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Research Article

Phytochemical fingerprinting and evaluation of *in silico* anti-thrombotic properties of *Justicia adhatoda* L. and *Cordia dichotoma* Frost.

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Abstract

The study aimed to characterize hydro-methanolic (25%) extracts of *Justicia adhatoda* L. (stem and flower) and *Cordia dichotoma* Frost. (Stem and fruit) and evaluate the *in silico* thrombolytic properties of the major phytocompounds present in the plants. In the preliminary fluorescence imaging through treatment by different reagents, both plants were found to be pharmacologically active. Further qualitative screening of phytochemicals, spectroscopy-based techniques, namely, UV-Vis Spectroscopy and FTIR, revealed various classes of compounds such as polyacetylenes, aglycones, quercetin, anthocyanins, anthraquinones, alkaloids, chalcones and aurones, flavanols, carotenoids, and flavanones. Further, by the application of Thin Layer Chromatography, phenols and flavonoids, namely Catechol, Kaempferol, Quercetin, and Lutein, along with other compounds like Chlorophyll b, Glutamic Acid, and Tryptophan were identified from the extracts. Finally, in the molecular docking study, three compounds, Datiscoside and Robinin of *C. dichotoma* and Daucosterol of *J. adhatoda* showed high binding energies (-10.224, -9.547 and -9.262 kcal mol^{-1} respectively) towards the G-protein coupled thrombotic platelet aggregation receptor P2Y1 when compared to that of the control MRS2500 (-7.148 kcal mol^{-1}).

Keywords: analytical characterization; molecular docking; phytochemicals; thrombosis

Introduction

Plants have been extensively used in many traditional systems of medicines like Unani, Ayurveda, Siddha, Naturopathy etc. since ages (Sen and Chakraborty, 2017). Even though both synthetic and plant-based drugs are effective in curing diseases there are some advantages in using plant-based drugs over the synthetic ones. Phytomedicines have fewer side effects, they are more sustainable, affordable and can provide a long-term cure. India accounts for 4500 species of flora and over a thousand of them possess both pharmaceutical and pharmacological relevance. Secondary metabolites of plants contribute to its therapeutic properties (Kabera *et al*., 2004; Yogeesha and Krishnakumar, 2022). Some of the major groups of the secondary metabolites include flavonoids, phenolics, alkaloids, terpenoids and glycosides that are used as bioactive metabolites for treating ailments as well as a to serve as nutraceuticals with modern medicines (Chiocchio *et al*., 2021; Leicach and

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Chludil, 2014). Considering the diversity of phytochemicals present in the plants, analytical characterization of these metabolites is very important in discovering potent chemical compounds that can be useful for treating various diseases.

Thrombosis is a disease characterized by formation of blood clots within the blood vessels which in turn obstruct the blood flow and the oxygen supply to vital body organs. Severe thrombosis may lead to life threatening conditions like heart attacks and strokes (Ashorobi *et al*., 2023). Platelet aggregation plays a very crucial role in the formation of thrombus and thrombotic disorders and P2Y1 is a key receptor involved in the platelet aggregation (Gachet, 2008). P2Y1 is a G-protein coupled receptor present on the surface of platelets which gets activated by ADP and ATP that are produced during platelet aggregation process (Tomaiuolo *et al*., 2017). Therefore, P2Y1 is a potential target for the treatment of thrombosis.

The plants for our study namely *Justicia adhatoda* L. and *Cordia dichotoma* Frost are reported to have medicinal and nutraceutical properties. *J. adhatoda* is a perennial evergreen shrub belonging to the Acanthaceae family, which is also popularly known as Adosa, Vasaka, Arusha, Atalotakam etc. Due to its high therapeutic benefits, all the parts of the plant are used for the treatment of various diseases asthma, swelling, rheumatism, malaria, joint pain, etc (Agnihotri, 2022; Biharee *et al*., 2022). *C. dichotoma*, of the botanical family Boraginaceae, is a deciduous tree that bears edible fruits with a sticky fleshy mass. Commonly known as Indian Cherry, it has high nutritive value and the whole plant is edible including fruits (Murthy *et al*., 2019). The pharmacological benefits of *Cordia* were analyzed and it was found to have analgesic, cardiovascular, gastrointestinal, antimicrobial, and immunomodulatory properties (Ma *et al*., 2021; Raghuvanshi *et al*., 2022). Although both are reported to have high therapeutic values, however literature is scarce on their thrombolytic properties.

In our present study, we extensively characterized hydro-methanolic extracts of different parts of *J. adhatoda* (stem and flower) and *C. dichotoma* (stem and fruit) by using multiple techniques such as qualitative phytochemistry, fluorescence microscopy, FTIR, and thin layer chromatography (TLC) to understand the prevailing phytochemicals/classes present in the plants. *In silico* molecular docking is considered as an advanced tool in the field of modern drug discovery. Application of this technique can effectively reduce screening time of the candidate drugs and significantly less expensive than conventional techniques (Giordano *et al*., 2022). Considering this, major compounds of the plants, selected both from the literature as well as identified through this study, were subjected to anti-thrombosis study *in silico*, targeting the P2Y1 as the receptor.

Materials and Methods

Plant materials and chemicals

The stem (JAS) and flower (JAF) of *Justicia adathoda* L. & stem (CDS) and flower (CDF) of *Cordia dichotoma* Frost., were collected from Salem, Tamil Nadu (11.6643° N,78.1460° E) and Uttarahalli, Karnataka (12.9070°N, 77.5521°E), respectively. The collected plants were separated into parts, after washing and shade dried under the ambient temperature. The dried plant parts were powdered into fine powder and was stored into airtight bags at a cold temperature (4 °C) until further use. All analytical standards (kaempferol, catechol, gallic acid and quercetin) were purchased from Sigma-Aldrich, USA. Other chemicals are of analytical grade, and obtained from SRL, India.

