RESEARCH ARTICLE

In-silico Study of Methyl Beta D-xylopyranoside: A Spectroscopically Screened Small Molecule from *Aganosma dichotoma*

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ABSTRACT

Background: To develop scientific evidence of unexplored traditional plants.

Methods: Standardization of leaf powder was done as per World Health Organization (WHO) guidelines, followed by fourier-transform infrared spectroscopy (FTIR) analysis to identify basic phytoconstituents. Preliminary phytochemical analysis and spectroscopic analysis was done to validate the identified phytoconstituents. Molecular docking was done to identify a small molecule, methy beta D-xylopyranoside.

Results: The air-dried leaves and barks were powdered and subjected to extraction based on the polarity of solvents through soxhlation, namely, methanol, ethanol, and chloroform, to obtain four different extracts. Further, preliminary phytochemical tests and qualitative determination of the different biologically active compounds from leave powder *Aganosma dichotoma* using FTIR revealed the presence of different phytoconstituents. And gas chromatography-mass spectrometry of crude extracts revealed different chemical entities with varying quantities and followed by *in-silico* molecular docking studies of hub genes against the photochemical small molecule. Thus, the identification of different biologically active compounds in the extracts of leaves *A. dichotoma* warrants further biological and pharmacological studies.

Conclusion: Small molecules identified from *A. dichotoma* have robust activity against myocardial infarction, but need to be validated through *in-vitro* and *in-vivo* studies.

Keywords: Myocardial infarction, Small molecules, Methyl beta D-xylopyranoside.

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INTRODUCTION

Traditional medicinal plants have been used to treat various illnesses for centuries. The World Health Organization (WHO) has noted that a significant portion of the global population on every continent has turned to herbal medicine because of its affordability and minimal adverse effects. These time-tested botanical remedies are well known for their effectiveness, safety, and quality.[1] One such example, *Aganosma dichotoma*, has held a place of importance in traditional medicine for many generations. This plant is recognized for its antiseptic properties, soothing effects (used in massage oils for conditions such as paraplegia, neuralgia, and sciatica), and its potential as an anthelmintic and emetic agent, as indicated by literature reviews. *A. dichotoma* is commonly known as Malati in Hindi and in Telugu. *A. dichotoma* is a common house plant in India, China, and Indonesia. It belongs to family Apocynaceae.[2] *A. dichotoma* leaves contain different phytoconstituents such as alkaloids, phenols, and steroids. The main chemical structure of *A. dichotoma* leaves is kaempferol, quercetin, rutin etc. which are known for its anti oxidant activity.[3]

Phytochemical small molecules hold great promise as a new class of drugs, harnessing the therapeutic potential found within plants. These compounds are organic, naturally occurring substances that play essential roles in the plant's defense mechanisms and overall metabolic processes. Over the years, extensive research has unveiled their vast pharmacological activities, making them attractive candidates for drug development.

One of the main advantages of phytochemical small molecules is their diverse chemical structures, which allow for a wide array of biological activities. These compounds have shown remarkable potential in treating various diseases, including cancer, cardiovascular disorders, neurological conditions, and inflammatory ailments. Additionally, they often possess fewer side effects compared to traditional drugs, making them safer alternatives for patients.

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The current study was conducted to identify the potential glycoside phytochemical small molecules from *A. dichotoma* using spectroscopical analysis followed by computational approach studies.

MATERIALS AND METHODS

Plant Material

A.dichotoma plant leaves were collected from Seshachalam hills and Tirumala hills, Chittoor district, Andhra Pradesh, South India in 2020. Plant taxonomists confirmed the taxonomic identification of the plant material. Dr. Madhav Chetty, in the Department of Botany, Sri Venkateswara University (S. V. University). The voucher specimen number 0619 has been deposited at the Herbarium of the Department of Botany, Sri Venkateswara University (S.V. University), Tirupati, Andhra Pradesh, India.

Standardization of Leaf Powder

Standard procedures as per WHO guidelines, determination of ash values, loss on drying along with bulk density and tapped density were done to identify the impurities of leaf powder.

Fourier Transform Iinfrared Analysis for Leaf Powder

Analysis of the powdered sample was conducted using a Bruker Alpha II FTIR spectrometer equipped with a diamond crystal attenuated total internal reflectance (ATR) accessory. About 0.2 mg of leaf powder was carefully positioned on the germanium component of the infrared spectrometer. Consistent pressure was maintained during this process. Infrared absorbance data were gathered across a wave number range spanning from 3500 to 675 cm^{-1}. The Opus 7.8 software was employed for the analysis, as outlined in the reference.[4]

Preparation of the Extracts

For extraction ethanol, methanol, chloroform, and petroleum ether was used in decreasing the order of polarity. The air-dried leaves of the plant were extracted by using a soxhlet extractor for 5 hours with 250 mL of ethanol. Excess solvents in the extracts were evaporated using a rotary evaporator to obtain the concentrated extracts.

