Research Article

Study on the Therapeutic Potential of *Pimenta dioica* Phytocompounds Isolated from Stem Extract and its Analysis through GCMS Profiling

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ABSTRACT

Aim: This study aimed to determine the phytochemical constituents present in the various extracts of *Pimenta dioica* stem through the standard qualitative biochemical and GCMS analysis. **Materials and Methods:** The phytochemical analysis was carried out according to the standard procedures. The total phenol and tannin was estimated using Folin ciocalteau method. The profiling of phytoconstituents present in the ethanolic extract of *Pimenta dioica* was done by Gas Chromatography-Mass Spectrometric (GCMS) analytic technique (Agilent Technologies). **Result:** The Phytochemical screening of *Pimenta dioica* stem extract revealed existence of various phytochemicals. The total phenolic and tannin content of the ethanolic extract was higher than aqueous extract. GCMS profiling indicated the presence of 21 bioactive compounds including Eugenol, 7(1H)-Pteridinone, Phenol, 4-(2-propenyl), 8-Methylnonanoic acid, methyl ester and Benzeneacetonitrile. The eugenol was the key component. **Conclusion:** The phytochemical and GCMS profiling of *Pimenta dioica* stem extract revealed the presence of bioactive constituents with vital therapeutic properties. Hence, isolation of individual phytochemical compounds may lead to the discovery of a novel therapeutic drug.

Keywords: *Pimenta dioica* (Allspice), Phytochemicals, Phenol, Tannin, GCMS

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INTRODUCTION

Plant materials encompass potentially active chemical substances termed phytochemicals that are necessary for typical metabolic processes. Plants contain a diverse range of biologically active substances. Phytochemicals found in spices have long been utilized as flavouring, aromatic and colouring agents in addition to being used medicinally. Numerous in vivo and *in vitro* investigations have hypothesised that dietary spices sustain health via their antioxidative, chemopreventive, antimutagenic, anti-inflammatory and immunological modulatory properties.[1]

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Pimenta dioica is a spice plant of family Myrtaceae and it is a native species of Central America and the West Indies. It is commonly known as the Allspice tree, as it resembles the aromatic flavour of the condiments such as cinnamon, cloves and nutmeg.[2] It is a small evergreen tree that usually grows up to the height of 13 m and its leaves possess pellucid glands which release the peculiar aroma of the spices when it is crushed. It bears small white flowers arranged as a group of cymes. It is cultivated as an ornamental plant across different geographical areas.[3]

The berries, commonly referred to as pimento, they have a rich history of being used as a flavor enhancer in many culinary traditions worldwide. It also plays a pivotal role in an ancient medicinal system to treat bronchitis, menstrual cramps, flatulence, dental aches, muscle aches, diabetes, depression, arthritis, fatigue and viral infections.[4] The other names of *Pimenta dioica* includes Jamaica pepper, Pimento, Pimenta, Pimenta jamaique, Pimenta Gorda, Dulce and English spice.[3]

The essential oil extracted from the leaves and fruits exhibited various pharmacological activities such as antimicrobial, anesthetic, analgesic, antioxidant, antiseptic, acaricidal, carminative, stimulant and muscle relaxant. The leaves are similarly used for culinary purposes like flavouring rice, which is widely practiced in India.[5] In the current study, we have investigated the phytochemicals present in the ethanol extract of the *Pimenta dioica* stem via qualitative biochemical and GCMS analysis.

Taxonomic Classification

Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Pimenta
Species: *P. dioica*

MATERIALS AND METHODS

Plant Identification

The stem sample was collected from in and around the local areas of Mannancherry, Alappuzha, Kerala, India (9º34'46.1"N 76º21'07.3" E) on March 2022. It was identified and verified by Dr. M. U. Sharief, Scientist E and Head, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India (Specimen No. BSI/SRC/5/23/2022). The voucher specimen (SRCAS/BT/H-03) was deposited in the Department of Biotechnology, Sri Ramakrishna College of Arts and Science, Coimbatore, Tamil Nadu, India.

Sample collection and processing

The stems of *Pimenta dioica* were collected and washed with sterile distilled water and it was shade dried for 2 weeks at room temperature and it was pulverized using a mechanical grinder. The powdered stem sample of *Pimenta dioica* was kept in a sealed container for extract preparation.