Visualization of plant powders for fluorescence properties under UV illumination

The plant powders of JAS, JAF, CDS and CDF were treated with different freshly prepared chemical reagents (methanol, aqueous NaOH, alcoholic NaOH, chloroform, aqueous $FeCl_3$, acetone, petroleum ether, 50% H_2SO_4, concentrated H_2SO_4, picric acid and 0.1 N HCl) for 2 min. The moist powders were spread over

glass slides and visualized under white light, short UV (254 nm) and long UV (365 nm) by using a UV transillumination system (Kumar *et al*., 2013; Siddiqui *et al*., 2017).

Extraction

Extractions from JAS, JAF, CDS and CDF were carried out using 25% methanol as the solvent along with 0.8 N HCl in order to facilitate acid digestion. All the samples were incubated under the above conditions at room temperature overnight and were centrifuged at 6000 rpm for 10 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper and was stored in glass vials at 4 °C until further use.

Preliminary qualitative phytochemical analysis

Protein

Preliminary analysis was carried out using ninhydrin reagent. Briefly, 1 mL of the plant extract was taken to which two-three drops of 0.2% of the ninhydrin reagent were added to the extract. This reaction mixture was incubated in a water bath. The formation of a purple colour indicates the presence of protein (Yadav and Agarwala, 2011).

Carbohydrates

The plant extract (1 mL) was treated with an equal volume of Benedict's reagent (alkaline solution which contains a cupric citrate complex). The reaction mixture was incubated in a water bath. Formation of a reddish-brown precipitate indicated the presence of carbohydrates (Bhandary *et al*., 2012).

Alkaloid

Preliminary test for alkaloid was carried out using Wagner's reagent. Briefly, 1 mL of the plant sample was taken, and to this a few drops of Wagner's reagent were added. The presence of an orange-brown precipitate confirms the presence of alkaloids (Tiwari *et al*., 2023).

Saponins

For the preliminary analysis of saponin 0.5 g of the plant powder was taken and 2 mL of the distilled water was added to this and incubated in a boiling water bath for 10 min. This mixture was filtered while it was hot and the filtrate was used for conducting further analysis (Singh *et al*., 2022; Theodora *et al*., 2023).

Demonstration of frothing

The analysis was carried out by taking 1 mL of the prepared extract and to this 3 mL of distilled water was taken and the mixture was vigorously shaken for two min. Frothing indicates the presence of saponins. (Mohammed *et al*., 2022).

Flavonoid

The presence of flavonoids was determined by taking 1 mL of the plant extract and this was treated with sodium hydroxide solution. The positive result indicated the presence of yellow colour that becomes colourless after the addition of dilute acid (Singh *et al*., 2022; Valsan and Bose, 2022).

Phenol

The plant extract was treated with $FeCl_3$ solution and the presence of phenol is indicated by the appearance of deep blue colour (Valsan and Bose, 2022).

Spectral analysis for characterization of plant extracts

UV-visible spectral analysis

Spectral analysis of the plant extracts was done by using an UV-Visible spectrophotometer (UV-1800, SHIMADZU). The samples were diluted with 50% methanol in 1:9 ratio before taking reading. The characteristic peaks were then recorded within the spectral range of 200 to 400 nm and further analysed for identification of phytochemical groups (Dhivya and Kalaichelvi, 2017; Uncu *et al*., 2019).

FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique that is used to identify organic and inorganic molecules based on their infrared absorption spectra. They act as one of the most efficient tools in identification of different functional groups (Chemical bonds) as different functional groups absorb light at different wavelength. Therefore, analysing the infrared spectrum enables us to identify different functional groups. A low rpm centrifugation followed by filtration was carried out in order to preparation the extract. Plant extracts of JAS, JAF, CDS, and CDF were loaded to the FTIR spectroscopy (Perkin Elmer, Model: L1600300) with a scan range of 700 to 4000 cm^{-1} (Johnson *et al*., 2020; Sahoo and Umashankara, 2022).

Identification of major phytochemical by thin layer chromatography (TLC)

The analysis was carried out on Aluminium TLC plates pre-coated with Silica gel 60 (20 × 20 cm) with fluorescent indicator F_{254} purchased from Sigma-Aldrich. 10 µL of standard solution mixtures and plant extracts were spotted on separate TLC plates. Butanol, Methanol, Acetic acid and Water were used as mobile phase in the ratio of 4:2:1:1 (Patra *et al*., 2012). The TLC plates were removed from the developing chambers and were air-dried at room temperature and were visualised in 254 nm. To identify the phytochemical constituents of the extracts, the retention factor (Rf) for each of the sample spots in the TLC plates were compared to that of the Rf of the standards. Rf values were calculated by using following formula:

$$\frac{\textit{Distance tarvelled by the solute}}{\textit{Distance travelled by the mobile phase}}$$

Evaluation of anti-thrombotic activity of plant extracts, in silico

Preparation of the protein receptor

The three-dimensional protein structure of the P2Y1 receptor (P2Y1R) protein (PDB id 4XNW, Chain A and B, X-ray Diffraction, Resolution 2.70 Å) with the bound receptor antagonist MRS2500 was obtained from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (*https://www.rcsb.org/*). All the structural optimization of the protein was performed by Swiss PDB viewer and Maestro 13.3 (Schrodinger) software as per our previously published work (Nag *et al*., 2022). Briefly, water molecules, bound ligands and nonpolar hydrogens were removed from the protein structure and the energy minimization was done at the physiological pH (7.4).

