PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 991-1003
https://doi.org/10.14719/pst.3120

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RESEARCH ARTICLE

# Phytochemistry of *Petroselinum crispum* (Mill.) Fuss, *Murraya koenigii* (L.) Spreng. and *Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm. and *in silico* studies of the role of their bioactive components against cancer

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OPEN ACCESS

ARTICLE HISTORY
Received: 22 November 2023
Accepted: 03 August 2024

Available online
Version 1.0 : 22 October 2024
Version 2.0 : 24 October 2024



**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Daimari E, Dutta U, Tochhawng L. Phytochemistry of *Petroselinum crispum* (Mill.) Fuss, *Murraya koenigii* (L.) Spreng. and *Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm. and *in silico* studies of the role of their bioactive components against cancer. Plant Science Today. 2024; 11(4): 991-1003. https://doi.org/10.14719/pst.3120

## Abstract

Phytochemical profiling using reliable equipment and validated methods helps us know the medicinal value of the plants. Since natural compounds have fewer side effects, they can serve as replacements for synthetic drugs that are being used in the treatment of challenging chronic diseases like cancer. The present study focuses on the bioactive phytochemical profiling of *Petroselinum crispum* (Mill.) Fuss, *Murraya koenigii* (L.) Spreng., and *Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm. and *in silico* studies to check the anticancer potential of their bioactive components. In thin-layer chromatography (TLC) plate analysis, it was found that the methanolic extracts of plants contained the maximum number of components. Gas chromatography–mass spectrometry (GC-MS) analysis of the methanolic extracts of plants showed the presence of 22 bioactive components. High-performance thin-layer chromatography (HPTLC) analysis of the extracts of these plants showed the important chromatographic peak, apigenin. *In silico* studies showed the binding efficacy of selected bioactive components observed in the analysis of plant extracts. Amongst them, apigenin was found to be most effective at binding to the receptors of targeted cancer cells, viz., hepatocellular carcinoma, lung, and breast cancer. After the analysis of the study, it was arrived at the conclusion that the plants, viz., *P. crispum*, *M. koenigii*, and *C. tamala*, possess various bioactive components, and some of these components have anticancer potential. Therefore, *in vivo* and *in vitro* studies should be essentially conducted for the development of cancer-preventive drugs.

## Keywords

phytochemical profiling; treatment; bioactive; anticancer; analysis; apigenin

## Introduction

Many plants which have been used for culinary purposes for centuries have therapeutic value. In stressful environmental conditions, plants undergo physiological adaptation and alter their biochemical profile, which leads to the production of a variety of secondary metabolites (1). The unique pharmacophores and biological activities of these metabolites have attracted the attention of medicinal researchers. Secondary metabolites such as polyphenols, alkaloids, and terpenes are known to be useful in the maintenance of health and chronic disease prevention. Flavonoids, a subclass of polyphenols, play important roles in the maintenance of plant health as well as

human health. Flavonoids have the ability to modulate our body's response to carcinogens, allergens, and microbes (2). An important example of flavonoids is apigenin.

Apigenin is chemically known as 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (C15H10O5). Since apigenin is a naturally occurring compound, its toxicity level is quite low (3). Apigenin can potentially modulate many important molecular signalling pathways, namely Phosphoinositide 3-kinase/Serine/threonine-specific protein kinase/Mammalian target of rapamycin (PI3K/AKT/mTOR), Mitogen-activated protein kinase/Extracellular signal-regulated kinase (MAPK/ERK), Wingless-related integration site/Beta-catenin (Wnt/β-catenin), and Janus kinases/Signal transducer and activator of transcription (JAK/STAT), which are involved in cancer cell proliferation, invasion, metastasis, cell cycle arrest, autophagy, and apoptosis, so it can effectively prevent various types of cancer, including liver, lung, colon, prostate, breast, thyroid, and skin cancer (3, 4).

All three selected plants, namely parsley [*Petroselinum crispum* (Mill.) Fuss], curry leaf [*Murraya koenigii* (L.) Spreng.], and Indian bay leaf [*Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm.], are known to show biological activities (5). *P. crispum* is an aromatic plant that belongs to the family Apiaceae. It is a biennial, hardy plant. It possesses branched stems and dark green curled or flat leaves arranged alternately on the stem (6). *M. koenigii* belongs to the family Rutaceae. It is a deciduous small tree or shrub reaching up to 6 m in height. The leaves are bipinnately compound, each having 11–15 leaflets alternately arranged on rachis (7). *C. tamala* belongs to the family Lauraceae. It is an evergreen perennial shrub. The leaves are simple, ovate with pinnate venation, and alternately arranged (8).

In the last few decades, there has been a revival of interest in the use of natural compounds as potential drug candidates to treat chronic diseases. Phytochemical profiling and *in silico* studies of the plants that have medicinal value can help us know how effectively these plants can be used in the treatment of diseases. Computational biology has opened a wonderful avenue for a better understanding of the interactions between target receptors and ligands. *In silico* studies not only save money and time but also help in the better design of drugs with low toxicity while retaining all the required functionalities (9). The use of instruments such as gas chromatography–mass spectrometry (GC-MS), high-performance thin-layer chromatography (HPTLC), etc. in the analysis of plant extracts has many advantages, such as sensitivity in detection, simplicity, and high resolution of complex mixtures (10, 11). In the present study, GC-MS analysis was performed to determine the phytoconstituents of the methanolic extracts of the three plants. Additionally, HPTLC was used to evaluate the presence of an important anticancer flavonoid compound, apigenin, in the fractioned extracts of *P. crispum*, *M. koenigii*, and *C. tamala* since culinary herbs are known to contain flavonoids. Further, a computational study, i.e., *in silico* molecular docking, was performed to elucidate the molecular mechanism of the anti-cancer activity of some of the detected compounds.

The rates of global cancer incidence and mortality are consistently high; therefore, research into discovering novel therapeutic drugs is always of continuous interest. Although lots of therapeutic techniques have been developed and hundreds of anticancer drugs have been discovered, the problem still remains unsolved. Many drugs are found to be quite efficient in killing tumour cells, but these drugs simultaneously affect the normal cells of the patients. This results in undesired side effects, such as patients becoming weak, depressed, and immunologically compromised (12). Resistance developed by cancer cells against chemotherapeutic agents also leads to problems such as cancer relapse, increased DNA repair capacity, etc. (13). In addition to all these, the late-stage presentation and late diagnosis of the disease hamper treatment. Hence, looking for measures to prevent cancer and finding effective therapeutic drugs that will have fewer side effects are important. Keeping all these facts in mind, the present study focussed on the bioactive phytochemical profiling of *P. crispum*, *M. koenigii*, and *C. tamala*, which are commonly used in cooking, and *in silico* studies to check the anticancer potential of their bioactive components.

## Materials and Methods

### *Plant materials*

*M. koenigii* and *C. tamala* were collected from the natural vegetation of Assam by the local people. *P. crispum* was obtained from the local vendors in Aizawl, Mizoram. These plant materials were identified by Dr. Souravjyoti Borah, Curator, Herbarium of the Botany Department of Guwahati University (GUBH), Guwahati. A sample of *P. crispum* (Reference No. *Herb./GUBH/2023/027*), *M. koenigii* (Reference No. *Herb./GUBH/2022/031*), and *C. tamala* (Reference No. *Herb./GUBH/2023/017*) were submitted to GUBH for future reference.

### *Thin-layer chromatography (TLC)*

#### Preparation of extracts

The leaves collected from *P. crispum*, *M. koenigii*, and *C. tamala* were washed and initially dried under a shaded drying condition. The dried leaves were ground, and extracts were prepared successively by maceration using a series of solvents with increasing polarity, namely petroleum ether, chloroform, methanol, and water. In each case, filtration was done using Whatman filter paper no. 1, and the filtrates were allowed to concentrate (14). Hence, a total of 12 extracts were obtained and stored for further studies.

#### Equipment and conditions for TLC

The equipment used was TLC plates pre-coated with silica gel 60 F254, beakers that could fit the TLC plates, petri dishes for covering the mouths of the beakers, and capillary tubes. A mobile phase suitable for the study was used (15). The chromatographic conditions maintained are shown in Table 1.