Preparation of extract

The powdered sample (25 g) was extracted using 250 mL of ethanol and sterile distilled water respectively in a sterile Erlenmeyer flask and it was kept in the orbital shaker for 72 hr at room temperature. After that, it was filtered through Whatman No. 1 filter paper and the resulting filtrate was kept for further investigation.[6,7]

Phytochemical Screening

The preliminary phytochemical analysis of a ethanolic and aqueous extract of *Pimenta dioica* stem was carried out as reported by Pandey and Tripathi (2014) and Shaikh, *et al*. (2021)[8,9] to ascertain the presence of various secondary metabolites such as alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, carbohydrates, diterpenes, phenols, resin, phytosterols, anthraquinones, volatile oils, cardiac glycosides, phlobatannins, lignins and coumarins.

Quantification of phytochemicals

Quantification of phenol

The total phenolic content in the cold derived extract of Pimenta dioica stem were determined using Folin Ciocalteau method. Each test tube was filled with ethanol and aqueous extract at varying concentrations respectively and make up to 4ml using distilled water. Each test tube was added with Folin-Ciocalteau reagent (0.5ml) and then 20 ml of Sodium Carbonate (20%) was added. The test tubes with the reaction mixture were left to remain at room temperature for 3 minutes, they were placed in boiling water bath for a minute. The absorbance was measured spectrophotometrically at the wavelength of 650 nm. The total phenolic content present in the Pimenta dioica stem extract was expressed as mg of gallic acid equivalent per g of dry weight.[10]

Quantification of tannin

Folin's method was performed to assess the total tannin content present in the ethanolic and aqueous extract of Pimenta dioica stem. Various aliquots of extracts were combined with Folin's reagent (0.1ml), and make up to 2 ml using distlled water. The test tubes with reaction mixture were incubated at room temperature for 3 minutes and then 35% sodium carbonate solution before (0.2ml) was added. The test tubes were incubated for another 30 mins at room temperature. The optical density was measure at the wavelength λ=725nm using a UV-visible spectrophotometer.[11]

GCMS analysis

GCMS analysis of ethanolic extract of *Pimenta dioica* was performed using GCMS Agilent Technologies (GC: 8890; MS: 7000D GC/TQ). GCMS capillary column used was HP 5MS Ultra inert (30 mx250 μmx0.25 μm). Helium (UHP Grade) was used as the carrier gas with a flow rate of 1.516 mL/min. The source temperature was 280ºC and the GC oven temperature begins from 80ºC and held for 2 min and it was raised up to 200ºC at the rate of 15ºC/min, with 2 min of holding time. Then, the temperature was increased to 240ºC at the rate of 4ºV/min and the holding minute was 2 min. Finally, Holding was allowed at 280ºC for 2 min with a program rate of 15ºC/min. The total running time was 38 min. The GCMS spectrum of the unknown compound in contrast with the spectrum of the known compound available in the NIST MS search V.2.3: 2017.

RESULTS

Phytochemical Screening

The phytochemical screening of ethanolic extract of *Pimenta dioica* stem exposed the existence of phytoconstituents such as alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, carbohydrates, diterpenes, phenols, phytosterols, anthraquinones, volatile oils, cardiac glycosides, phlobatannins and lignins. The phytochemicals like alkaloids, terpenoids, tannins, saponins, phytosterol, phenol, diterpenes, phlobatannins, carbohydrates and flavonoid were found in the aqueous extract. Table 1 represents the phytochemical screening of the ethanolic and aqueous extract of *Pimenta dioica* stem.

Table 1: Phytochemical screening of *Pimenta dioica* stem extracts.

Sl. No.	Phytochemical	Test	Ethanol	Aqueous
1.	Alkaloids	Meyer's test	++	+
2.	Glycosides	Libermann's test	+	_
3.	Flavonoids	Lead acetate test	++	+
4.	Terpenoids	Salkowski test	++	++
5.	Tannins	Braymer's test	++	+
6.	Saponins	Froth test	+	+
7.	Carbohydrates	Benedict's test	++	+
8.	Diterpenes	Copper acetate test	++	+
9.	Phenols	Ferric chloride test	++	+
10.	Resin	Turbidity test	_	_
11.	Phytosterols	Salkowski's test	++	+
12.	Anthraquinones	Borntrager's test	++	_
13.	Volatile oils	NaOH-HCl test	+	_
14.	Cardiac glycosides	Keller killani test	+	_
15.	Phlobatannins	Precipitate test	++	+
16.	Lignins	Labat test	+	_
17.	Coumarins	NaOH test	_	_

(-): absence of compound; (+): Presence of low compound content. (++): Presence of High compound content.