Preparation of ligand

Based on the literature, twenty (20) phytochemicals of *J. adathoda* and *C. dichotoma* (ten each), were selected as ligands for this study. The literature details are provided in Table S1, supplementary file. The bound ligand MRS2500 was selected as a control for this study. MRS2500 is a nucleotide antagonist, bind to the specific site of the P2Y1R as demonstrated by Zhang et al., 2015. All the three-dimensional structures of the ligands including the control were downloaded from the PubChem database (*https://pubchem.ncbi.nlm.nih.gov/*) and geometrically optimized through Avogadro 1.95 software with the physiological pH 7.4. Energy minimization of the ligands were performed utilizing a universal force field (UFF)

algorithm as per our previous method (Nag et al., 2023). Two- dimensional structures of the ligands are represented in Figure 1.

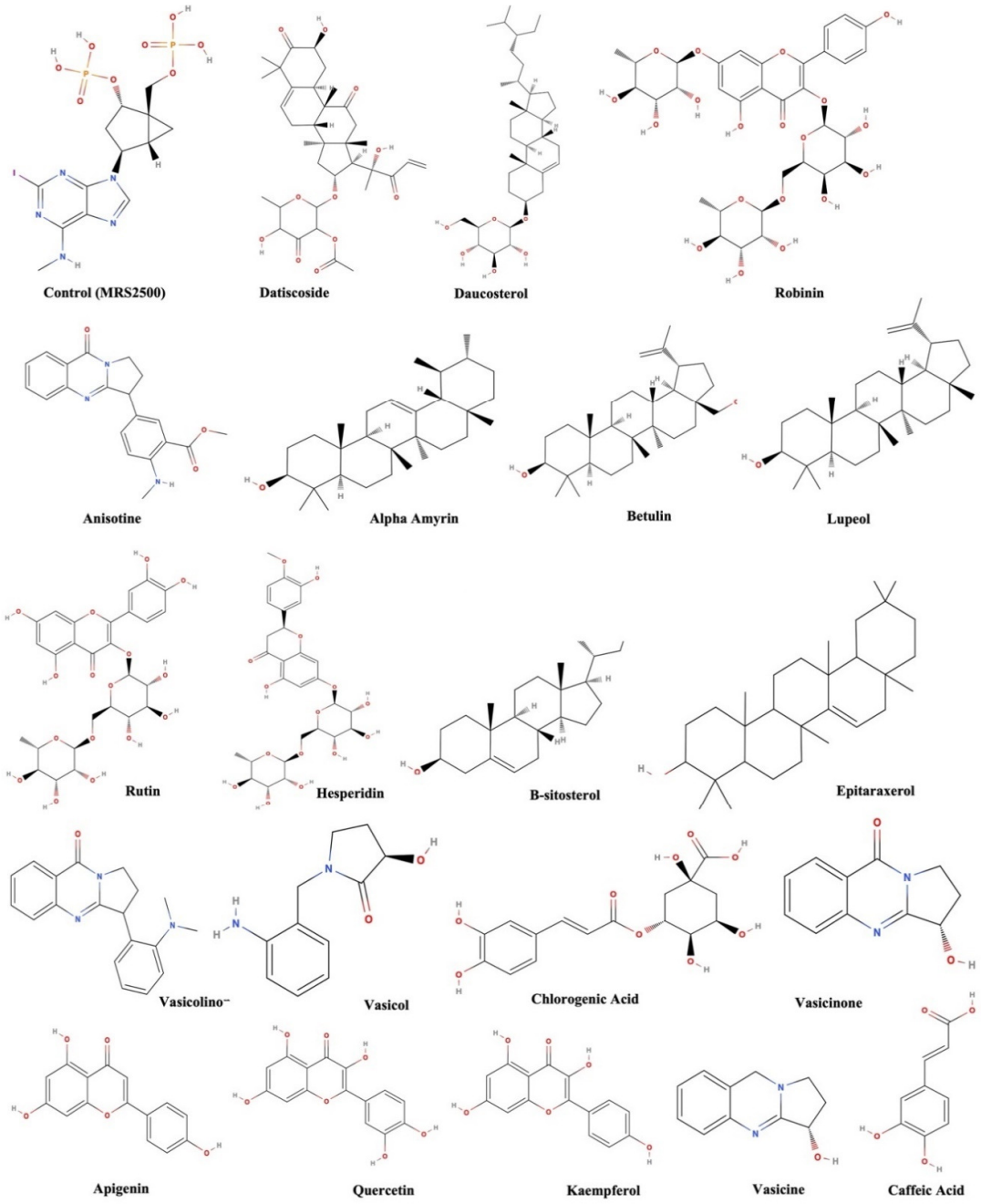

Figure 1. Two dimensional structures of twenty selected phytochemicals along with the control MRS2500

Molecular docking

The binding pocket of the protein P2Y1R was predicted by using the binding site of the co-crystallized ligand MRS2500. The ligand binding site was set as per the grid coordinate centre and size as 20.31 × 21.88 × 8.64 (x X y X z) and 20 × 20 × 20 (x X y X z) Å respectively by using Discovery Studio 2021 (BIOVIA, San Diego, USA). The protein-ligand molecular docking was performed by DockThor web server

(*https://www.dockthor.lncc.br/v2/*). Based on Document supercomputer, DockThor utilizes tools like PdbThorBox and MMFF94S53 force field (LeGrand *et al*., 2020). The docking results were expressed in terms of binding affinity (kcal mol^{-1}) and the phytochemical results were compared to that of the control MRS2500.