**Table 1.** Chromatographic conditions maintained for TLC.

| Sl. No. | Phase | Requirements |
|---|---|---|
| 1. | Stationary phase | TLC plates pre-coated with silica gel 60 F254 |
| 2. | Mobile phase | Toluene:ethyl acetate:formic acid (6:4:0.3, v/v/v) |

### TLC procedure

Separate TLC plates were used for *P. crispum* extracts (petroleum ether, chloroform, methanol, and water), *M. koenigii* extracts (petroleum ether, chloroform, methanol, and water), and *C. tamala* extracts (petroleum ether, chloroform, methanol, and water) to check the splitting of bands following the Harborne method (16). On each plate, a straight line was drawn with the help of a pencil (about 2 cm from the bottom of the plate). Four equidistant points were marked with a pencil on the line for loading the extracts. These points were annotated 1, 2, 3, and 4. A small drop of petroleum ether, chloroform, methanol, and aqueous extracts were loaded at points 1, 2, 3, and 4, respectively, using capillary tubes. The solvent (mobile phase) was kept in the beakers at room temperature. The TLC plate prepared for each plant was placed inside the beaker in an apparently straight position. When the solvent front traversed almost 80% of the plate, the plate was taken out. The bands formed on the plates were then made prominent by keeping the plates in a beaker saturated with iodine vapour.

## *GC-MS*

### Preparation of extracts

Leaves of *P. crispum*, *M. koenigii*, and *C. tamala* were collected, washed, and dried under a shaded drying condition. The dried leaves were ground, and extracts were prepared for each plant by maceration (17). Methanol was used as the solvent since the TLC results showed that bands were formed in the methanol extract of each plant. For each plant extract, filtration was done using Whatman filter paper no. 1. The extraction was repeated twice for each plant. The filtrates collected were allowed to concentrate.

### Equipment and conditions for GC-MS

The analysis of the phytochemicals of methanol extracts was done using GC-MS equipment (Model: Perkin Elmer Clarus 680 GC/600C MS). The GC-MS machine used was capable of autocalibration. The machine was checked regularly to maintain the sensitivity parameter. Blank solvents were run periodically to know if residual impurities from previous analyses were left behind. Elite-5 MS capillary column (Perkin Elmer, USA) of size 60 m × 250 µm was used. Status of the column was checked periodically to avoid column bleeding. Helium was used as a carrier gas in this experiment. Other instrumental conditions include: injection port temperature = 280°C, split = 10:1, solvent delay = 8 min, transfer temperature = 180°C, and source temperature = 150°C.

### GC-MS procedure

One µL of filtrate was injected. The initial oven temperature was 60°C for 1 min, then increased to 200°C at a rate of 7°C/min, held for 3 min, increased to 300°C at a rate of 10°C/min, and held for 5 min. Scanning was done for the range 50–600 Da. The peaks generated were identified using the National Institute of Standards and Technology (NIST) library.

## *HPTLC*

### Equipment and conditions for HPTLC

The equipment used were TLC plates pre-coated with silica gel 60 F254 (10 × 10 cm); Linomat V sample applicator (Chemieerzeugnisse & Adsorptionstechnik AG (CAMAG), Switzerland); 100 µL syringe (Hamilton, CAMAG, Switzerland); hair dryer; glass twin trough developing chamber (20 × 10 cm, CAMAG, Switzerland); ultraviolet (UV) chamber (CAMAG, Switzerland); and TLC scanner 3 (scanning speed: 20 mm/sec and slit dimension: 5 × 0.45 mm, CAMAG, Switzerland) where winCATS software was installed.

Reference standard apigenin was obtained from Sigma Aldrich Ltd. Standard solutions of apigenin at two different levels of concentration, i.e., 50 µg/mL and 100 µg/mL, were prepared. A mobile phase suitable for the study was used (15). The chromatographic conditions maintained are shown in Table 2.

**Table 2.** Chromatographic conditions maintained for HPTLC.

| Sl. No. | Phase/ Parameters | Conditions |
|---|---|---|
| 1. | Stationary phase | TLC plates pre-coated with silica gel 60 F254 |
| 2. | Mobile phase | Toluene : ethyl acetate : formic acid (6 : 4 : 0.3, v/v/v) |
| 3. | Saturation time | 45 min |
| 4. | Wavelength | 254 nm |
| 5. | Lamp | Deuterium and halogen |

### HPTLC procedure

Three separate TLC plates were used to detect the presence of apigenin in the *P. crispum* extracts (petroleum ether, chloroform, methanol, and water), *M. koenigii* extracts (petroleum ether, chloroform, methanol, and water), and *C. tamala* extracts (petroleum ether, chloroform, methanol, and water). Each plate contained 6 tracks produced by spotting bands (8 mm in length). A Linomat V sample applicator (equipped with a 100 µL syringe) was used to apply 2 µL of solutions to create those bands. The distance of the first track from the left edge of the plate was 15 mm; the distance between the tracks was 14 mm; and the distance of the tracks from the bottom was 5 mm. On the plate of each plant, the first two tracks were for 50 µg/mL and 100 µg/mL apigenin standard solution, and the remaining tracks were for the extracts prepared using the four types of solvents (Fig. 1A–1C).

The plates were put in a twin trough chamber presaturated with the mobile phase. The mobile phase was allowed to migrate to a distance of about 7.5 cm. The plates were then dried using a hair dryer and visualized in a UV chamber. Scanning of the plates was done using TLC scanner 3. winCATS software installed in TLC scanner

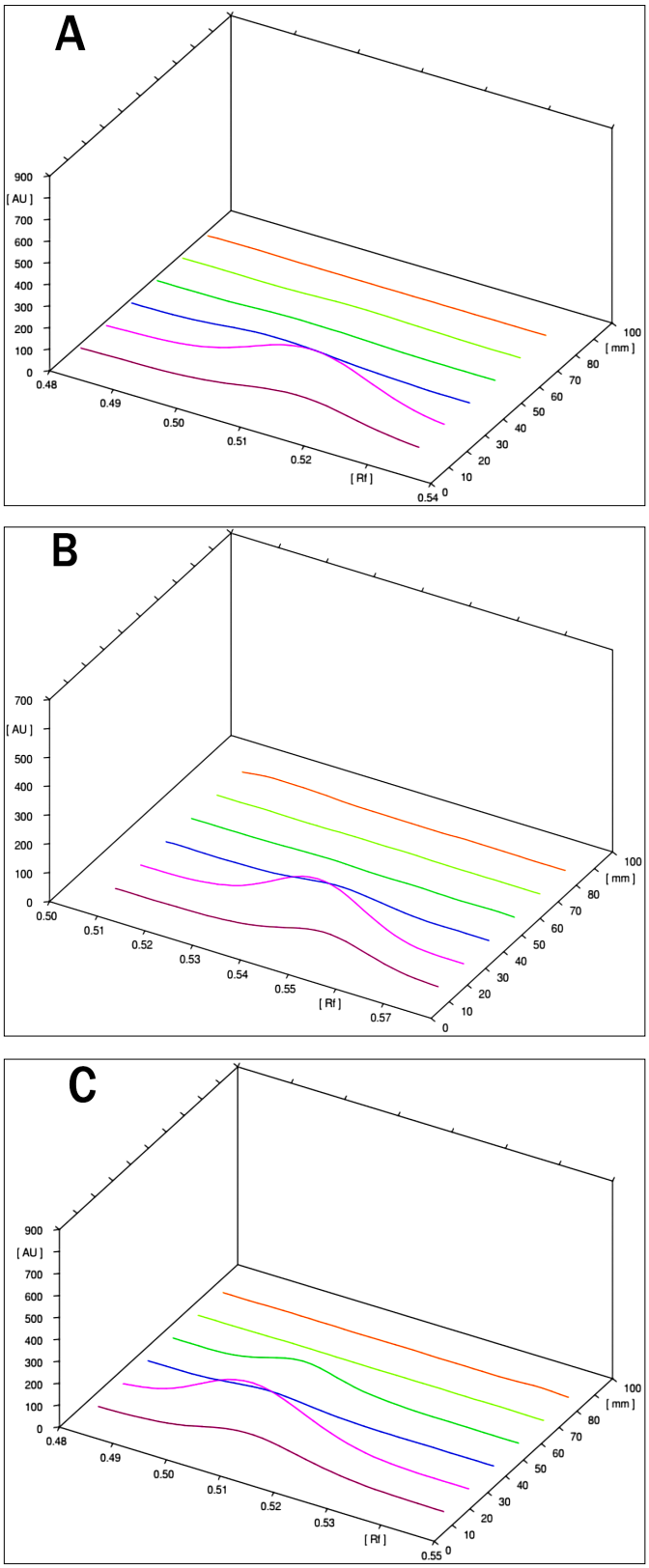



**Fig. 1**. Chromatogram showing the tracks produced on TLC plates. (**A**) TLC plate used for *P. crispum* extracts, (**B**) TLC plate used for *M. koenigii* extracts, (**C**) TLC plate used for *C. tamala* extracts.

3 was used for densitometry measurements, spectra recording, and data processing.