Quantification of phytochemicals

Quantification of phenol

The linear regression equation (y=0.0096x+0.0617), which was employed as a standard, of gallic acid was utilised to determine the total phenolic compound content found in the extract of *Pimenta dioica* stem. The analysis showed that the ethanolic extract of the *Pimenta dioica* stem had a higher concentration of phenols (0.51 to 1.16 GAE/g of extract), compared to the aqueous extract (0.09 to 0.66 GAE/g of extract) (Figure 1).

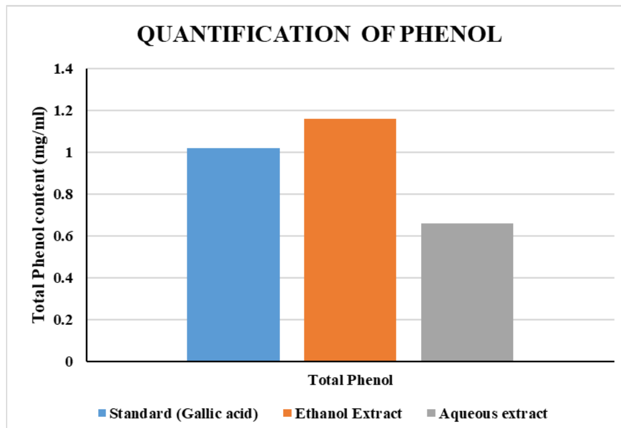


Figure 1: Quantification of Phenol.

Quantification of tannin

Tannic acid was used as a reference to determine the total tannins present in the ethanolic and aqueous extracts of *Pimenta dioica* stems (y=0.0078x+0.0192, r2=0.9978). In comparison to the aqueous extract (0.19 to 1.04 TAE/g of extract), the ethanol extracts (0.43 to 1.37 TAE/g of extract) were found to have higher tannins (Figure 2)

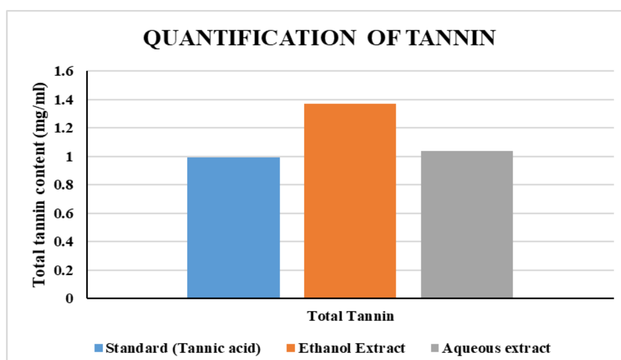


Figure 2: Quantification of Tannin.

GCMS analysis

GCMS profile of ethanolic extract of *Pimenta dioica* stem is shown in Figure 3, which indicated the presence of

21 bioactive compounds. Table 2 represents the list of phytoconstituents and their peak area, retention time, molecular formula and molecular weight, whereas Table 3 shows the structure and pharmacological properties of the detected phytochemicals. Few major bioactive compounds exhibited high peak area in the GCMS analysis are Eugenol, 7(1H)-Pteridinone, Phenol, 4-(2-propenyl), 8-Methylnonanoic acid, methyl ester, Benzeneacetonitrile, 3,4-dimethoxy-, Cyclohexylamine, N, N-dimethyl-1-phenyl-, etc. Acetamide, 2-chloro- was the first bioactive compound identified with less retention time (7.5466) and the longest retention time was 37.0680 acquired by the compound Chloroneb.