Results and Discussion

Visualization of plant powders for fluorescence properties under UV illumination

The powdered stem and flower samples of *J. adathoda* (JAS and JAF) and fruit samples of *C. dichotoma* (CDS and CDF) exhibited different colouration on treatment with various reagents as presented in Table S2, supplementary file. Under the visible wavelength all the samples exhibited different shades of brown colour which varied from dark greenish brown to light brown (Figure 2). The samples when illuminated with 254 nm, dominance of green fluorescence was observed for all four samples. However, *Cordia* samples also showed yellow fluorescence when treated with different reagents and observed under 254 nm. Blue to green fluorescence was prevalent for all the samples at 354 nm. Plant contains various pharmacologically active secondary metabolites such as phenolics, flavonoids, alkaloids etc and they characteristically show fluorescence at different wavelengths, as we observed in our study. Various researchers consistently used fluorescence-based technique to preliminary establish the presence of bioactive metabolites in the plants (Menpara *et al*., 2014; Suresh and Xavier, 2023).

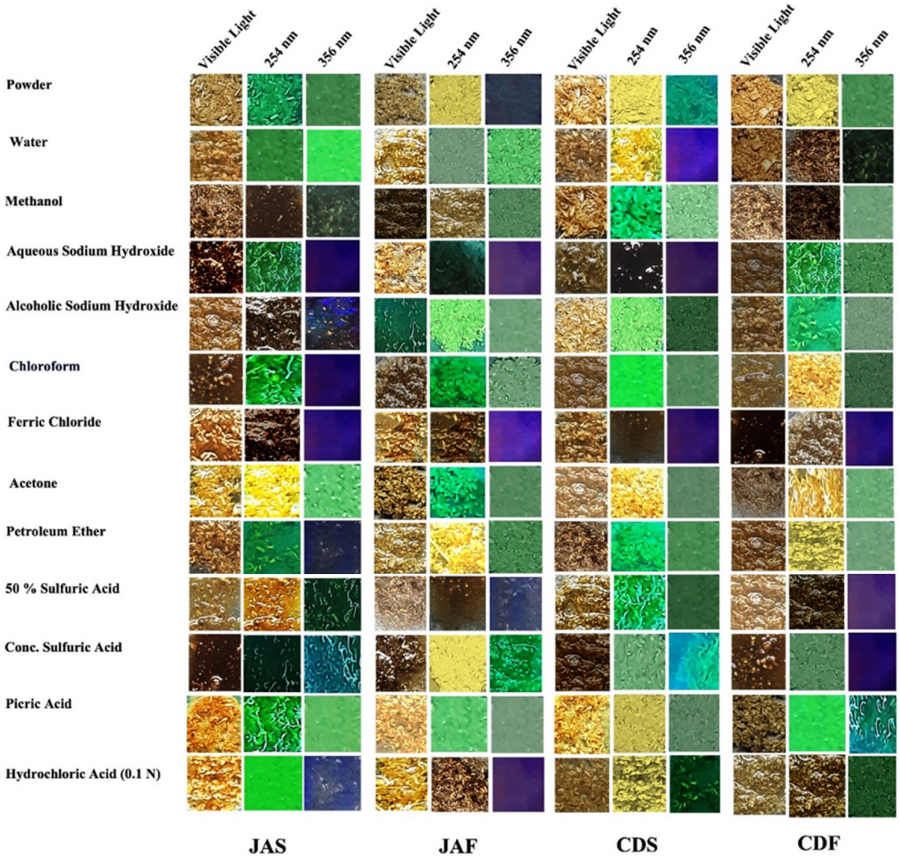

Figure 2. Fluorescence analysis of powdered samples of *Justicia adhatoda* (Stem:JAS and Flower:JAF) and *Cordia dichotoma* (Stem: CDS and Fruit: CDF) under visible light, and UV illumination (254 and 356 nm)

Preliminary qualitative phytochemical analysis

The qualitative result of the methanolic plant extracts exhibited positive results for protein, carbohydrates, alkaloids, saponins, flavonoids, and phenolics. When compared with *C. dichotoma*, *J. adhatoda* were found to have higher number of phytochemicals (Table 1). Plants are rich in various class of phytochemicals and are known to have significant health benefits, such as antioxidant, anti-cancer, anti-diabetes, etc (Ibrahim *et al*., 2019; Kumar *et al*., 2022; Rajurkar *et al*., 2012). Investigations carried out by Sudevan et al., 2019 suggested that the metabolites of *J. adathoda* namely flavonoids, saponins, alkaloids, amino acids, tannins and terpenoids possessed significant anti-inflammatory and anti-cancer properties. Anti-microbial properties *J. adathoda* was demonstrated by Shinwari *et al*. (2020). A number of *in vitro* and *in vivo* studies indicated that *C. dichotoma* contained quercetin that could improve cardiovascular functions (Prajapati *et al*., 2017). The results are shown in Figure 3.