For each track of standard apigenin solution, a point was plotted on a graph sheet using the values of the concentration of standard apigenin and peak area (Table 3). Using these points, a line graph and calibration curve of apigenin were obtained (Fig. 2).

**Table 3**. The values of the concentration of standard apigenin and peak area for the tracks of standard apigenin solution.

| Sl. No. | Track | Concentration of standard apigenin (µg/mL) | Peak area |
|---|---|---|---|
| 1. | Track 1 of plate used for *P. crispum* | 50 | 1035.5 |
| 2. | Track 1 of plate used for *M. koenigii* | 50 | 950.9 |
| 3. | Track 1 of plate used for *C. tamala* | 50 | 1246.1 |
| 4. | Track 2 of plate used for *P. crispum* | 100 | 2376.3 |
| 5. | Track 2 of plate used for *M. koenigii* | 100 | 2024.3 |
| 6. | Track 2 of plate used for *C. tamala* | 100 | 2690.6 |

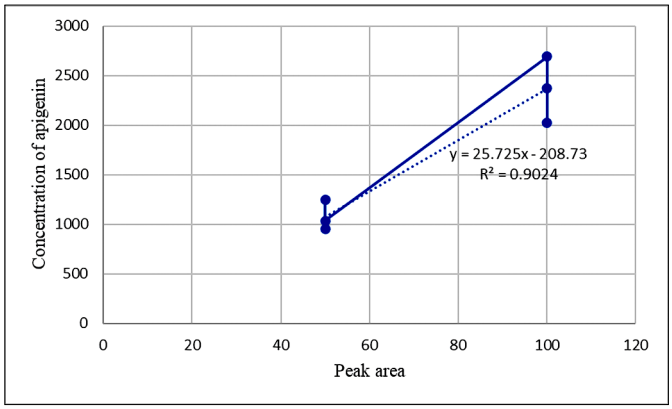



**Fig. 2**. Line graph and calibration curve of apigenin.

## *In silico studies of anticancer components*

### Receptors and ligands

The receptors for *in silico* studies were selected on the basis of their active role in the development of common types of cancer, such as hepatocellular carcinoma, breast cancer, colorectal cancer, etc. For the present study, nuclear factor kappa B (NF-κB) and platelet-derived growth factor (PDGF) were chosen as receptors. These receptors were searched in the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org/), and structures in .pdb format were obtained.

Based on the GC-MS and HPTLC analysis results, the list of compounds present in plant extracts were known. Through the study of available literature, the biological activities of these compounds were known. The compounds that show anticancer or antitumour activities were selected. In the present study, the selected ligands were apigenin, phytol, citronellol, alpha-pinene, 3-carene, hydroquinone, alpha-terpineol, and limonene. These ligands were searched in the Zinc15 database (https://zinc15.docking.org/), and structures in .mol2 format were obtained.

### Docking

Docking was done using Molegro Virtual Docker (MVD 2013.6.0.0) software for Windows. Using the different ligand binding conformations and estimating the energy of such bindings, the software predicted the possible receptor-ligand interactions. The receptor-ligand interactions were visualized using BIOVIA Discovery Studio (v21.1.0.20298), a free software developed by Dassault

Systèmes. Hence, the different types of interactions of ligands with the amino acid residues of the receptors were revealed. The interactions were observed in 2D as well as 3D forms.

## Results

### *TLC analysis*

The bands were observed in most of the cases (Fig. 3A–3C). This signifies that the solvent prepared by mixing toluene, ethyl acetate, and formic acid (6 : 4 : 0.3, v/v/v) is suitable to be used as the mobile phase for analysing these extracts. Hence, this mobile phase was considered for HPTLC analysis. As the methanol extract of each plant showed bands, methanol extracts were considered for GC-MS analysis.

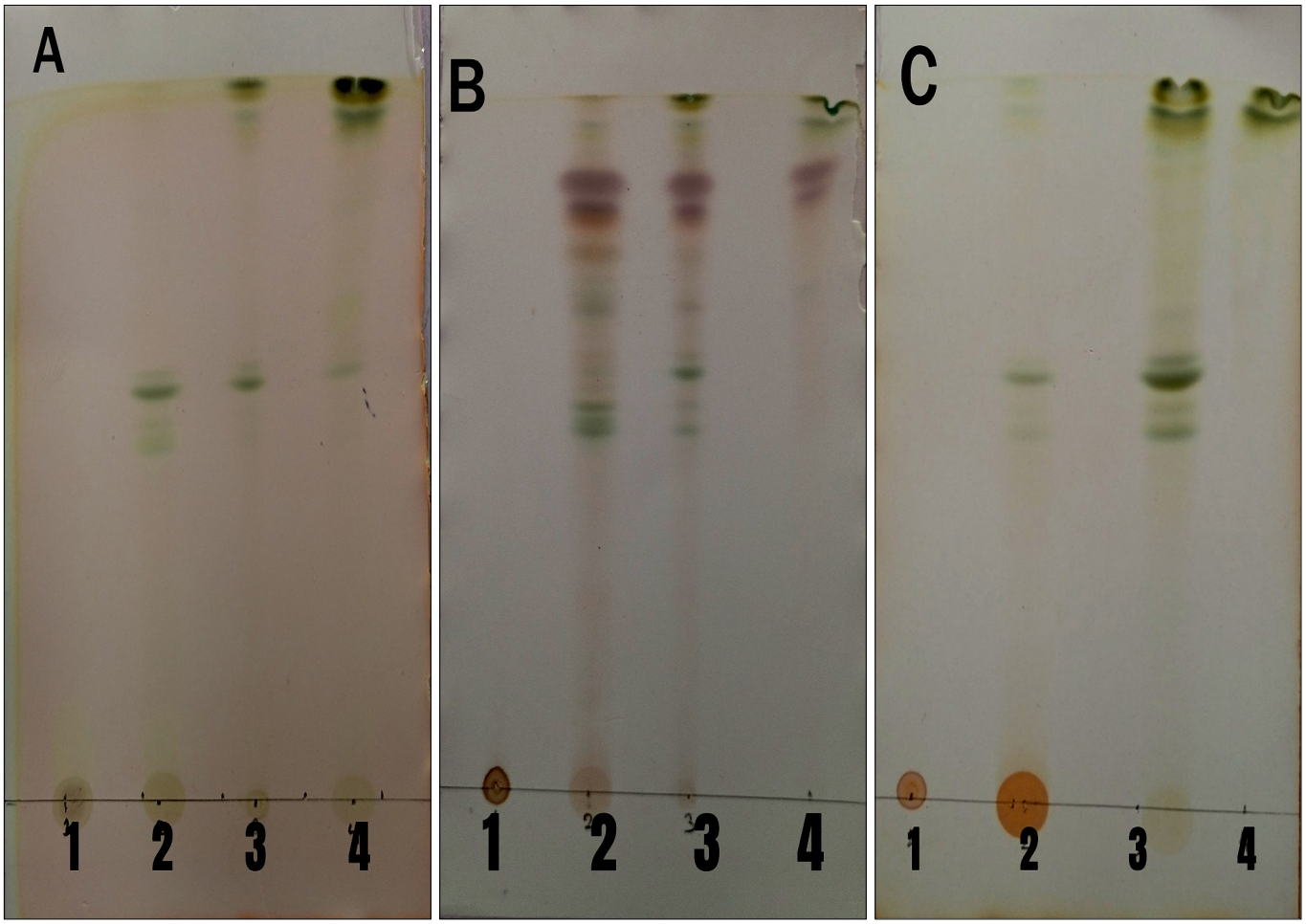



**Fig. 3**. TLC showing the formation of bands. (**A**) TLC plate used for *P. crispum* extract, (**B**) TLC plate used for *M. koenigii* extract, (**C**) TLC plate used for *C. tamala* extract.

### *GC-MS analysis*

Many peaks of different heights were obtained by performing GC-MS analysis of plant extracts (Fig. 4–6). The peaks indicated the presence of various compounds in the extracts. Studying the available literature, the detected compounds that show biological activities were known and listed (Table 4). The chemical structures of these compounds are shown in Fig. 7.

### *HPTLC analysis*

After running the chromatogram for HPTLC on the three TLC plates, the retardation factor ($R_f$) value for the apigenin standard was found to be 0.52 ± 0.01 (mean ± standard error). The standard apigenin showed a single peak in all the cases (Fig. 8A, 8B, 9A, 9B, 10A and 10B).