Preliminary Phytochemical Analysis

Standard methods with little modifications were used for screening and identification of chemical constituents of extracts. The phytochemicals such as, saponins, phenolic compounds, tannins, terpenoids, flavonoids, alkaloids, cardiac glycosides, carbohydrates, and phenolic compounds were determined.[5]

Identification of Glycoside Small Molecule through Spectroscopical Analysis

The *A.dichotoma* extracts, including ethanol, methanol, chloroform, and petroleum ether, underwent Gas chromatography–mass spectrometry (GC-MS) analysis. The analysis was conducted by sending the extracts to IICT Hyderabad. A GC-trace utilizing a standard non-polar column was employed for the analysis. The GC-MS analysis was conducted at low resolution, with a single injection. The injector temperature was set at 260°C, and 1-µL of the sample was injected into the instrument. To identify the components, the obtained spectra were compared with the database of known component spectra stored in the GC-MS NIST (2008) library. This comparison aided in identifying and characterizing the various compounds present in the *A. dichotoma* extracts. The further methanolic extract was sent to SAIF, IIT Bombay for LC-MS analysis with HPLC + PDA detector + Mass Spectrometer with database for small molecules. The sample was filtered to remove any particulate matter and the HPLC column was connected to the system and equilibrated it with the mobile phase. The sample was injected into LC-MS and data was acquired simultaneously using the PDA detector and mass spectrometer.[6]

Molecular Docking Studies of Identified Glycoside Small Molecule

Molecular docking is done to identify the binding affinity of selected traditional plant extracts with their top 10 hub genes which were identified in using an artificial intelligence approach. The virtual screening tool CB dock was utilized. Target receptors of identified glycoside small molecule was downloaded in PDB format from the protein data bank and preparation of proteins was done using Discovery studio visualizer, water molecule and hetero atoms was deleted and saved as pdp_prep format for further study. Structures of ligand was downloaded from PubChem in SDF format. Each ligand was uploaded in CB dock webserver and docked individually with each of the ten targets (hub genes). Docked complexes was downloaded and Discovery Studio 2020 (BIOVIA) was used to capture best poses of the protein-ligand complex in 2D diagram.[7,8]

RESULTS

Standardization of Leaf Powder

The standardization procedure helps to find out the inorganic content in the powder, bulk density, tapped density values are 0.33, 0.41 gm/cm^3 and Carr's index of *A. dichotoma* leaf powder is 11%. Total ash value, acid-soluble ash value and water-insoluble ash value has been calculated, values are 7.3, 0.4 and 2.6%, respectively (Table 1). These are within the limits as prescribed by the WHO and values are 7.3, 0.4 and 2.6%, respectively.

FTIR Analysis

FTIR data was collected over the wave number ranging from 3500 to 675 cm^{-1} primary amines, aliphatic compounds, secondary amines, alkyl compounds, proteins, sulfate ions and alcohol Figure 1 are observed while aromatic compounds and isopropyl alcohol are observed in Table 2 in fingerprint region.[9]

Preparation of the Extracts

Extracted solvents in the extracts were evaporated using rotary evaporator and the percentage of yield was calculated 20, 24, 17, 16% (w/w), respectively and then stored at 4°C for further use.

Table 1: Standardisation leaf powder

Bulk density (gm/ cm^3)	*0.33 ± 0.45*
Tapped density (gm/ cm^3)	0.41 ± 0.3
Carr's Index (%)	11 ± 0.13
Loss on drying (%)	1.4 ± 2
Total Ash value (%)	7.3 ± 1.8
Acid soluble ash (%)	0.4 ± 0.57
Water insoluble ash (%)	2.6 ± 0.13

Each value is mean of triplicates, ± indicates the standard deviation

Table 2: FTIR analysis of *A.dichotoma* leaf powder

Absorbance (cm-^{1})	*Functional groups*	*Possible type of compound*
3344	Imino compounds, =N-H stretch	1° amine
2970	CH stretching of CH3, CH2/NH stretching	Aliphatic compounds
1466	CH stretching of CH3, CH2/NH stretching	Aliphatic compounds
1378	Aromatic amine, CN stretch	Secondary amine
1341	Aromatic amine, CN stretch	Secondary amine
1306	Dialkyl/aryl sulfones	Alkyl/aryl compounds
1160	C-O of proteins and carbohydrates, stretching modes of the C-OH groups of serine, threonine, and tyrosine residues of cellular proteins, hydrogen-bonded stretching mode of C-OH groups	Protein (serine, threonine, and tyrosine) and collagen
1128	Sulfate ion	Organic material
1108	Secondary alcohol, C-O stretch	Alcohol
951	Aromatic C-H in-plane bend	aromatic compound
817	C-C-0 Symmetric stretch	isopropyl alcohol

Preliminary Phytochemical Analysis

Four extracts have been screened for preliminary phytoconstituents and flavonoids, cardiac glycosides, tannins, phenols, triterpenoids and coumarins are present in ethanolic extract. While, flavonoids, glycosides, protein, amino acids, triterpenoids, quinones and coumarins are present in methanolic extract and glycosides, cardiac glycosides, carbohydrates, triterpenoids, quinones and coumarins are present in chloroform extract. Cardiac glycosides, tannins, triterpenoids, quinones and coumarins are present in Petroleum ether extract Table 3.