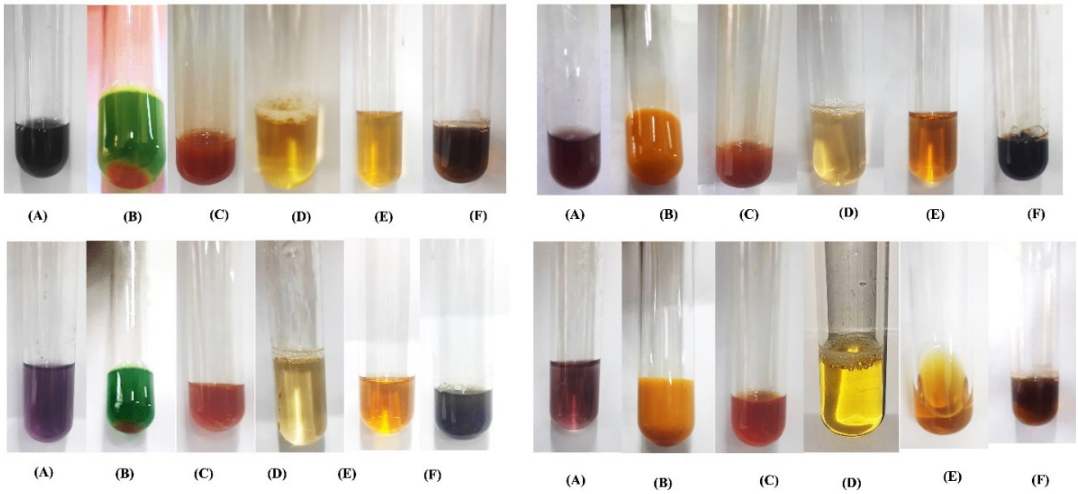

Figure 3. Qualitative estimation of the phytochemical classes of JAS (*Justicia adhatoda* stem) JAF (*J. adhatoda*) flower), CDS (*Cordia dichotoma* stem), and CDF (*C. dichotoma* fruit) extracts represented in the order A: Protein b: carbohydrate: C: alkaloid D: saponin E: flavonoid and F: phenolics

Table 1. Qualitative estimation of phytochemicals of JAS, JAF, CDS and CDF

S. No	Phytochemical Test	JAS	JAF	CDS	CDF
1.	Protein	+++	++	++	++
2.	Carbohydrate	+	+++	+	+++
3.	Alkaloid	+++	+++	++	++
4.	Saponin	+++	+	++	+++
5.	Flavonoid	+++	+++	+++	+++
6.	Phenol	+++	+++	+++	+++

*JAS= *Justicia adathoda* stem, JAF= *J. adathoda* Flower, CDS= *Cordia dichotoma* stem, CDF= *C. dichotoma* Fruit; "+" = Presence of the phytochemical, as the number of "+" increases the colour intensity and the presence of the specific phytochemical increases

Spectral analysis for characterization of plant extracts

UV- visible spectral analysis

Spectroscopic methods act as a powerful tool for understanding and evaluating the qualitative and quantitative analysis of the biological and pharmaceutical materials. The peaks obtained from each of the samples, are summarised in Table 2. Both the *C. dichotoma* extracts showed high number of peaks in the spectral analysis, twelve and eight for CDS and CDF, respectively. While, JAS showed eight peaks, JAF only

had four spectral peaks (Figure S1, supplementary file). The results indicated that among four extracts, JAF had the least variety of phytochemical classes. Spectral identification of eleven (11) classes of phytochemicals namely, polyacetylenes, aglycones, quercetin, anthocyanins, anthraquinones, alkaloids, chalcones and aurones, flavanols, carotenoids, and flavanones were performed as per Harborne (1989). In agreement to our findings such phytochemical classes from *J. adhatoda* and *C. dichotoma* were reported elsewhere in the literature. Earlier work on *J. adathoda* have reported the presence of anthraquinones, aglycones, quercetin, and anthocyanins (Godghate and Sawant, 2013; A. Singh *et al*., 2015; Umashankar *et al*., 2021; Wilson *et al*., 2021). Similarly experiments carried out by Hussain et al., 2021 suggested the presence of aglycones and different flavonoids from the methanolic bark sample of *C. dichotoma*.

Table 2. UV-Visible spectroscopy peak values and class of compounds identified from the methanolic extracts of *Justicia adhatoda* and *Cordia dichotoma*

Peak No.	Sample name	Wavelength (nm)	Absorbance	Class of compound
a.1	JAF	207.5	4.0	Polyacetylenes
a.2		214		Aglycones
a.3		228		Flavanol
a.4		275.50	2.37	Anthocyanins
b.1	JAS	222.5	4.0	Anthraquinones
b.2		248.50		Chalcones and Aurones
b.3		303		Alkaloid
b.4		306		Flavanols
b.5		308	3.83	
b.6		310.50	3.59	
b.7		312	2.91	Aglycones
b.8		313.50	3.02	Polyacetylenes
c.1	CDF	202	4.0	
c.2		203.50		
c.3		206.50		
c.4		211	4.0	Aglycones
c.5		217.50	3.94	Carotenoids
c.6		219.50	4.0	
c.7		223.50	3.30	Flavanones
c.8		278.50	1.10	Flavanols
d.1	CDS	203	4.0	Polyacetylenes
d.2		205		
d.3		207.50		
d.4		210.50		
d.5		222.50		Anthraquinones
d.6		225.50		
d.7		238	3.97	
d.8		241	3.38	Chalcones and Aurones
d.9		276	2.50	Flavanols
d.10		284	2.46	Alkaloid
d.11		287	2.31	Anthraquinones
d.12		334	1.33	Polyacetylenes

JAS= *Justicia adathoda* stem, JAF= *J. adathoda* Flower, CDS= *Cordia dichotoma* stem, CDF= *C. dichotoma* Fruit

FTIR analysis

Chemical characterization of various organic molecules present in the plant extract was done with the help of Fourier transform infrared (FTIR) spectroscopy. FTIR is adaptable, strong and non-destructive analytical technique for the identification of molecular structures of different phytochemicals (Kainat *et al*., 2022). The Infrared spectra of all four methanolic extracts (JAS, JAF, CDS, and CDF) are represented in **Figure 4**. Within the frequency range of 4000-700 cm^{-1}, FTIR analysis revealed 16 different peaks of varying intensities (strong, broad, medium, and weak). The analysis revealed seven different functional groups namely, phenols, alkanes, aldehydes, amino acids, carboxylic acids, esters and ethers. The presence of O-H and C-H stretching were detected in both the plants (*J. adathoda* and *C. dichotoma*) at the absorbance range of 3600-2695 cm^{-1}. C-H bending vibrations were observed in two different intensity ranges i.e., 2000-1650 cm^{-1} (weak) and 1450-1375 cm^{-1} (medium). JAS exhibited C=O stretching vibrations at 1740-1720 cm^{-1} with medium and broad intensity peaks whereas CD (S&F) samples showed same vibrations with the weak intensity. Both extracts displayed C-O stretching vibrations at 1320-1000 cm^{-1} with strong to medium intensity peaks. The obtained peak values and corresponding functional groups were tabulated and presented in Table 3.