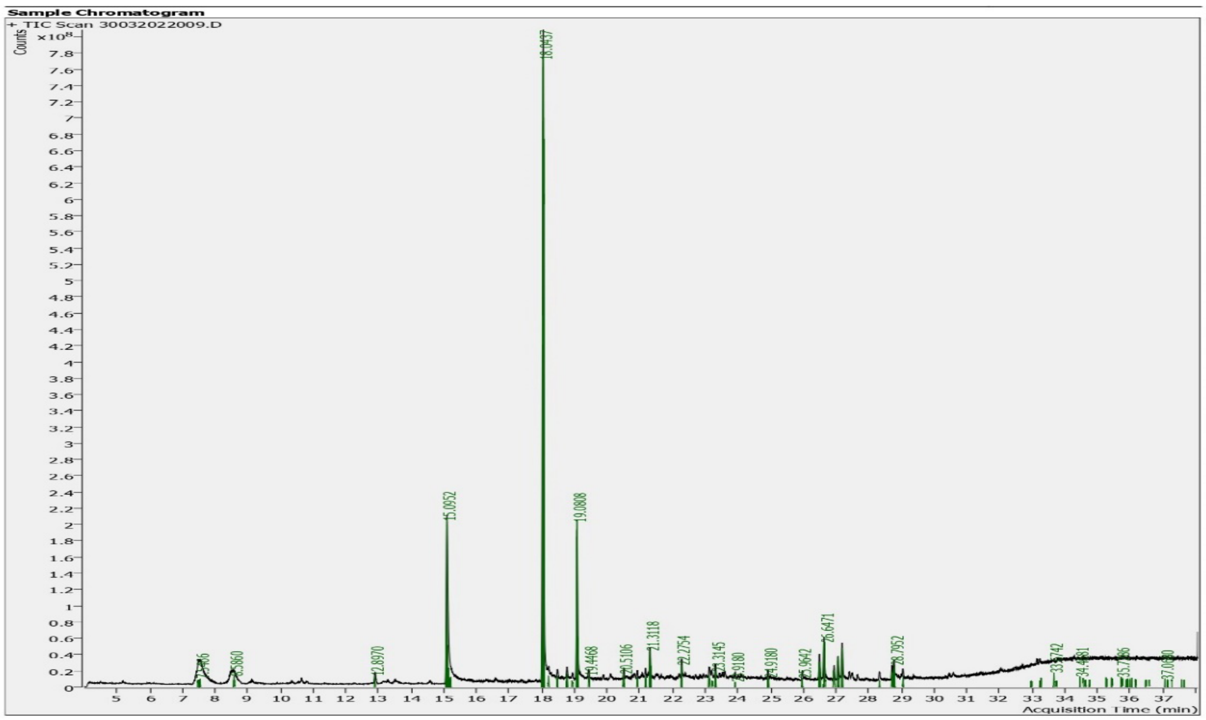


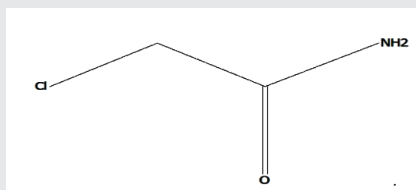
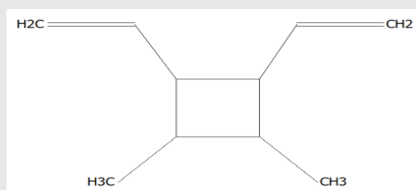
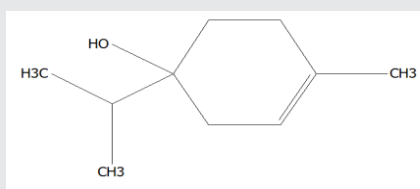
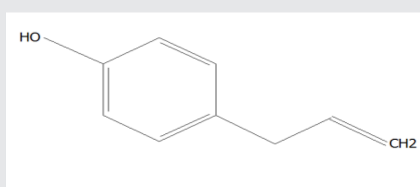
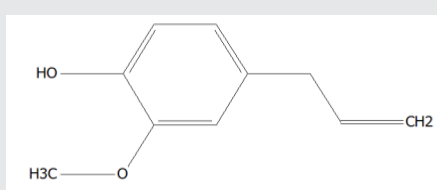
Figure 3: GCMS analysis of ethanolic extract of *Pimenta dioica* stem.

