMMES IQ application. With a second-degree polynomial, 11 data points, and the Savitzky-Golay method, the mean of each sample's range was computed. The spectra were normalized by dividing the intensity of each band by the average intensity within the 1800-600 cm^-1 region.[11]

Next, Centering around the mean (MC) and applying Savitzky-Golay first-derivative. preprocessing (using a second-degree polynomial and 13 data points), and cross-validation approach were used to represent the 1800-600 cm-1 IR region using principal component analysis (PCA). Using a second- A polynomial of a specific degree with 11 data points, employing the Savitzky-Golay technique. was used to compute and smooth the range for each case. By dividing each band's intensity by utilizing its average intensity within the range of 1800-600 cm^-1., the spectra were normalised. Then, using mean centering, Savitzky-Golay first-derivative preprocessing (using a second-degree polynomial and 13 data points), and cross-validation approach, PCA analysis was carried out on this IR region. [12,13,14]

Nuclear magnetic resonance of protons (H1-NMR):

The chemical shifts provide details on the electronic and spatial states of the protons, while the integral , Analyzing the signals beneath the peaks provides insights into the molecule's proton count.. The coupling patterns provide details about the protons that are adjacent (vicinal or geminal). Peak intensities are shown by the ordinate, and chemical shift values () for particular proton types are represented by the abscissa. Clusters of methyl, methylene, and methine groups joined to

saturated carbon atoms are frequently found to contain protons. Olefinic methine protons are shown as peaks between 4.8 and 5.9 ppm. Additionally, aldehyde groups exhibit signals in the 9.0 to 10.0 ppm range. [15,16,17]