Identification of Glycoside Small Molecules through Spectroscopical Analysis

To validate the preliminary analysis four extracts was screened using GC-MS analysis. Nearly 78, 60 and 77 compounds were traced in methanolic, ethanolic and chloroform extracts respectively. The molecular weight of compounds was noted from PubChem and categorized into small molecules. Out of

Table 3: Preliminary phytochemical screening of ethanolic extract of *A. dichotoma*

Phytoconstituents	*ADE*	*ADM*	*ADC*	*ADPE*
Alkaloids	-	-	-	-
Flavonoids	+	+	-	-
Glycosides	-	+	+	-
Cardiac glycosides	+	+	+	+
Tannins	+	-	-	+
Carbohydrates	-	-	+	-
Protein and amino acids	-	+	-	-
Phenolic compounds	+	-	-	-
Triterpenoids	+	+	+	+
Quinones	-	+	+	+
coumarins	+	+	+	+

ADE- *A. dichotoma* Ethanolic extract, ADM- *A. dichotoma* Methanolic extract, ADC- *A. dichotoma* Chloroform extract, ADPE- *A. dichotoma* Petroleum Ether extract, '+'- indicates Positive and '-' indicates negative.

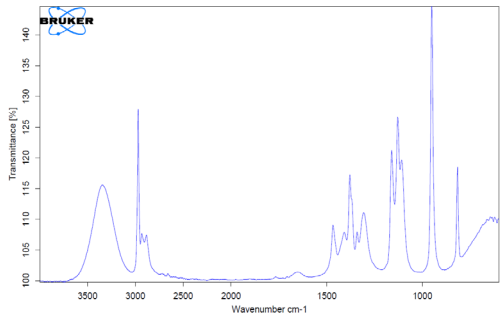


Figure 1: FTIR spectroscopic analysis of *A. dichotoma* leaf powder

which 13 small molecules in ethanolic extract, 32 in methanolic extract and 23 in chloroform extract are found.

In Table 4 13 phytochemical small molecules were detected and few among them were thiophene, tetrahydro-2-methyl-, urea, N'-(4-chlorophenyl)-N-methoxy-N-methyl-,2-propanone, dodecanoic acid, 1,3-dihydroxy-,2- isothiocyanate, heptane 9-octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester, 4-O-methylmannose, oleic acid, n-hexadecanoic acid, phytol, cyclotrisiloxane, hexamethyl-, cinnamyl cinnamate Figure 2.

Meanwhile, more than 70 components were detected in the methanolic extract and 31 were small molecules among them Figure 3 and few important compounds Table 5 were Hexadecane- cyclo alkene, 4,6-dichloro-5-nitropyrimidine, methyl (methyl 4-O-methyl-alpha-d-mannopyranoside) uronate, fucosterol, lanosterol, taraxasterol. One glycoside is identified which methyl beta D-xylopyranoside is a glycoside derivative composed of a methyl group.

Seventy-seven compounds were detected in Figure 4 and 23 are small molecules which has few compounds like Table 6 cycloheptasiloxane tetradecamethyl, gamma. -Ionone, alpha. -Gurjunene, Coumarin, 3,4-dihydro-4,4,6,8-tetramethyl-, phytol, tirucallol. In this study of *A. dichotoma*, the identified