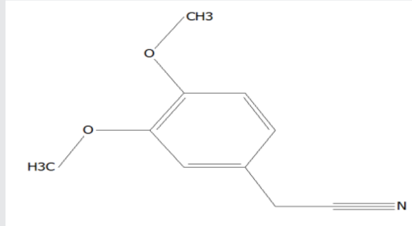
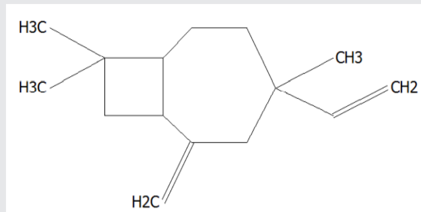
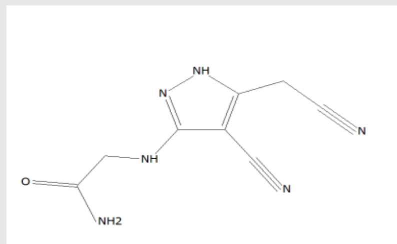
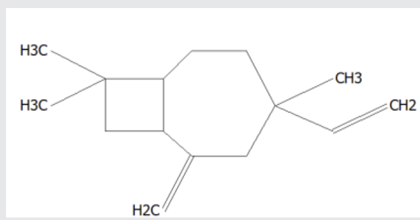
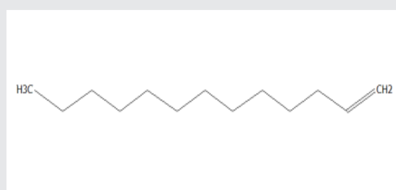
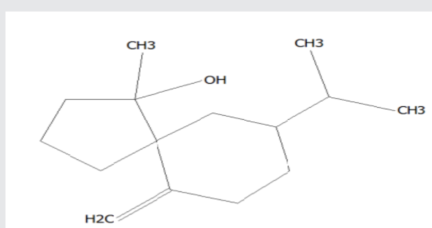
Table 2: List of phytoconstituents identified in the ethanolic extract of *Pimenta dioica* stem by GCMS analysis

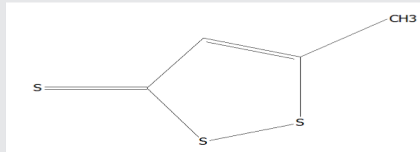
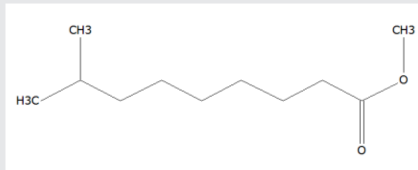
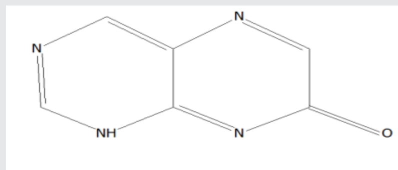
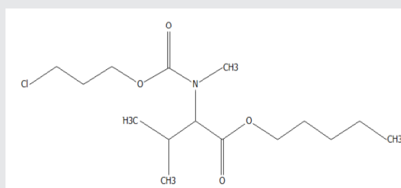
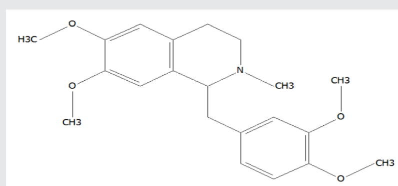
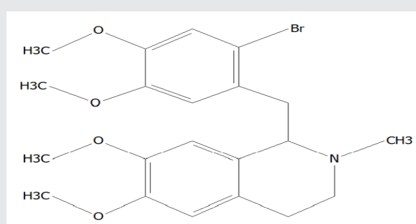
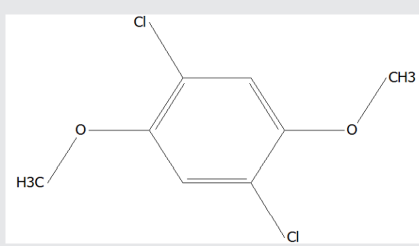
Sl. No.	Compound name	Molecular Formula	Retention time	Peak area (%)	Molecular weight (g/mol)
1.	Acetamide, 2-chloro-	C_2H_4ClNO	7.5466	0.37	93.512
2.	Cyclobutane, 1,2-diethenyl-3,4-dimethyl-	$C_{10}H_{16}$	8.5860	0.81	136.23
3.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,	$C_{10}H_{18}O$	12.8970	0.75	154.2493
4	Phenol, 4-(2-propenyl)-(Chavicol)	$C_9H_{10}O$	15.0952	12.30	134.1751
5	Eugenol	$C_{10}H_{12}O_2$	18.0437	58.02	164.2011
6	Benzeneacetonitrile, 3,4-dimethoxy-	$C_{10}H_{11}NO_2$	19.0808	11.31	177.1998
7	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-	$C_{15}H_{24}$	19.4468	0.97	204.35
8	Acetamide, 2-[[4-cyano-5-(2-nitriloethyl)-1Hpyrazol-3-yl] amino]-	$C_8H_8N_6O$	20.5106	1.05	204.19
9	Cyclohexylamine, N,N-dimethyl-1-phenyl-,	$C_{14}H_{21}N$	21.3118	2.56	203.3232
10	1-Tridecene	$C_{13}H_{26}$	22.2754	1.03	182.3455
11	Spirojatamol	$C_{15}H_{26}O$	23.3145	0.45	222.3663