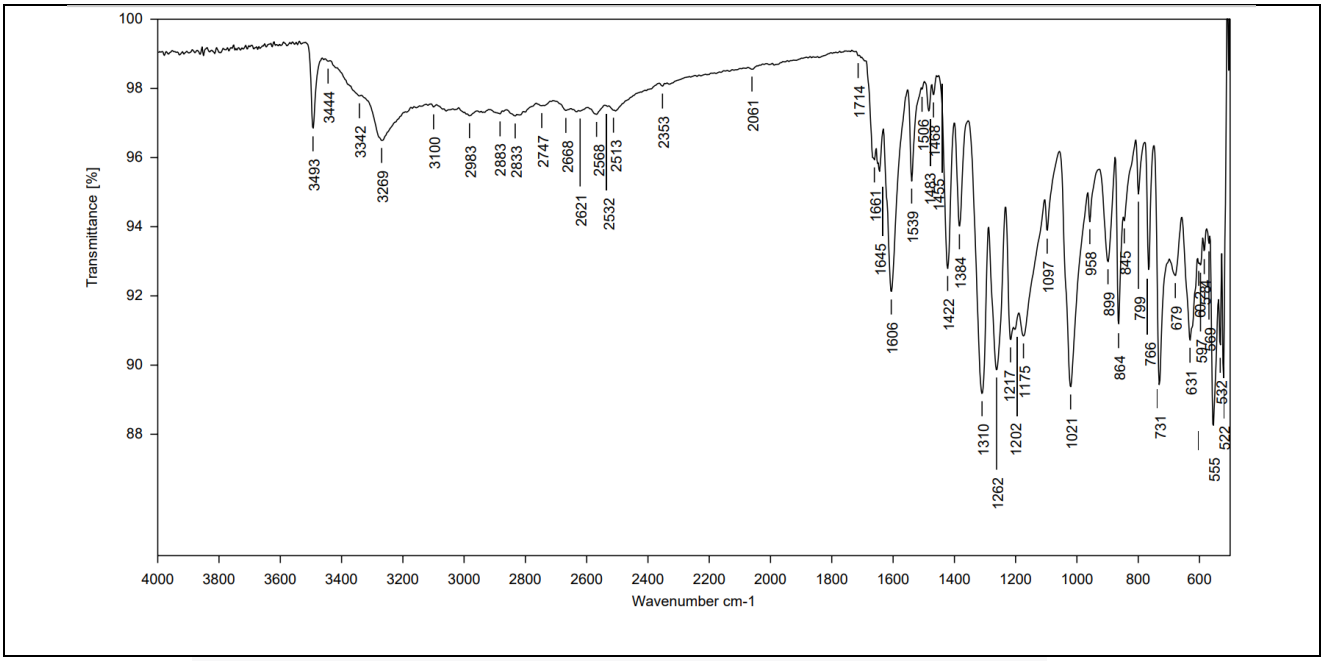
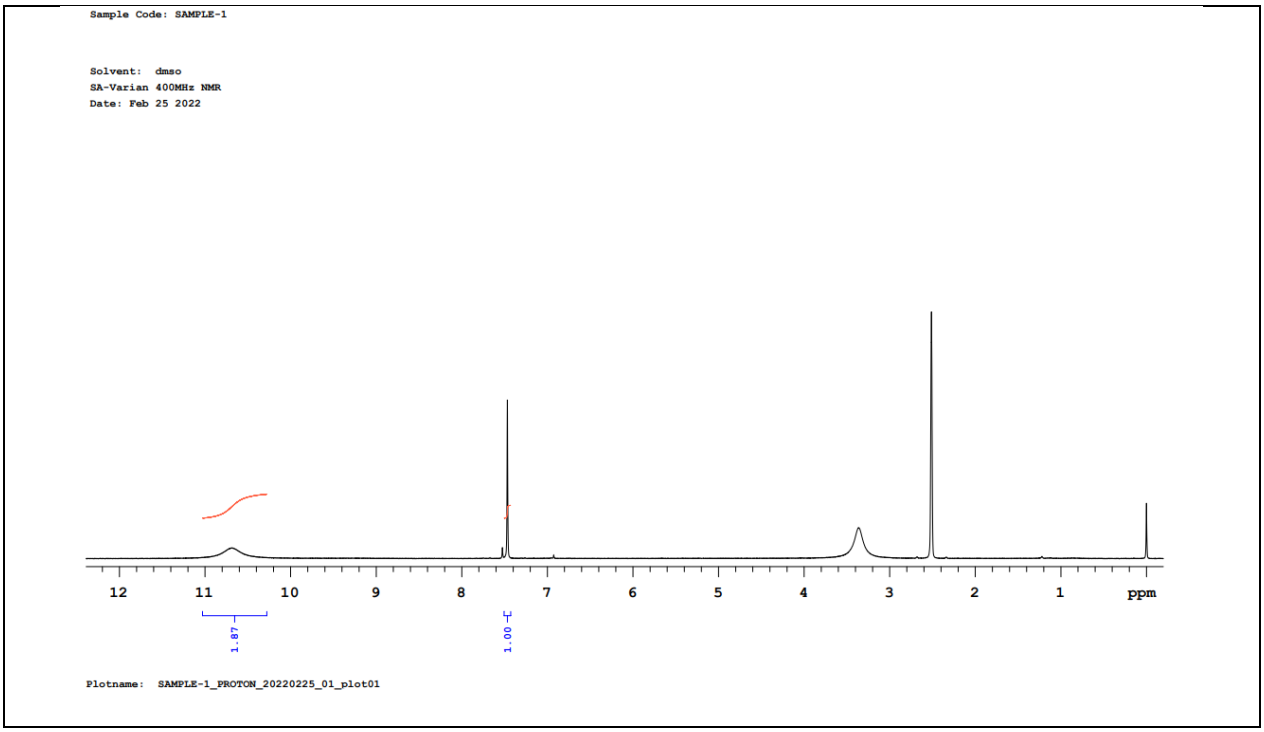


Figure 1 Displays Spectra obtained from Fourier Transform Infrared Spectroscopy.