### Detection of apigenin in the plant extracts

HPTLC analysis showed that the chromatogram for petroleum ether, chloroform, and methanol extracts of *P. crispum* had a peak at the same $R_f$ value as the apigenin standard (Fig. 8C–8E), hence apigenin was present in these extracts. Similarly, the peak in the chromatogram for the methanol extract of *M. koenigii* and the methanol and chloroform extracts of *C. tamala* at the same $R_f$ value as the apigenin standard indicated the presence of apigenin in those extracts (Fig. 9C, 10C and 10D). The area of these peaks is shown in Table 5.

### Quantification of apigenin present in the plant extracts

Using the calibration curve of apigenin obtained by plotting the concentration of standard apigenin versus peak area, the concentration of the apigenin present in the different extracts of *P. crispum*, *M. koenigii*, and *C. tamala* was determined (Table 6).

### *In silico* studies

The mean of the re-rank scores generated by docking was calculated for each receptor-ligand binding. For generating these re-rank scores, different energy parameters, viz.,

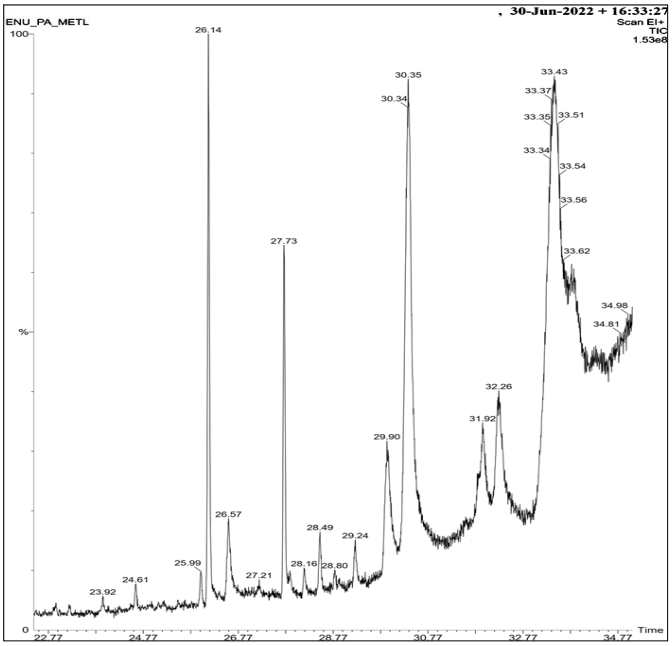



**Fig. 4**. GC-MS chromatogram of methanol extract of *P. crispum*.

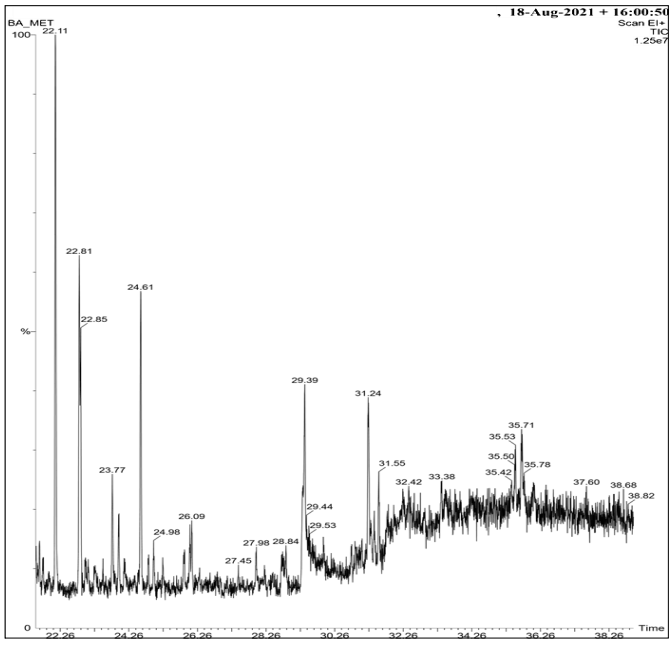



**Fig. 6**. GC-MS chromatogram of methanol extract of *C. tamala*.

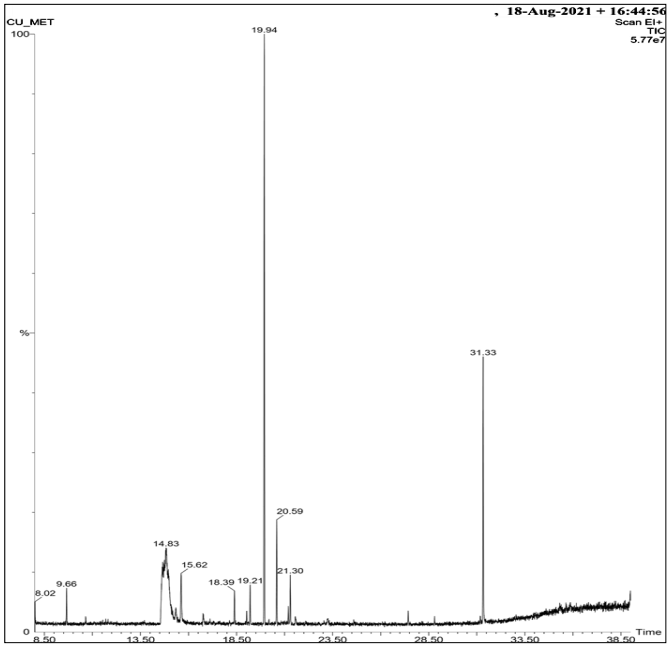



**Fig. 5**. GC-MS chromatogram of methanol extract of *M. koenigii*.

E-inter (protein-ligand), E-inter total, Van der Waal's, steric, H-bond energy, etc., were used by the software. The mean re-rank scores were then used to prepare graphs (Fig. 11 and 12). These re-rank scores, which are negative values, signify the binding potential of different ligands when interacting with the active sites of receptors. In this study, prostaglandin J2 and sunitinib were taken as the reference ligands for the cases of NF-κB and PDGF, respectively (40, 41). Among the selected bioactive compounds, apigenin had the lowest re-rank scores (-75.40 when interacting with NF-κB and -65.69 when interacting with PDGF). Hence, the binding potential of apigenin was highest for the interaction with NF-κB as well as PDGF. While analysing the interactions of apigenin with the receptors, different types of interactions, viz. Van der Waal's, conventional hydrogen bond, carbon-hydrogen bond, Pi-Pi T-shaped, Pi -alkyl, and alkyl, were found (Fig. 13 and 14).

**Table 4**. Bioactive compounds detected in the methanol extracts of *P. crispum*, *M. koenigii*, and *C. tamala*.

| Sl. | Name | Chemical | Biological activities | RT | Area% | Plant |
|---|---|---|---|---|---|---|
| 1. | Oleic acid | $C_{18}H_{34}O_2$ | Antibacterial (18) | 26.567 | 1.326 | *P. crispum* |
| 2. | Undecanoic acid | $C_{11}H_{22}O_2$ | Antifungal (19), antibacterial (19), antiviral (19) | 26.567 | 1.326 | *P. crispum* |
| 3. | Hentriacontane | $C_{31}H_{64}$ | Anti-inflammatory (20), antitumour (20), antimicrobial (20) | 15.197 | 0.125 | *P. crispum* |
| 4. | Neophytadiene | $C_{20}H_{38}$ | Antipyretic (21), analgesic (21), anti-inflammatory (21) | 27.732 | 2.864 | *P. crispum* |
| 5. | Phytol | $C_{20}H_{40}O$ | Antitumour (22), anti-inflammatory (22), immune-modulating (22) | 27.732 | 2.864 | *P. crispum* |
| 6. | Citronellol | $C_{10}H_{20}O$ | Anti-inflammatory (23), antimicrobial (23), anticancer (23) | 27.732 | 2.864 | *P. crispum* |
| 7. | Gamolenic acid | $C_{18}H_{30}O_2$ | Anti-inflammatory (24), antimicrobial (24) | 29.903 | 2.660 | *P. crispum* |
| 8. | N-hexadecanoic acid | $C_{16}H_{32}O_2$ | Antibacterial (25), antioxidant (25) | 30.353 | 0.129 | *P. crispum* |
| 9. | Dodecanoic acid | $C_{12}H_{24}O_2$ | Antibacterial (26) | 30.353 | 0.129 | *P. crispum* |
| 10. | Tetradecanoic acid | $C_{14}H_{28}O_2$ | Antifungal (27), antioxidant (27) | 30.353 | 0.129 | *P. crispum* |
| 11. | Tridecanoic acid | $C_{13}H_{26}O_2$ | Anthelminthic (28), anti-inflammatory (28), antimicrobial (28) | 30.353 | 0.129 | *P. crispum* |
| 12. | Alpha-pinene | $C_{10}H_{16}$ | Antitumour (29) | 9.659 | 0.787 | *M. koenigii* |
| 13. | 3-Carene | $C_{10}H_{16}$ | Anticancer (30) | 9.659 | 0.787 | *M. koenigii* |