Table 4: Small molecules present in ethanolic extract

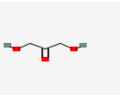
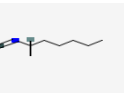
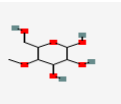
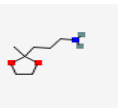
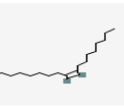
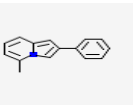
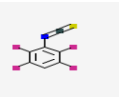
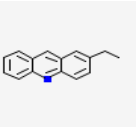
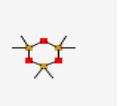
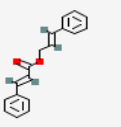
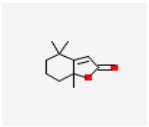
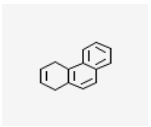
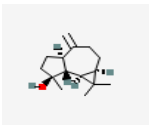
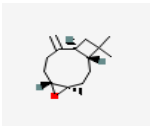
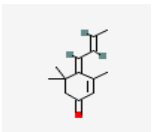
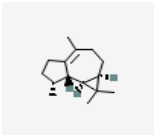
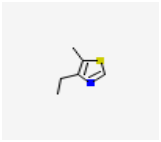
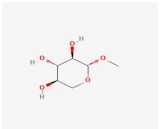
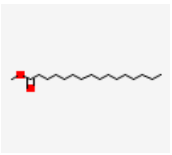
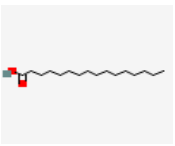
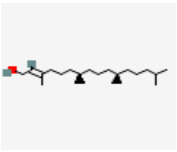
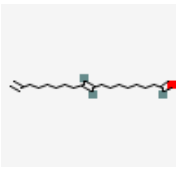
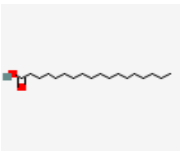
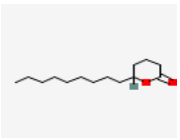
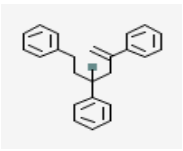
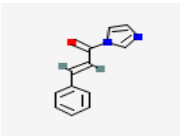
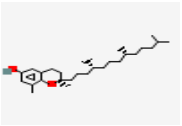
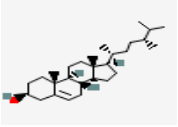
S. No.	*RT time*	*Name of the compound*	*Activity*	*Structure*	*Molecular weight (gmol)*	*Phytochemical nature*
1	5.404	2-Propanone, 1,3 dihydroxy-	Cosmetic ingredient		90.08	Ketone
2	13.725	2-Heptane isothiocyanate	Anti-cancer		157.28	Isothiocyanate
3	15.234	Dodecanoic acid	Anti-microbial		200.32	Fatty acid
4	16.457	4-O-Methylmannose	Antibacterial and antifungal		194.18	Carbohydrate
5	16.781	4,4-Ethylenedioxy-1-pentylamine	Stimulation of neuronal cells, biological sensor		145.2	Amine
6	17.260	Oleic acid	Anti-inflammatory, cardioprotective		282.5	Fatty acid
7	19.570	n-Hexadecanoic acid	Anti-inflammatory and antimicrobial activity		256.42	Fatty acid
8	21.033	Phytol	Antimicrobial, anti-inflammatory anti-cancer, antioxidant and antiarthritic		296.5	Terpene
9	22.606	5-Methyl-2-phenylindolizine	Anti-microbial		207.27	Alkaloid
10	22.852	2,3,5,6-Tetrafluorophenyl isothiocyanate	Anti-microbial		207.15	Isothiocyanate
11	24.257	2-Ethylacridine	Antimicrobial and antitumor		207.27	Alkaloid
12	24.335	Cyclotrisiloxane, hexamethyl-	Antibacterial activity, antioxidant		222.46	Silicon compound
13	26.639	Cinnamyl cinnamate	Fragrance		264.3	Phenolic compound

Table 5: Small molecules present in methanolic extract

S. No	*RT time*	*Name of the compound*	*Activity*	*Structure*	*Molecular weight (g/mol)*	*Phytochemical nature*
1	12.392	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Antibacterial, nematicide		180.24	Benzofuranone
2	12.845	1,4-Dihydrophenanthrene	Anti-cancer		180.24	Phenanthrene
3	12.968	Spathulenol	Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities		220.35	Sesquiterpene
4	13.039	Caryophyllene oxide	Anticancer and analgesic activities		220.35	Sesquiterpene
5	13.195	Hexadecane	Antimicrobial and antioxidant activity		226.44	Hydrocarbon
6	13.518	Megastigmatrienone	Aroma		190.28	Terpenoid
7	14.159	Tridecane	Fragrance		184.36	Hydrocarbon
8	14.198	Viridiflorene	Anti-inflammatory, anti-microbial, anti-oxidant, cytotoxic and Tumor		204.35	Sesquiterpene
9	14.315	Heptadecane	Anti-inflammatory, anti-oxidant		240.5	Hydrocarbon
10	15.493	4-Ethyl-5-methylthiazole	Antibiotic		127.21	Thiazole
11	15.629	Methyl beta D-xylopyranoside	Anti-oxidant		164.16	Glycoside derivative

12	16.399	n-Pentacosane	Antibacterial		352.7	Aldehyde
13	16.671	Methyl palmitate	Anti-inflammatory and antifibrotic		270.5	Terpene
14	17.033	Palmitic acid	Anti-inflammatory and antifibrotic		256.42	Fatty acid
15	18.431	Phytol	Antinociceptive, antioxidant activities, anti-inflammatory and antiallergic effects		296.5	Hydrocarbon
16	18.697	9,17-Octadecadienal	Antimicrobial		264.4	Fatty acid ester
17	18.917	Octadecanoic acid	lubricating agent		284.5	Ester
18	20.509	2H-Pyran-2-one, tetrahydro-6-nonyl-	Flavouring agent		226.35	Pyranone
19	21.241	2,4,6-Triphenyl-1-hexene	Anti-bacterial		312.4	Hydrocarbon
20	22.878	trans-1-Cinnamoylimidazole	Anti-cancer, anti-microbial, anti-fungal, anti-protozoal		198.22	Imidazole
21	24.904	delta. -Tocopherol	Food antioxidant		402.7	Vitamin E
22	27.150	Campesterol	cholesterol-lowering agent and act in cancer prevention		400.7	Phytosterol