Table 3. FTIR analysis of methanolic extracts of *Justicia adathoda* and *Cordia dichotoma*

S. No	Wavenumber (cm^{-1}) reference article)	Wavenumber (cm^{-1}) test sample	Functional group	Intensity	Phytocompounds identified and relevant chemical compound	Detected
1.	3600-3200 (Chandra, 2019; Kainat *et al*., 2022)	3350.61	O-H stretch, H-bonded	Strong and broad in *J. adathoda*, Strong broad in *C. dichotoma*	Alcohols and phenols, aromatic compound	JAS, CDS, JAF, CDF
2.	3000-2850 (Chandra, 2019; Kainat *et al*., 2022)	2920.53	C-H stretch	Weak	Alkanes, aliphatic compound	JAS, JAF
3.	2830-2695 (Kainat *et al*., 2022)	2812.34	C-H stretching	Medium in *J. adathoda* and *C. dichotoma*	Aldehyde	JAS, CDS, JAF, CDF
		2800.53	C-H stretching	Weak		CDS, CDF
		2789.23	C-H stretching	Medium in *J. adathoda* and *C. dichotoma*		JAS, CDS, JAF, CDF
4	1740-1720 (Chandra, 2019)	1725.48	C=O stretching	Medium and broad in *J. adathoda*, weak and broad in *C. dichotoma*		JAS, CDS, JAF, CDF
5.	2250-2700 (Pharmawati and Wrasiati, 2020)	2400.71	NH component	Weak, Broad	Amino acid, amino-related component	CDS, CDF
6.	2250-2700 (Pharmawati and Wrasiati, 2020)	2450.57	NH component	Weak	Amino acid, amino-related component	JAS, JAF

S. No	Wavenumber (cm^{-1}) reference article)	Wavenumber (cm^{-1}) test sample	Functional group	Intensity	Phytocompounds identified and relevant chemical compound	Detected
7.	2250-2700 (Pharmawati and Wrasiati, 2020)	2300.14	NH component	Weak	Amino acid, amino-related component	JAS, JAF
8.	2000-1650 (Kainat *et al*., 2022)	1982.37	C-H bending	Weak	Aromatic	JAS, CDS, JAF, CDF
		1885.58	C-H bending	Weak		CDS, CDF
9.	1450-1375 (Kainat *et al*., 2022)	1406.32	C-H bending	Medium	Alkanes	JAS, CDS, JAF, CDF
10.	1342-1266 (Kainat *et al*., 2022)	1325.27	C-N stretching	Medium	Aromatic amine	JAS, CDS, JAF, CDF
11.	1320-1000 (Kainat *et al*., 2022)	1200.23	C-O stretching	Medium	Alcohols, carboxylic acids, esters, ethers	JAS, CDS, JAF, CDF
		1115.2	C-O stretching	Medium		JAS, CDS, JAF, CDF
		1000.21	C-O stretching	Strong		JAS, CDS, JAF, CDF

JAS= *Justicia adathoda* stem, JAF= *J. adathoda* Flower, CDS= *Cordia dichotoma* stem, CDF= *C. dichotoma* Fruit