| | | | | | | |
|---|---|---|---|---|---|---|
| 14. | Sabinene | $C_{10}H_{16}$ | Antifungal (31), anti-inflammatory (31) | 10.649 | 0.205 | *M. koenigii* |
| 15. | Beta-myrcene | $C_{10}H_{16}$ | Antidiabetic (32), antiinflammatory (32), antibacterial (32) | 10.649 | 0.205 | *M. koenigii* |
| 16. | Beta-pinene | $C_{10}H_{16}$ | Antimicrobial (33), antidepressant (33), cytotoxic (33) | 10.649 | 0.205 | *M. koenigii* |
| 17. | Piperidine | $C_5H_{11}N$ | Anti-allergic (34), anti-inflammatory (34), analgesic (34) | 14.661 | 5.373 | *M. koenigii* |
| 18. | Hydroquinone | $C_6H_6O_2$ | Antioxidant (35), immunomodulatory (35), anticancer (35) | 14.996 | 0.078 | *C. tamala* |
| 19. | Alpha-terpineol | $C_{10}H_{18}O$ | Antioxidant (36), anticancer (36), analgesic (36) | 15.256 | 0.078 | *C. tamala* |
| 20. | Limonene | $C_{10}H_{16}$ | Antioxidant (37), antidiabetic (37), anticancer (37) | 15.256 | 0.078 | *C. tamala* |
| 21. | Myrcenol | $C_{10}H_{18}O$ | Antimicrobial (38) | 15.256 | 0.078 | *C. tamala* |
| 22. | Eugenol | $C_{10}H_{12}O_2$ | Anti-inflammatory (39), antioxidant (39) | 18.458 | 87.399 | *C. tamala* |