12	Dodecane, 1-iodo-	$C_{12}H_{25}I$	23.9180	0.23	268.18
13	2-Dodecanol	$C_{12}H_{26}O$	24.9180	0.42	186.33
14	3H-1,2-Dithiole-3-thione, 5-methyl-	$C_4H_4S_3$	25.9642	0.22	148.270
15	8-Methylnonanoic acid, methyl ester	$C_{11}H_{22}O_2$	26.6471	3.87	186.2912
16	7(1H)-Pteridinone	$C_6H_4N_4O$	27.1953	2.60	163.1368
17	Trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	28.7952	0.98	296.5
18	DL-Valine, N-methyl-N-(3-chloropropoxycarbonyl)-,	$C_{15}H_{28}ClNO_4$	33.6742	0.94	321.84012
19	dl-Laudanosine	$C_{21}H_{27}NO_4$	34.4681	0.39	357.44
20	Papaveroline, 2'-Bromo-2-methyl-,tetramethyl(ester)	$C_{21}H_{26}BrNO_4$	35.7286	0.41	436.33944
21	Chloroneb	$C_8H_8Cl_2O_2$	37.0680	0.32	207.054

Table 3: Structure and Pharmacological activities of phytocompounds present in the ethanolic extract of *Pimenta dioica* stem

Sl. No.	Compound name	Structure	Pharmacological activities	Reference
1	Acetamide, 2-chloro-		Antimicrobial Activity.	Katke *et al*., 2011
2	Cyclobutane, 1,2-diethenyl-3,4-dimethyl-		antitumor, microbicidal, antimalarial, Anticoagulant, antioxidant, anti-inflammatory, anti-leishmania and analgesic activity.	Joshi *et al*., 2020
3	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,		Anti-inflammatory, anti-microbial, Analgesic, Laxative and Anti-Depressant activity.	Kumar *et al*., 2022
4	Phenol, 4-(2-propenyl)-(Chavicol)		Antioxidant, antiestrogen, anticonvulsant and neuroprotective activity.	Kushwaha *et al*., 2019
5	Eugenol		Anti-cancer, antioxidant, antidiabetic, antimicrobial, anti-inflammatory activity.	Nisar *et al*., 2021

6	Benzeneacetonitrile, 3,4-dimethoxy-		Cytotoxic activity.	Tawfik *et al*., 2017
7	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-		Anti-inflammatory, antioxidant antihyperlipidemic and antimicrobial	Prakasia *et al*., 2015
8	Acetamide, 2-[[4-cyano-5-(2-nitriloethyl)-1Hpyrazol-3-yl]amino]-		Not reported.	Zukin and Zukin (1979)
9	Cyclohexylamine, N,N-dimethyl-1-phenyl-,		Binds in the rat central nervous system (reducing PCP binding to rat brain homogenates).	Arrabal *et al*., 2011
10	1-Tridecene		Sedative, hypotensive, anti-inflammatory,	Kalpesh *et al*., 2017
11	Spirojatamol		anti-stress, CNS depressant and analgesic activity.	
12	Dodecane, 1-iodo-		Antineoplastic, Testosterone 17beta-dehydrogenase (NADP+) inhibitor, Antineoplastic activity.	Kumaresan *et al*., 2015
13	2-Dodecanol		Antimicrobial activity.	Fatima *et al*., 2017