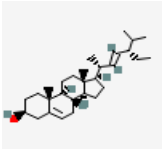
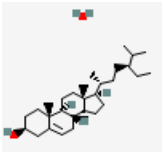
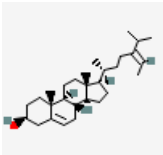
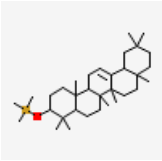
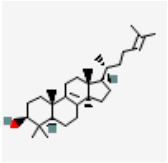
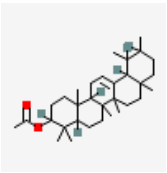
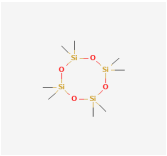
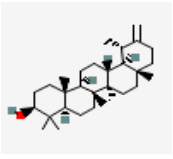
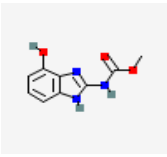
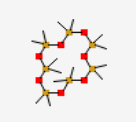
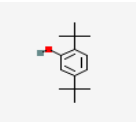
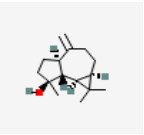
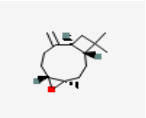
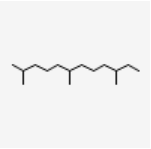
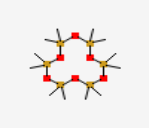
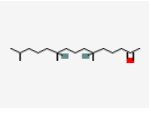
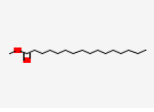
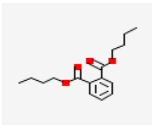
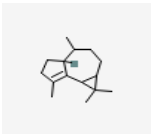
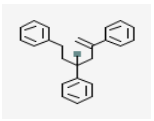
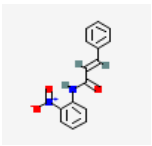
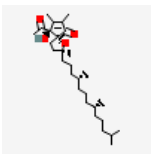
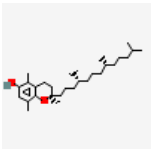
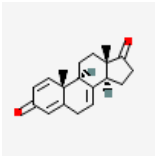
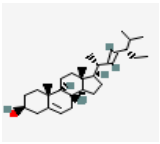
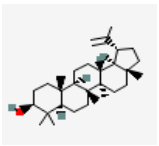
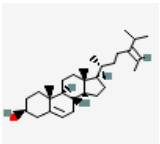
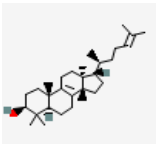
23	27.493	Stigmasterol	Anti-osteoarthritic		412.7	Phytosterol
24	28.134	Gamma. -Sitosterol	Cholesterol-lowering agent		432.7	Triterpene
25	28.348	Fucosterol	Anticancer, antidiabetic, antioxidant, hepatoprotective, antihyperlipidemic, antifungal,		412.7	Phytosterol
26	28.471	Beta. -Amyrin	Analgesic, anti-inflammatory, anticonvulsant, antidepressive		426.7	Phytosterol
27	28.574	Lanosterol	cholesterol lowering		426.7	Sterol
28	29.040	Alpha. -Amyrin	Analgesic, anti-inflammatory, anticonvulsant, antidepressive, gastroprotective, hepatoprotective,		426.7	Triterpene
29	29.953	Cyclotetrasiloxane, octamethyl-	manufacture of polymeric materials breast implants		296.61	Silicon compound
30	31.668	Taraxasterol	Phytosterol, antioxidant		426.7	Sterol
31	31.681	Methyl (7-hydroxy-1H-benzimidazol-2-yl) carbamate	Preservative in paints		207.19	Benzimidazole derivative

Table 6: Small molecules present in chloroform extract

S.No	*RT time*	*Name of the compound*	*Activity*	*Structure*	*Molecular Weight (g/mol)*	*Phytochemical nature*
1	11.991	Cycloheptasiloxane, tetradecamethyl-	Anti-bacterial		519.07	Silicon compound
2	12.185	Phenol, 2,5-bis(1,1-dimethylethyl)-	Anti-phytopathogenic		206.32	Phenolic compound
3	12.975	Spathulenol	Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities		220.35	Sesquiterpene
4	13.040	Caryophyllene oxide	Anticancer and analgesic activities		220.35	Sesquiterpene
5	13.188	Tridecane	Fragrance		184.36	Hydrocarbon
6	14.308	Heptadecane	Anti-Inflammatory, anti-oxidant		240.5	Hydrocarbon
7	14.373	Farnesan	Muscle relaxer, calming and sedative effects, anti-inflammatory, anti-fungal, and antibacterial properties		212.41	Sesquiterpene
8	15.383	Octadecane	Lubricant, transformer oil and anti-corrosion agents		254.5	Hydrocarbon
9	15.745	Cyclohexasiloxane, dodecamethyl	Used in personal care products such as hair/skin care products, antiperspirants and deodorants.		444.92	Silicon compound
10	15.862	6,10,14-Trimethyl-2-pentadecanone	Antibacterial, anti-nociceptive and anti-inflammation activities		268.5	Ketone
11	16.405	Nonadecane	Antimicrobial, antioxidant, anticancer		268.5	Hydrocarbon
12	16.677	Methyl palmitate	Anti-inflammatory and antifibrotic		270.5	Fatty acid ester