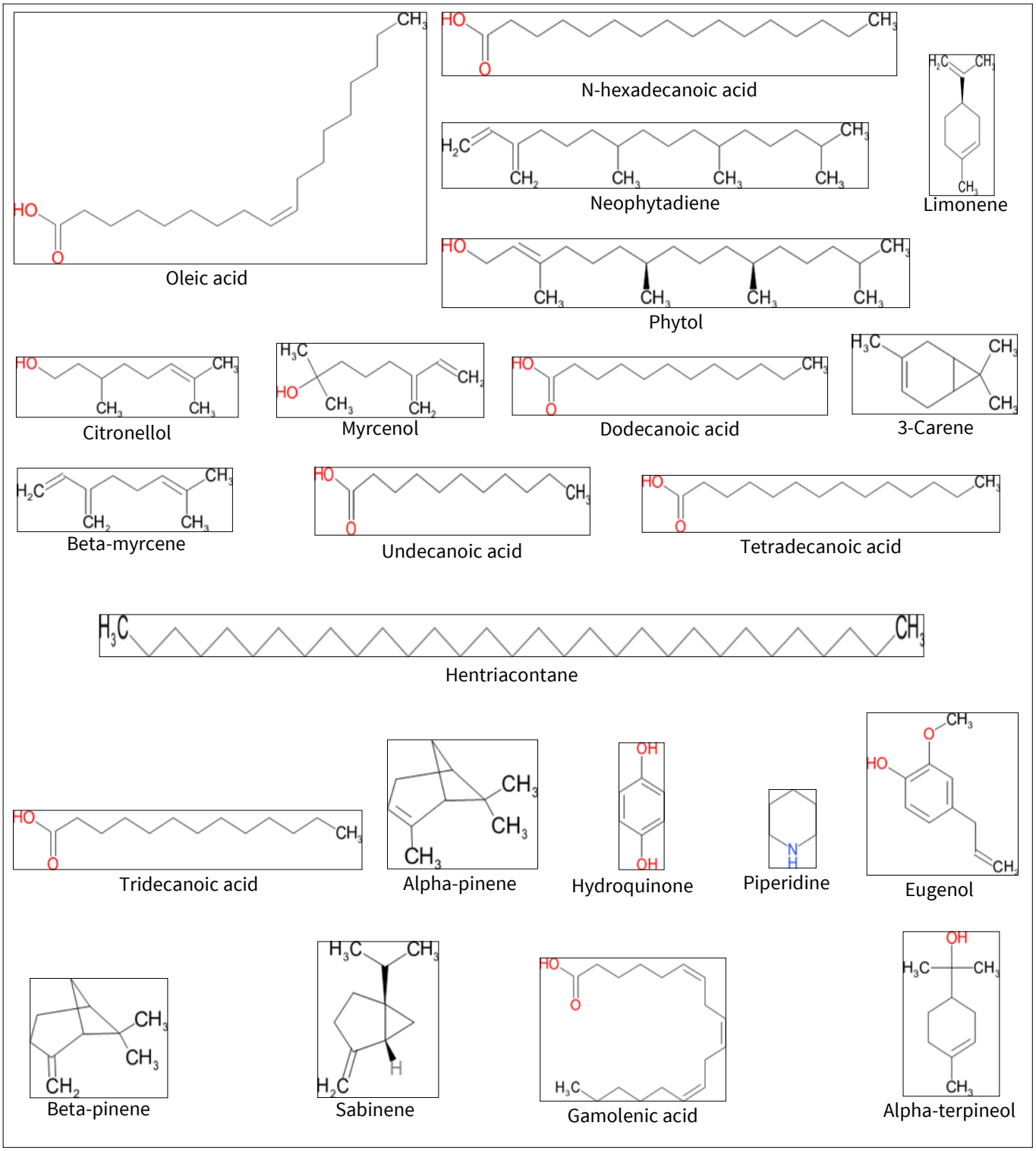



**Fig. 7.** The chemical structures of the detected compounds which show biological activities.

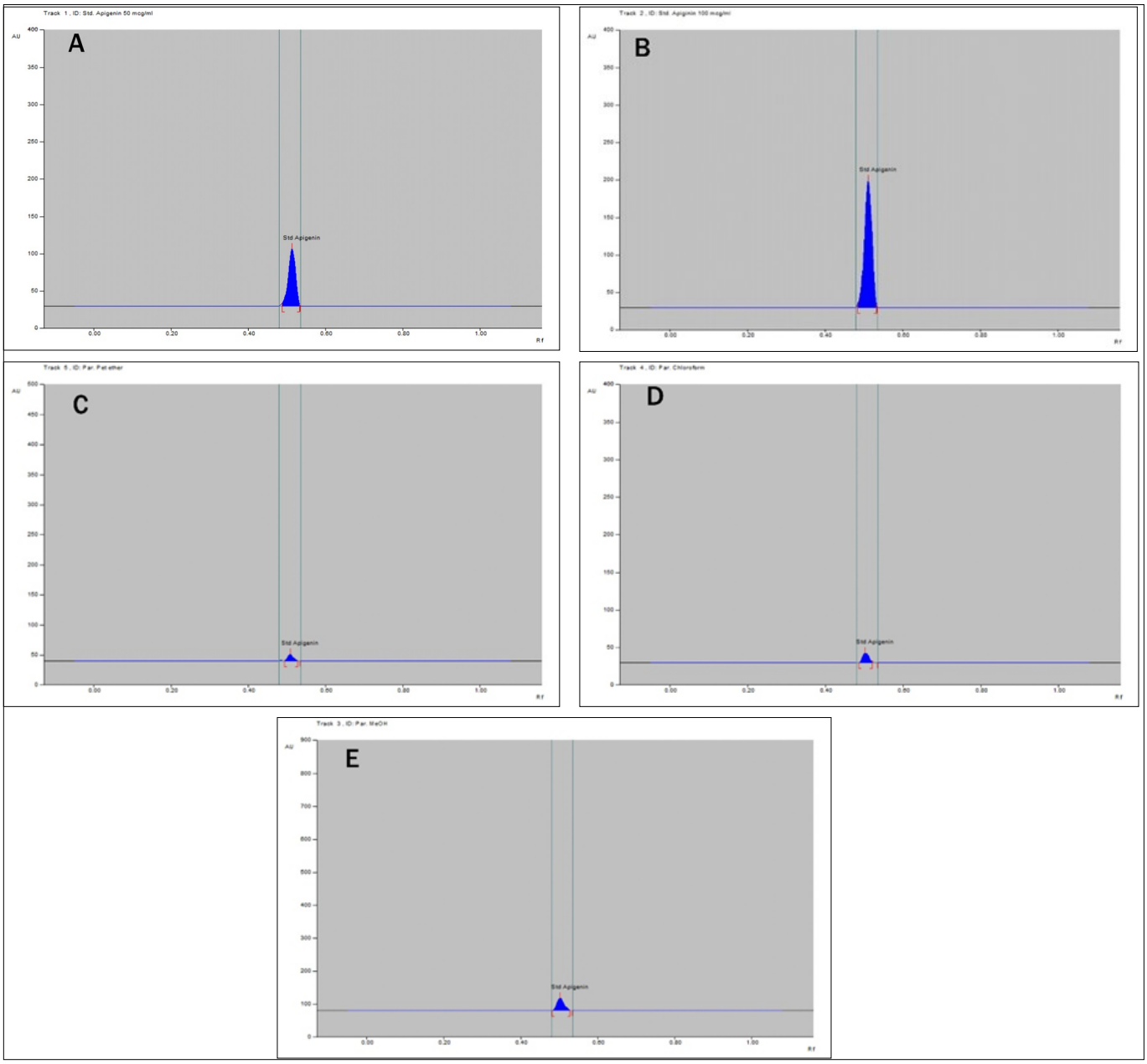



**Fig. 8.** Detection of peak in the chromatogram of the TLC plate used for *P. crispum* extracts. (**A**) 50 µg/mL – apigenin standard solution, (**B**) 100 µg/mL – apigenin standard solution, (**C**) Petroleum ether extract, (**D**) Chloroform extract, (**E**) Methanol extract.

## Discussion

Medicinal plants are rich sources of phytochemical compounds, which can be crucial in treating various chronic illnesses. In recent times, a large number of potent biomolecules have been extracted from a wide range of medicinal plants (42). It is thought that these potent chemical components from natural sources can be used to treat a broad range of illnesses with fewer side effects. Natural compounds can interfere with the molecular mechanisms of diseases and prevent them from occurring.

A few chronic illnesses are very critical, and there are no particular medications for them. Hence, looking for novel therapeutic compounds from natural sources by applying modern and reliable techniques has become important. In this regard, an important instrument is GC-MS, which can provide a highly selective analysis, enhancing chromatographic results and allowing for the characterization of compounds that have similar mass spectral data. In the current study, GC-MS analysis of plant extracts showed the presence of different types of components. Studying the available literature on phytochemicals, it was known that some of these components have important bioactive properties which help in the treatment and prevention of diseases. In a similar study using GC-MS, plants used for culinary purposes were shown to have the same bioactive properties, which help in the prevention of diseases (43). Another important instrument is HPTLC, well known for its automation, scanning, and full optimization for providing chromatographic information on complex mixtures of substances. In the present study, HPTLC analysis of different extracts of *P. crispum*, *M. koenigii*, and *C. tamala* showed peaks in the chromatogram, which indicated the presence of apigenin in those extracts. Among those peaks, the peak in the chromatogram of the chloroform extract of *C. tamala* was the highest, and it had the maximum area. Using the calibration curve of apigenin

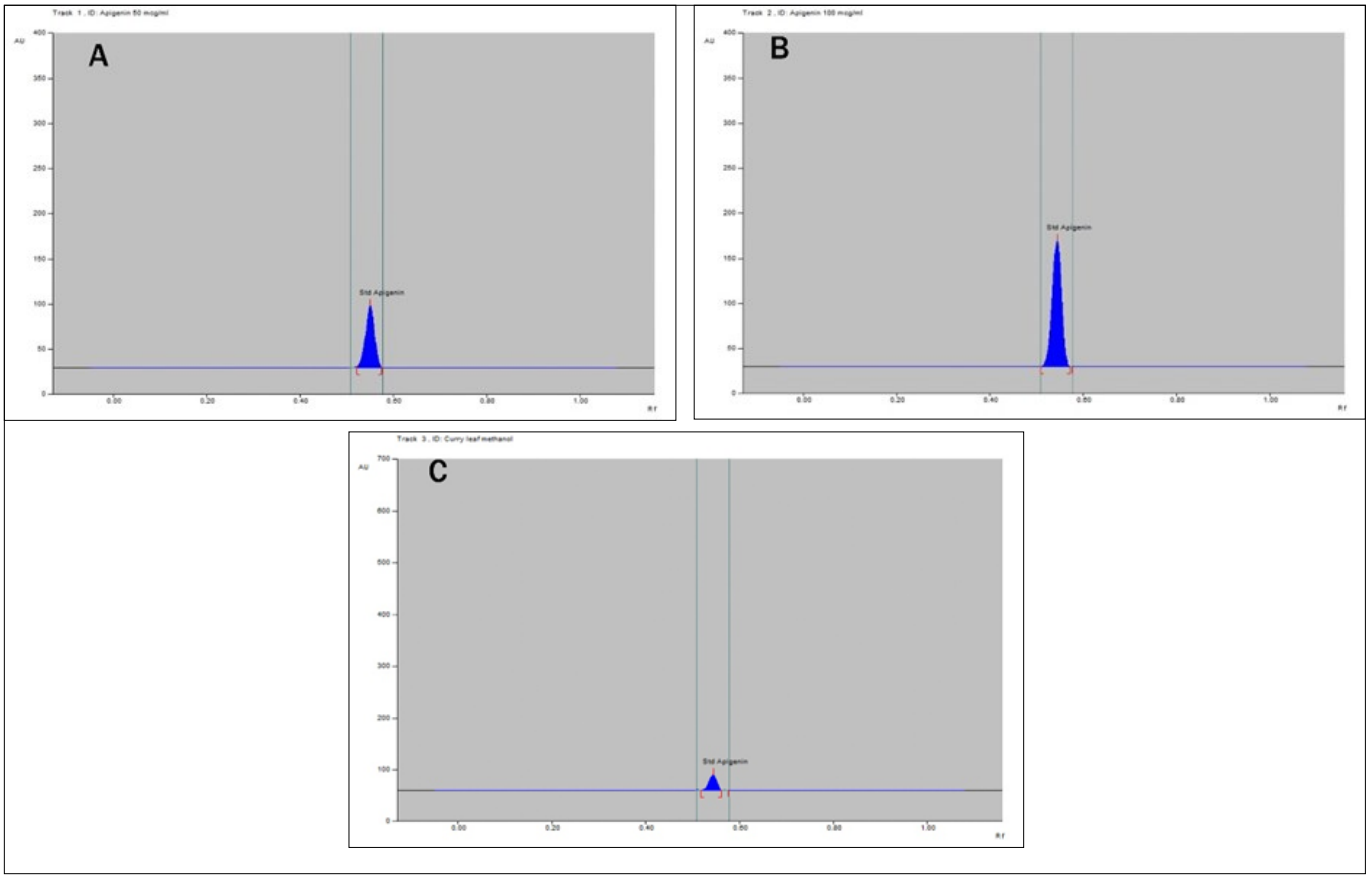



**Fig. 9.** Detection of peak in the chromatogram of the TLC plate used for *M. koenigii* extracts. (**A**) 50 µg/mL – apigenin standard solution, (**B**) 100 µg/mL – apigenin standard solution, (**C**) Methanol extract.