Figure 2 SPECTRA FPR Proton Nuclear Magnetic Resonance (H^1-NMR):



RESULTS: The various extracts of the sample contained a variety of ingredients based on the findings of the phytochemical screening. Alkaloids, phenols, and flavonoids were present in alkaloids, ethyl acetate, Chloroform, and aqueous extracts, yet proteins and amino acids were only found in the methanol extract. The presence of terpenoids, flavonoids, tannins, and proteins was demonstrated by the methanol extract. Table 1 provides a summary of these findings. FTIR spectroscopy was employed for the identification of functional groups, and the spectrum was acquired using a diffused reflectance mode. The FT-IR spectra shown in Figure 1 showed two high wave area IR absorption bands at 3456 cm-1 and 2939 cm-1, which were linked to the vibrations involving the asymmetric and symmetric stretching of -OH and -CH groups and -CH2 groups,

respectively. There were also more spectral bands Table 2 lists the Bending oscillations of the CH, C-O-C, and CH3 groups, along with the bending of the skeletal bonds and Figure 1 depicts them graphically. The retrieved compounds were compared to a standard chart in order to determine which chemicals were active. The results from the H1 NMR testing are presented in Table 3. The exocyclic double bond was represented by two olefinic protons in the spectrum from 5.34 to 7.26. Additionally, there were small tertiary singlets at 0.63.0.65, 0.68, 0.74, 0.90, and 0.97 for a second secondary hydroxyl group. [16,17,18]

TABLE 2 Compounds retrieved from IR SPECTRUM

Spectrum	Compounds
3273.02	Alcohols (O-H stretch)
2922.16	Alkanes and alkyles (C-H stretch)
2852.72	Alkanes and alkyles (C-H strech)
1631.78	C=C-C(O)-OH or Ar-C(O)-OH. C=O stretch
1573.27	Amides (N-H)
1386.62	Alkanes and Alkyles (CH3 C-H bond)
1236.37	Alkyl halides (C-F stretch or ether)
1029.99	Ethers (Ar-O-R =C-O-C)
659.66	-
582.50	-
497.63	-
449.41	-

Table 3 Compounds retrieved from H^1 NMR

PPM	Cor_pounds
7.26	Aromatic phenol or OH
5.35	Vinylic or amide
5.34	Vinylic or amide
3.87	α to oxygen
3.66	Alcohol ,aniline
3.78	α to oxygen ,OH ,aniline
2.77	α to nitrogen
2.75	α to nitrogen
2.36	Benzylic
2.34	Benzylic
2.32	Benzylic
2.30	Benzylic
2.09	α to carbonyl
2.05	α to carbonyl
2.04	Alkyl
2.02	Alkyl
1.63	Alkyl
1.61	Alkyl
1.30	Alkyl
1.25	Alkyl

DISCUSSION AND CONCLUSION

To extract the dried Neurocalynx Calcinus plant powder, four solvents were utilized, selected based on their increasing polarity: chloroform, ethyl acetate, methanol, and water. The obtained sample extracts underwent phytochemical screening, unveiling the existence of Alkaloids, saponins, proteins, amino acids, terpenoids, and flavonoids were identified using FTIR and H1 NMR spectroscopy for the characterization of chemical compounds. FTIR analysis of the Neurocalynx Calcinus seed revealed, The existence of various functional groups such as carboxylic acids (-COOH), ketones (C=O), methylene (-CH2-), alkenes (C=C), and alcohols (OH).H1 NMR was used to determine proton counts and their electronic states across different compounds. Moreover, further exploration of A range of functions including antibacterial, antifungal, anti-inflammatory, and antioxidant activities was conducted to discern potential therapeutic applications of the isolated compounds.[19,20]

Ethical Approval: The Institutional Animal Ethics Committee (IAEC) 606/20/c/CPCSEA at the College of Pharmaceutical Sciences, DSU, Bangalore, granted approval for the experiment and its associated procedures.

Conflicts of Interest: The authors assert the absence of any conflicts of interest.

Authors' Contribution: Contributions from the authors are as follows: Mrs. Anita conceived and designed the research idea, as well as carried out practical experiments. She also collected and analyzed the data under guidance. The reformatting, drafting, and preparation of the revised manuscript were performed by her . The finalization of the manuscript and its submission, in accordance with journal requirements, were overseen by Dr. Kalpana Divekar and Dr. Geetha K.M.

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