13	17.040	Dibutyl phthalate	Antibacterial		278.34	Phthalate Ester
14	19.959	alpha. -Gurjunene	Aroma		204.35	Sesquiterpene
15	21.254	2,4,6-Triphenyl-1-hexene	Anti-bacterial		312.4	Hydrocarbon
16	23.571	2-Nitrophenyl cinnamamide	Anti-inflammatory		268.27	Aromatic compound
17	24.309	alpha. -Tocospiro A	Antioxidant		462.7	Triterpene
18	26.354	Vitamin E	Food antioxidant		430.7	Vitamin
19	26.749	1,4,7-Androstatrien-3,17-dione	Aromatase inhibitor		282.4	Steroid
20	27.506	Stigmasterol	anti-osteoarthritic		412.7	Phytosterol
21	27.869	Lupeol	Anti-inflammatory and anti-cancer		426.7	Triterpene
22	28.367	Fucosterol	anticancer, antidiabetic, antioxidant, hepatoprotective, antihyperlipidemic, antifungal,		412.7	Phytosterol
	29.196	Tirucallol	Anti-inflammatory		426.7	Triterpene

compounds have been found to be present in other plant species as well, based on previous research. For example, in the leaves of *Cleistanthus collinus*, 17 compounds were identified, and n-hexadecanoic acid was recognized as the major compound.[10,11] Furthermore, heptadecanoic acid, ethyl ester, tricosanoic acid, and stearic acid have been reported in the hexane extract of the leaves of *Desmodium elegans*.[12] These findings suggest that the compounds identified in *A. dichotoma* are not unique to this species and are also present in other plants, indicating some level of similarity in their chemical composition. Based on the results of LC-MS we have identified few potent phytocompounds that can act against our disease area of interest from *A. dichotoma* methanolic extract. Further, we have sent a sample for LC-MS analysis, 10 compounds were identified[13]. Glycoside is our area of interest and we have used screened both gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) data using various artificial intelligence tools.