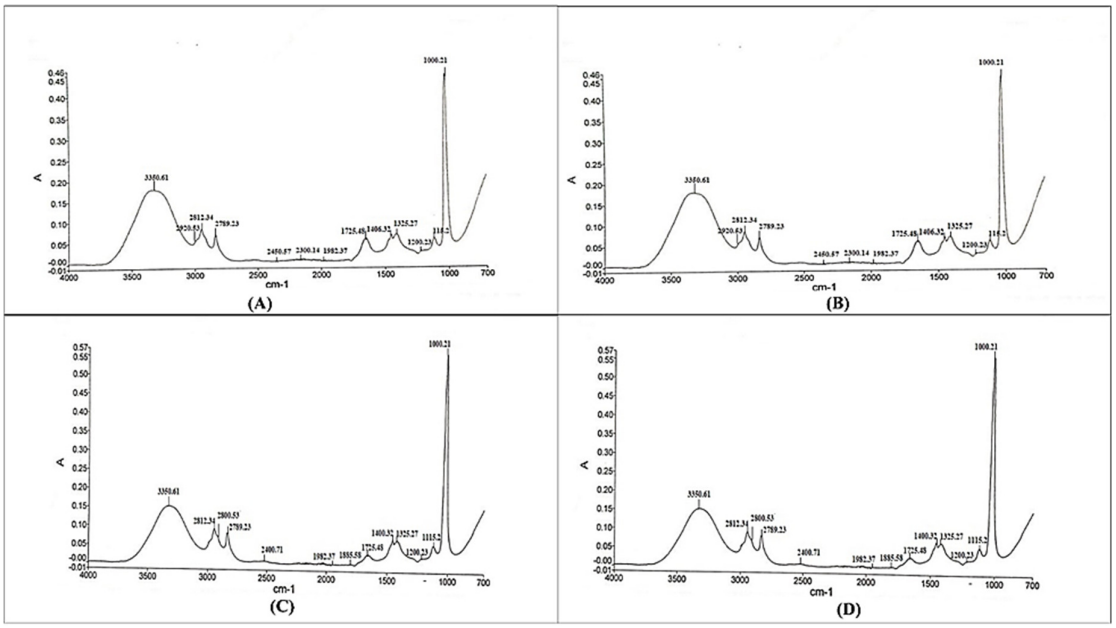

Figure 4. Fourier transform infrared (FTIR) spectroscopy analysis of the methanolic extracts of (A) JAF (*Justicia adhatoda* flower), (B) JAS (*J. adhatoda* stem), (C) CDF (*Cordia dichotoma* fruit), (D) CDS (*C. dichotoma* stem)

Identification of plant metabolites by thin layer chromatography (TLC)

The identified compounds through TLC are presented in Table S3, supplementary file. By matching the retention factors of reference standards, compounds namely catechol, kaempferol, and quercetin, were identified from the methanolic extracts of JAS and JAF respectively. These compounds were frequently reported in the literature (Senthilkumar *et al*., 2012; Singh *et al*., 2015). In a similar run, while kaempferol were found to be present in both the extracts of CDS and CDF, catechol and quercetin were detected from the CDS extract only (Figure 5). Researchers demonstrated the presence of all three compounds from the *C. dhichotoma* methanolic extracts elsewhere (El-Massry *et al*., 2021). Further, we were able to identify a few other compounds from the literature by matching the TLC condition (mobile phase butanol: methanol: acetic acid: water in the ratio of 4:2:1:1) and retention factors. Overall, we found the presence of lutein, chlorophyll b and amino acids glutamic acid, tryptophan in the extracts. The results of TLC could be well correlated with the findings of UV-spectroscopic and FTIR. For example, catechol in accordance to Dewar *et al*. (1958) was found in a range of 270-290 nm in UV-spectral analysis. All the four samples in UV-spectral analysis showed peaks within this range. Similarly, quercetin also falls under the same UV-spectral range and within the FTIR spectra of 3400-3200 cm^{-1} with O-H stretching vibration.

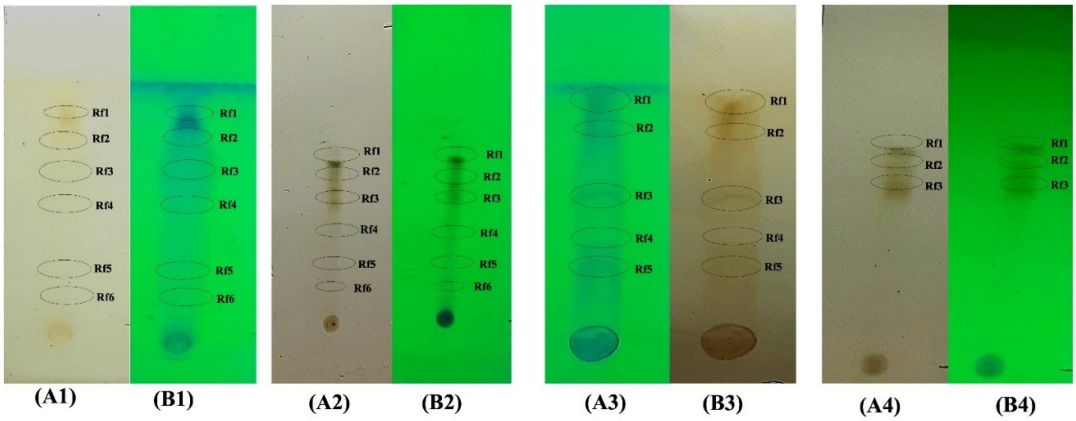

Figure 5. Identification of the compounds from the methanolic extracts of *Justicia adhatoda* (Stem:JAS and Flower:JAF) and *Cordia dichotoma* (Stem: CDS and Fruit: CDF) A: TLC plate under visible light, B: TLC plate under 254 nm

Molecular docking and interaction analysis

The results of molecular docking are shown in Table 4. The triterpene datiscoside, showed the highest affinity (-10.224 Kcal mol^{-1}) against the PY21R in the molecular docking study, when compared with the control MRS2500 (-7.148 kcal mol^{-1}). Another two compounds namely, daucosterol and robinin also showed high binding affinities towards the target proteins (-9.547 and -9.262 kcal mol^{-1}). PY21R is a G-protein coupled receptor and play pivotal role in the activation of platelets. Therefore, PY21R is common therapeutic target for the treatment of thrombosis (Yang *et al*., 2022; Yuan *et al*., 2016). All three top phytochemicals along with the control stabilized their interaction with the target protein through hydrogen and alkyl bonds (Figure 6). The amino acid residue ARG158A, mediated the most of the ligand-protein interactions. Other two amino acids such as TYR315A, and TYR166A shared the same binding pocket for both control and the ligands (Table 5).