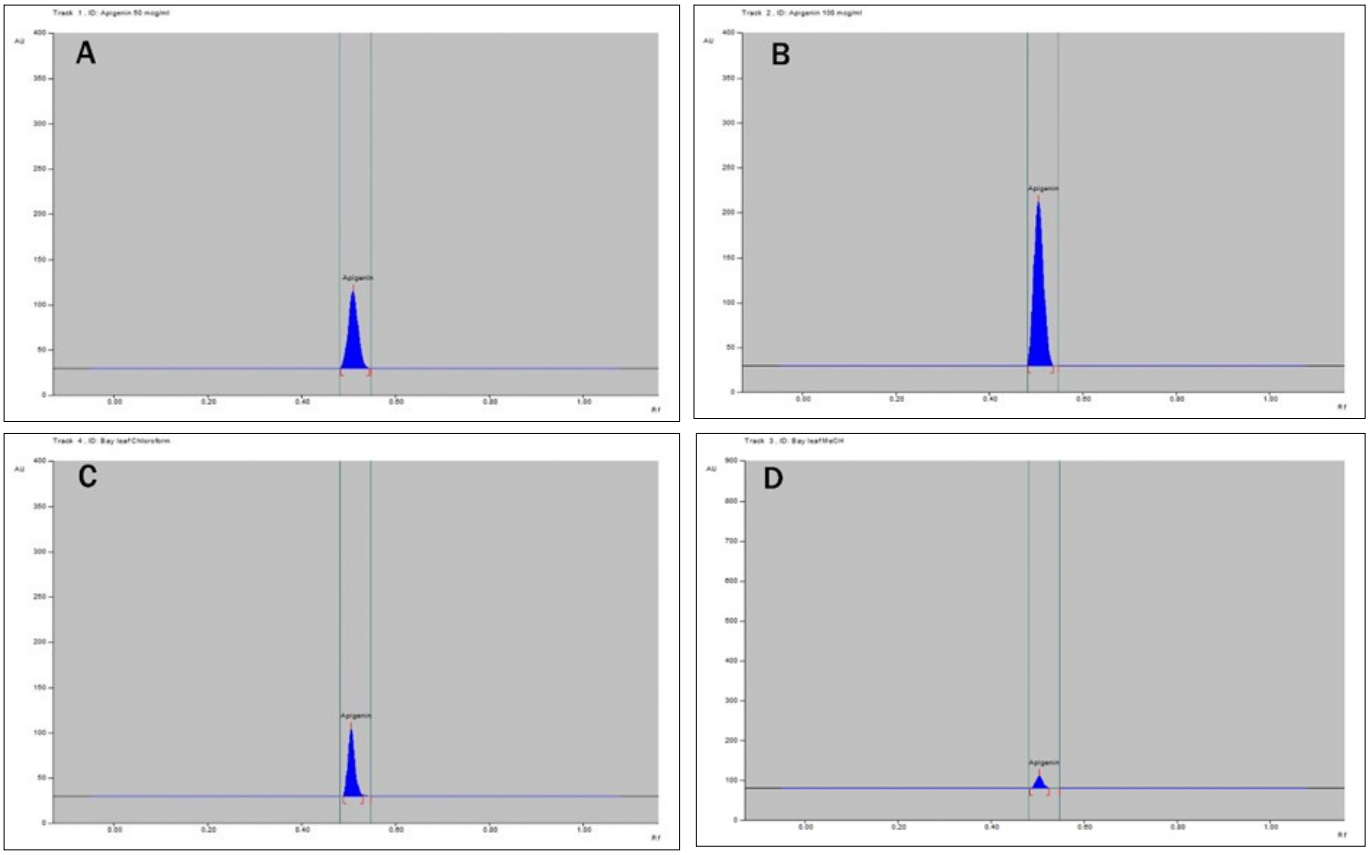



**Fig. 10.** Detection of peak in the chromatogram of the TLC plate used for *C. tamala* extracts. (**A**) 50 µg/mL– apigenin standard solution, (**B**) 100 µg/mL – apigenin standard solution, (**C**) Chloroform extract, (**D**) Methanol extract.

prepared by plotting standard apigenin concentration versus area of peak, the concentration of apigenin in this extract was calculated to be 38.07 µg/mL. Unlike other extracts, the methanol extract showed the presence of apigenin in each of these plants.

**Table 5**. Details of the peaks detected in the chromatogram at same $R_f$ value as apigenin standard.

| Sl. No. | Plant | Extract | Maximum height | Peak area |
|---|---|---|---|---|
| 1 | *P. crispum* | Petroleum ether extract | 11.3 | 119.8 |
| | | Chloroform extract | 13.1 | 156.0 |
| | | Methanol extract | 37.7 | 502.0 |
| 2 | *M. koenigii* | Methanol extract | 29.6 | 354.1 |
| 3 | *C. tamala* | Chloroform extract | 75.0 | 770.7 |
| | | Methanol extract | 31.1 | 325.1 |

**Table 6**. Determination of apigenin concentration using concentration versus peak area curve.

| Sl. No. | Plant | Extract | Concentration of apigenin (µg/mL) |
|---|---|---|---|
| 1 | *P. crispum* | Petroleum ether extract | 12.77 |
| | | Chloroform extract | 14.18 |
| | | Methanol extract | 27.63 |
| 2 | *M. koenigii* | Methanol extract | 21.88 |
| 3 | *C. tamala* | Chloroform extract | 38.07 |
| | | Methanol extract | 20.75 |

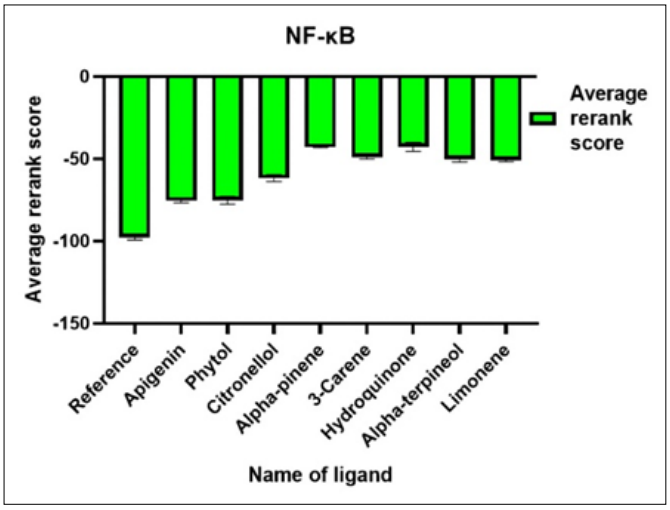



**Fig. 11.** Graphical representation of re-rank scores of ligands which signify

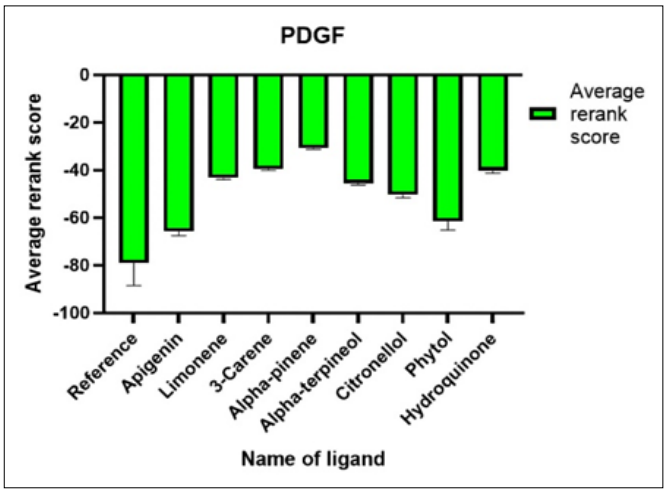
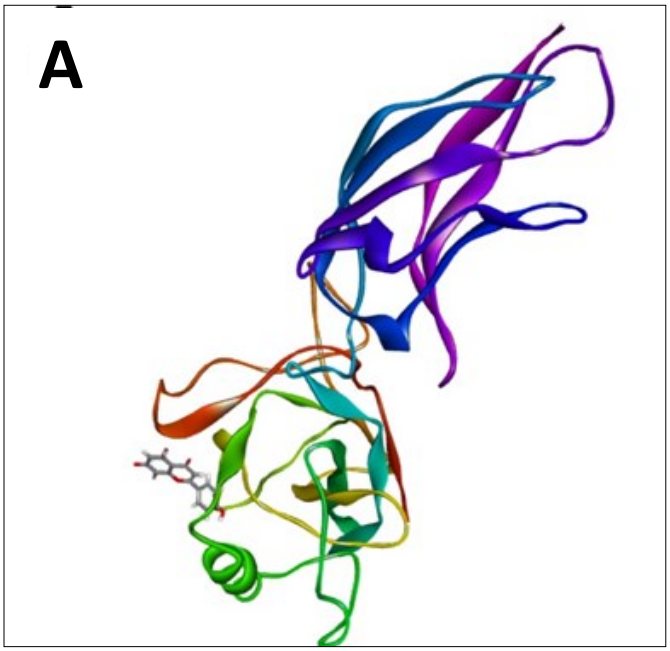



**Fig. 12**. Graphical representation of re-rank scores of ligands which signify the binding potential of these ligands against PDGF.