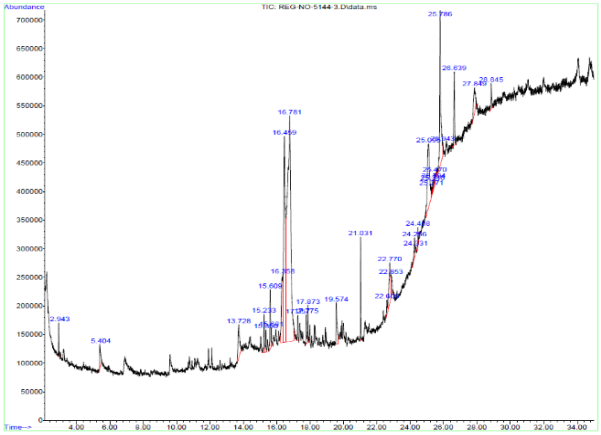


Figure 2: GCMS spectroscopic analysis of *A.dichotoma* ethanol extract

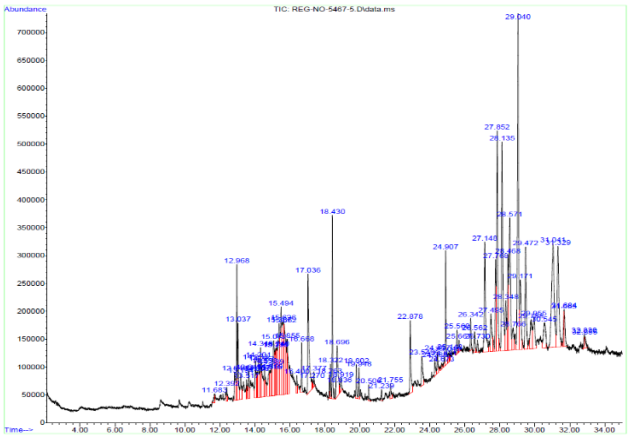


Figure 3: GCMS spectroscopic analysis of *A.dichotoma* methanol extract

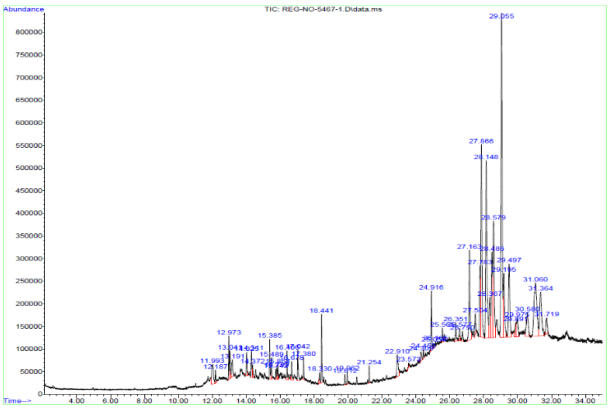


Figure 4: GCMS spectroscopic analysis of *A.dichotoma* chloroform extract

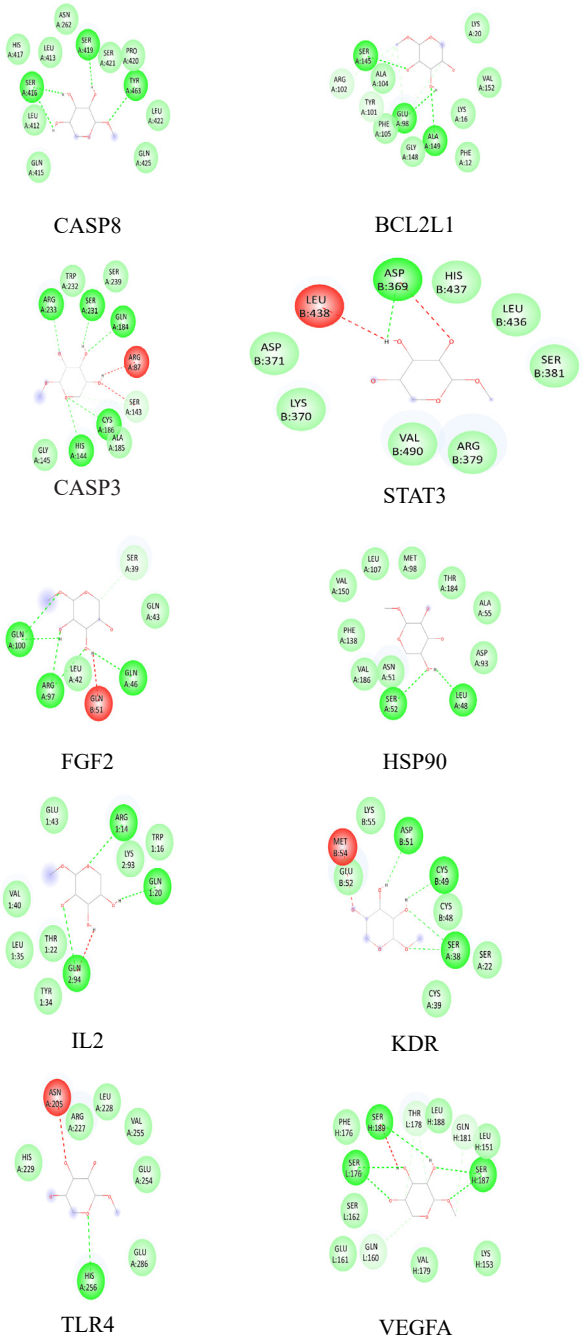


Figure 5: Molecular Docking of Hub genes with small molecule identified

Table 7: Biding affinity values of molecular docking

Name of genes	*Binding affinity values*
	A. dichotoma
STAT3	-5.4
HSP90AA1	-5.2
CASP3	-5.2
BCL2L1	-5.1
IL2	-5
TLR4	-4.8
VEGFA	-4.7
KDR	-4.7
CASP8	-4.5
FGF2	-4.5

Molecular Docking Studies of Identified Glycoside Small Molecule

According to results of GC MS data was considered for the identification of a potent phytochemical small molecule that is methyl glycoside, methyl beta D- xylopyranoside. We have identified top ten hub genes using various artificial intelligence approaches and identified a pathway against myocardial infarction.[14] Molecular docking of methyl beta D-xylopyranoside was done against 10 hub genes CASP3, STAT3, BCL2L1, VEGFA, IL2, HSP90AA1, CASP8, TLR4, FGF2, KDR. Lowest binding affinity was considered for further studies and 2D images are saved Figure 5.

DISCUSSION

Current study is focused on the identification glycoside small molecule from *A. dichotoma* , a plant with traditional significance and potential medicinal properties through spectroscopical analysis and computational approach. Standardization of powder is in the limit prescribed as per WHO guidelines; bulk density, and tapped density of leaf powder indicate good flow properties of powder, while ash values are also in the limit which indicates purity of powder.

The preliminary phytochemical analysis of *A.dichotoma* revealed the presence of various bioactive compounds,

Rationalized extraction was done using different solvents, and in preliminary analysis, we have identified the presence of various phytoconstituents, flavonoids, cardiac glycosides, tannins, and terpenoids. These findings validate the traditional use of *A. dichotoma* and provide a foundation for further investigation.

The application of advanced spectroscopic techniques, such as FTIR analysis, GC-MS, and LC-MS provided valuable insights into the chemical composition of *A. dichotoma* extracts. FTIR analysis revealed the presence of various functional groups, corroborating the presence of specific compounds. Through GC-MS analysis, we have identified numerous small molecules in the extracts, around 20 compounds in ethanolic extract, 30 in methanolic extract and 23 in chloroform extract. This comparative analysis enhances the credibility of the findings and suggests that *A. dichotoma* shares chemical similarities with plants known for their therapeutic effects. LC-MS analysis results identified 10 compounds, which are screened using different artificial intelligence tools, but these compounds exhibited are not meet the criteria of ideal drug molecule. We screened GCMS data and we have identified few phytochemical small molecules out of which methyl beta D-xylopyranoside is a glycoside[14].