Table 4. Result of molecular docking simulation and molecular interaction between P2Y1 receptor and different ligands selected from *Justicia adhatoda* and *Cordia dichotoma*

S/N	PubChem ID	Compound name	Class of compounds	Binding affinity (kcal mol $^{-1}$)
1	5382554	Datiscoside	Triterpene	-10.224
2	5742590	Daucosterol	Phytosterol	-9.547
3	5281693	Robinin	Flavone	-9.262
4	442884	Anisotine	Alkaloid	-9.033
5	73170	Alpha Amyrin	Triterpenoid	-9.009
6	72326	Betulin	Triterpene	-8.989
7	259846	Lupeol	Triterpenoid	-8.967
8	5280805	Rutin	Flavonoid	-8.563
9	10621	Hesparidin	Flavonoid	-8.527
10	222284	B-sitosterol	Phytosterol	-8.463
11	344467	Epitaraxerol	Triterpenoid	-8.327
12	627712	Vasicolinon	Alkaloid	-7.727
13	92470596	Vasicol	Alkaloid	-7.457
14	1794427	Chlorogenic Acid	Phenolic Acid	-7.352
15	442935	Vasicinone	Alkaloid	-7.313
16	44448831	MRS2500 (Control)	Nucleotide	-7.148
17	5280443	Apigenin	Flavone	-6.961
18	5280343	Quercetin	Flavanol	-6.909
19	5280863	Kaempferol	Flavanol	-6.898
20	667496	Vasicine	Alkaloid	-6.553
21	689043	Caffeic Acid	Cinnamic Acid	-6.344

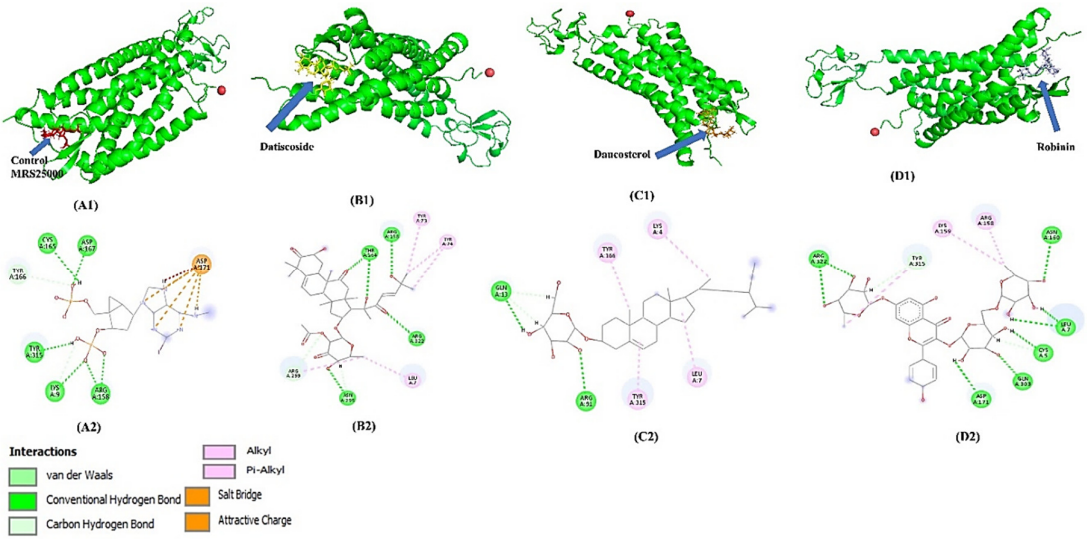

Figure 6. Three-dimensional interactions between P2Y1 receptor (PDB id 4XNW) with the control (A1) MRS2500 and the top three ligands (B1) Datiscoside, (C1) Daucosterol, (D1) Robinin, Two-dimensional interactions between P2Y1 receptor with the control (A2) MRS2500 and the top three ligands (B2) Datiscoside, (C2) Daucosterol, (D2) Robinin

Table 5. Amino acid residues interacting with the top three phytochemicals along with the control

Name	Amino acid interaction	Hydrogen bonds/ carbon hydrogen bonds	Alkyl bonds/Pi-Alkyl
MRS25000	ARG158A, LYS9A, TYR315A, ASP167A, CYS165A, TYR166A	ARG158A, LYS9A, TYR315A, ASP167A, CYS165A, TYR166A	_
Datiscoside	ASN295A, ARG322A, THR164A, **ARG158A**, ARG299A, LEU7A, TYR73A, TYR74A	ASN295A, ARG322A, THR164A, ARG158A, ARG299A	LEU7A, TYR73A, TYR74A
Daucosterol	GLN13A, ARG91A, **TYR315A**, LEU7A, LYS4A, **TYR166A**	GLN13A, ARG91A	**TYR315A**, LEU7A, LYS4A, **TYR166A**
Robinin	ASP171A, GLN303A, CYS5A, LEU7A, **ARG158A**, LYS159A	ASP171A, GLN303A, CYS5A, LEU7A, ASN160A, ARG322A, **TYR315A**	ARG158A, LYS159A

Conclusions

In the present study, we identified 11 different classes of phytochemicals namely, polyacetylenes, aglycones, quercetin, anthocyanins, anthraquinones, alkaloids, chalcones, aurones, flavanols, carotenoids, and flavanones from *J. adathoda* and *C. dichotoma* in combination with multiple techniques such as qualitative screening, UV-spectroscopy, FTIR and fluorescence imaging. Further, TLC identified some phenolics, such as Kaempferol, Quercetin, Catechol etc, in agreement with the findings of other techniques. Finally, the *in silico* molecular docking study with twenty major phytochemicals of *J. adathoda* and *C. dichotoma* targeting the thrombotic protein P2Y1R, revealed that both the plants could potentially be exploited for their thrombolytic properties through extensive *in vitro* and *in vivo* studies. Among these twenty compounds, Datiscoside and Robinin of *C. dichotoma* and Daucosterol of *J. adhatoda* showed high binding energies (-10.224, -9.547 and -9.262 kcal mol^{-1} respectively) towards P2Y1R. It is expected that, future research shall find secondary metabolites of *J. adathoda* and *C. dichotoma*, as effective drug candidates for the treatment of thrombosis and its underlying conditions.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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