*P. crispum*, *M. koenigii*, and *C. tamala* are known to show anti-inflammatory, antioxidant, antimicrobial, and antidiabetic activities, and in addition to these activities,



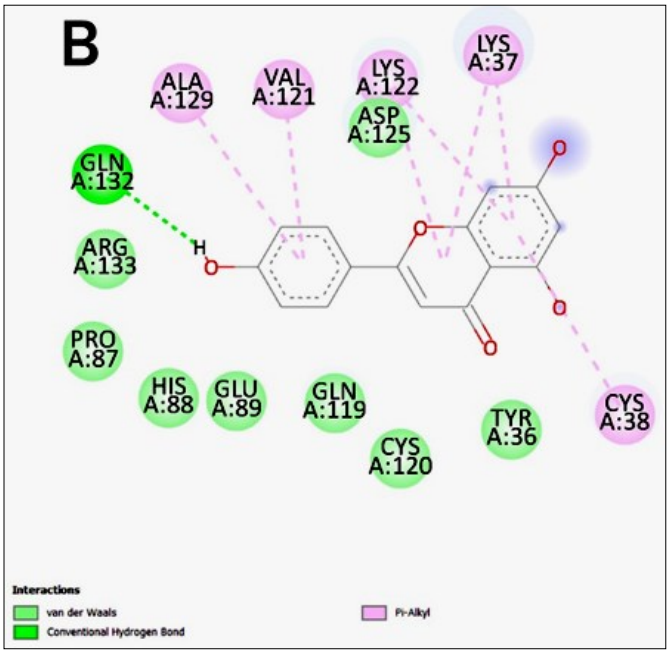




**Fig. 13**. Interaction of apigenin with the active site of NF-κB. (**A**) 3D repre-

these plants also show anticancer activity (44). The presence of anticancer components can be regarded as the reason behind it. In our study, *P. crispum* was found to be the source of three anticancer compounds, viz. apigenin, phytol, and citronellol; *M. koenigii* was found to be the source of three anticancer compounds, viz. apigenin, alpha-pinene, and 3-carene; and *C. tamala* was found to be the source of four anticancer compounds, viz. apigenin, hydroquinone, alpha-terpineol, and limonene.

Phytochemical profiling of plants is important in the discovery of new drugs through the characterization of active metabolites. Proper detection of the bioactive compounds of plant extracts and *in silico* analysis help us know how effectively the extracts can be used in the treatment of a particular disease. Molecular docking is a useful process for checking receptor-ligand interactions. The required structures of ligands for docking can be taken from the Zinc 15 database, which has a collection of com-

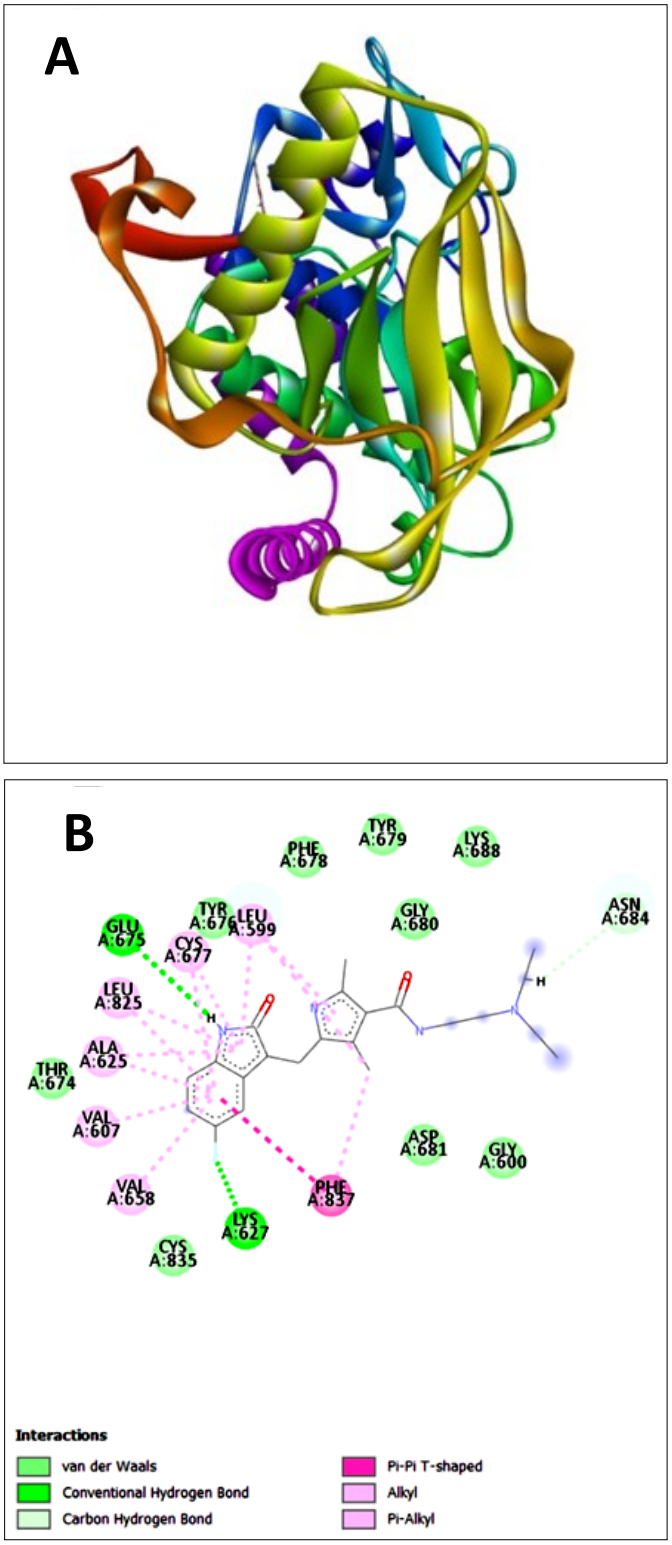



**Fig. 14.** Interaction of apigenin with the active site of PDGF. (**A**) 3D representation, (**B**) 2D representation.

pounds for virtual screening (45). Similarly, the 3D structures of receptors can be taken from the RCBS Protein Data Bank, which has a collection of structures of large molecules, such as nucleic acids and proteins (46). The affinity of a ligand to bind at the active site of the receptor determines its ability to disrupt the normal functioning of the receptor. Hence, such interruptions in the normal functioning of important receptors in the cell cycle cause subsequent inhibition of the growth and proliferation of cells. *In silico* studies narrow down the search for potential lead molecules from a wide range of phytochemicals and help us understand the molecular mechanisms responsible for biological activities (47). As a result, it helps us choose the potential plant extracts to be tested in a wet lab. In the present study, apigenin was found to have the highest binding potential while interacting with the selected receptors involved in causing cancer. The findings of our research are in agreement with the results of a computational and *in vitro* study on the anticancer property of apigenin (48). The results of our study are also supported by the results of a similar *in silico* study showing the anticancer properties of plants used for culinary purposes (49).

Phytoconstituents are seen to provide protection from cell injury and diseases caused by free radicals (50). In addition, the use of phytoconstituents is much safer than the use of synthetic drugs. This publication on plant science today is an early report of the presence of apigenin in *P. crispum*, *M. koenigii,* and *C. tamala* of the North Eastern region of India. To the best of our knowledge, no other literature has been published to date stating the presence of apigenin in these plants of the North Eastern region of India. The present study gives insight into how common culinary herbs can be useful in the mitigation of diseases. The presence of different types of bioactive compounds in these plants can be important for synergistic impact when the extracts of these plants are used in the treatment of disease. Hence, through standardization and properly combined formulations of plant extracts, drugs effective against particular diseases can be obtained.

## Conclusion

TLC analysis of the components of *P. crispum*, *M. koenigii*, and *C. tamala* showed that bands were formed for the methanol extract of each of these plants. Hence, methanol can be used as a solvent for the extraction of the metabolites of these plants. In the GC-MS analysis, a total of 22 bioactive components were detected in the methanolic extract of these plants. Similarly, in HPTLC analysis, the presence of the anticancer component apigenin was detected in the extracts of these plants. *In silico* studies showed that, while interacting with the cancer receptors, the binding affinity of apigenin was higher than that of other anticancer compounds taken for the analysis. After the present study, we arrive at the conclusion that the apigenin present in these plants can play a role in cancer prevention. Hence, it is important to study the extracts of these plants by performing *in vivo* and *in vitro* experiments. In order to properly understand the biochemical mechanism and the signal pathways of apigenin, further studies need to be done.

## Acknowledgements

The authors are thankful to Department of Zoology, Cotton University, Guwahati, Assam for allowing them to conduct this research. The authors acknowledge Guwahati Biotech Park, Guwahati, Assam for providing the facilities for GC-MS analysis of the prepared plant extracts and are thankful to Dr. Rajiv Chandra Dev Goswami of this research centre for his help. The authors sincerely thank Dr. Akalesh

Kumar Verma, Department of Zoology, Cotton University, Guwahati, Assam for allowing them to perform this study using Molegro Virtual Docker software purchased by him. The authors express their deep sense of gratitude to Diksha Dutta, Department of Zoology, Cotton University, Guwahati, Assam and Dr. Suraj Chetri, Department of Zoology, Assam Down Town University, Guwahati, Assam for their technical support while performing *in silico* analysis. The authors are grateful to Yasangam Umbon, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam for his technical support while performing HPTLC analysis.

## Authors' contributions

ED conducted the experiments, analysed the data, and wrote the manuscript. UD supervised the present study and did the reviewing and editing of the manuscript. TCL did the HPTLC part of the present study. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None.

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