Methyl beta-D-xylopyranoside is a glycoside, which means it consists of a sugar molecule (xylopyranose) bonded to another moiety (methyl group) through a glycosidic linkage. Its chemical structure comprises a xylopyranose ring (a type of pyranose sugar ring) connected to a methyl group. The "beta-D" designation indicates the stereochemistry of the glycosidic bond, specifying the orientation of the glycosidic linkage between the sugar and the methyl group[15]. Different artificial intelligence tools were used to identify the pharmacokinetic, toxicity and gene targets of the identified glycoside small molecule along with hub genes. Top 10 hub genes were considered for molecular studies.

Molecular docking studies further deepened the understanding of *A. dichotoma* potential health benefits. By targeting key hub genes associated with myocardial infarction, the study explored the binding affinity of methyl glycoside, methyl beta D-xylopyranoside, a prominent compound in the plant. The molecular simulations provided insights into the potential interactions between the phytochemical and the selected proteins. This approach offers a rational basis for understanding the molecular mechanisms underlying *A. dichotoma* cardioprotective effects. The compound's ability to bind to specific target proteins suggests its potential role in modulating key pathways associated with cardioprotective activity.

CONCLUSION

In conclusion, this study combined traditional knowledge with modern scientific techniques to explore the phytoconstituents and potential small molecules of *A. dichotoma* (Malati) leaves. Advanced spectroscopic methods, helped to identify potent glycoside small molecule of *A. dichotoma* extracts, revealing the presence of numerous potential health benefits. Among the other small molecules, methyl beta D-xylopyranoside, a glycoside derivative, stood out as a promising candidate. Molecular docking studies targeting hub genes associated with myocardial infarction highlighted the compound's potential cardioprotective effects. The binding affinity of methyl beta D-xylopyranoside to key proteins suggests its ability to modulate crucial pathways related to heart health. This research not only contributes to the understanding of *A. dichotoma's* phytochemical profile but also demonstrates the potential of phytochemical small molecules, such as glycosides, as valuable candidates for drug development. Further studies are required to validate the findings and explore the therapeutic applications of methyl beta D-xylopyranoside.

REFERENCES

1. Karunamoorthi K, Jegajeevanram K, Vijayalakshmi J, Mengistie E. Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. Journal of Evidence-Based Complementary & Alternative Medicine. 2013 Jan;18(1):67-74.
2. Maurya D, Adhikari C, Kumar T, Bishwas AJ. A review: *Aganosma dichotoma* (Roth) K. Schum.: Traditional use, phytochemical constituents, and pharmacological properties. International Journal of Botany Studies. 2021 Dec; 6(6):1276-1279.
3. Pandey D, Joshi A, Hemalatha SJ. Quality Control Standardization and In-Vitro Antioxidant Activity of *Aganosma dichotoma* K. Schum Root. Pharmacognosy Journal. 2015;7(1).
4. Bernardo Rincón-López L, Flores-Naveda A, Buenrostro-Figueroa J, Reyes Valdés MH, Martínez-Ávila GC, Rojas R, Tafolla-Arellano JC. FTIR Analysis Reveals Chemical Response during Sorghum (Sorghum bicolor)-Sugarcane Aphid, Melanaphis sacchari, Interaction. Southwestern Entomologist. 2021 Sep 1;46(3).
5. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020 Mar;8(2):603-8.
6. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. Journal of food science and technology. 2015 Feb; 52:1212-7.
7. Liu Y, Grimm M, Dai WT, Hou MC, Xiao ZX, Cao Y. CB-Dock: A web server for cavity detection-guided protein–ligand blind docking. Acta Pharmacologica Sinica. 2020 Jan;41(1):138-44.
8. Stanzione F, Giangreco I, Cole JC. Use of molecular docking computational tools in drug discovery. Progress in Medicinal Chemistry. 2021 Jan 1; 60:273-343.
9. Nandiyanto AB, Oktiani R, Ragadhita R. How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technology. 2019 Mar 7;4(1):97-118.
10. Parasuraman S, Raveendran R, Madhavrao C. GC-MS analysis of leaf extracts of *Cleistanthus collinus* Roxb. (Euphorbiaceae). Int J Pharm Sci 2009;1(2):284-6.
11. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J Pharm Clinical Res 2012;5(2):90-4.
12. Khan A, Usman R, Wang M, Rauf A, Muhammad N, Aman A, Tahir TH. *In vitro* biological screening of the stem of *Desmodium elegans*. Asian Pacific J Tropical Biomedicine 2013;3(9):711-5.
13. El Sayed AM, Basam SM, El-Naggar EM, Marzouk HS, El-Hawary S. LC–MS/MS and GC–MS profiling as well as the antimicrobial effect of leaves of selected Yucca species introduced to Egypt. Scientific reports. 2020 Oct 20;10(1):17778.
14. Kosanam, S., Pasupula, R. Artificial intelligence approach in identification of differentially expressed genes of methyl glycoside against myocardial infarction. ADV TRADIT MED (ADTM) (2023). https://doi.org/10.1007/s13596-023-00691-5.
15. Kytidou K, Artola M, Overkleeft HS, Aerts JM. Plant glycosides and glycosidases: A treasure-trove for therapeutics. Frontiers in Plant Science. 2020 Apr 7; 11:357.