14	3H-1,2-Dithiole-3-thione, 5-methyl-		Not reported.	
15	8-Methylnonanoic acid, methyl ester		Antimicrobial activity.	Sahin *et al*., 2015
16	7(1H)-Pteridinone		Anti-proliferative and Anti-tumour activity.	Hou *et al*., 2019
17	Trans-13-Octadecenoic acid, methyl ester		Antimicrobial, antioxidant and anti-inflammatory activity.	Abdullah *et al*., 2020
18	DL-Valine, N-methyl-N-(3-chloropropoxycarbonyl)-,		Not reported.	
19	dl-Laudanosine		Antimicrobial activity.	Díaz *et al*., 2019
20	Papaveroline, 2'-Bromo-2-methyl-,		Anti-inflammatory activity.	Selvi and Bhaskar, 2012
21	Chloroneb		Anti-fungal activity.	Knudsen *et al*., 2011

DISCUSSION

Pimenta dioica has remained using for culinary and medicinal purposes since ancient days. The essential oil extracted from various portions of *Pimenta dioica* was reported with antimicrobial activity, cytotoxic activity, antioxidant activity, anti-inflammatory and anti-quorum sensing activity[12-15]

For our part of familiarity, this report is the first description on the GCMS profiling of ethanolic extract of *Pimenta dioica* stem. In the contemporary study, the phytoconstituents present in the ethanolic extract were alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, carbohydrates, diterpenes, phenols, phytosterols, anthraquinones, volatile oils, cardiac glycosides, phlobatannins and lignins. Conclusively, the *Pimenta dioica* phytocompounds extracted were wide-ranging conferring to the type of solvent used and the solubility of the solvent employed. These findings stood align with Swamy *et al*. used different solvents for leaves of *Plectranthus amboinicus*.[16] However, the aqueous extract contained several diverse phytochemicals, with alkaloids, terpenoids, tannins, saponins, phytosterol, phenol, diterpenes and phlobatannins, as well as carbohydrates and flavonoids. The innumerable phytochemicals present in the *Pimenta dioica* stem will subsidize to the Plethora of biological actions. The phytochemical screening of acetone, aqueous and ethanolic extract of *Pimenta dioica* stem showed the existence of alkaloids, phenols, glycosides, flavonoids, terpenoids, tannins, steroids, phytosteroids, fats, gum and mucilage.[17] The *Pimenta dioica* bark ethanol extract possess further secondary metabolites than *Pimenta dioica* leaf extract.[18] The findings of Murali *et al*., 2021,[19] conveyed the presence of alkaloids, flavonoids, phenol, terpenoids, steroid and carbohydrate in the ethanol extract of *Pimenta dioica* leaves. Correspondingly, *Pimenta racemosa* leaves extracted with isopropanolic solvent comprised triterpenoids, reducing sugar, terpenoids, steroids and cardiac glycosides.[20] These bioactive substances may offer novel perspectives for in-depth inquiries intended at assessing the mechanisms of action, which are essential in paving the path for clinical trials.[21]

The existing study revealed that the cold derived ethanolic extract of *Pimenta dioica* stem holds a greater number of phytochemicals than the cold derived aqueous extract. Equally, the total phenolic and tannin content of ethanolic extract were substantial than the aqueous extract. However, a study found that the total phenolic content of aqueous extract of *Pimenta dioica* leaves was 0.0524 mg/g gallic acid equivalent.[22] The total phenolic content of *Pimenta dioica* stem was also observed by the Manorama and Sindhu.[17] According to reports, tannins such pedunculagin, 4,6-(S)-hexahydroxydiphenoyl-(/)-D-glucopyranose, nilocitin and casuarinin are present in the leaves of *Pimenta dioica*.[23]

GCMS profiling of ethanolic extract of *Pimenta dioica* stem displayed the existence of compounds such as Eugenol, 7(1H)-Pteridinone, Phenol, 4-(2-propenyl), 8-Methylnonanoic acid, methyl ester, Benzeneacetonitrile 3,4-dimethoxy-, Cyclohexylamine, N, N-dimethyl-1-phenyl-, etc. The major phytoconstituent is found to be Eugenol followed by Benzeneacetonitrile 3,4-dimethoxy and Phenol, 4-(2-propenyl)-. The essential oil prepared from the *Pimenta dioica* leaves also encompasses the highest amount of eugenol and revealed potent antioxidant properties.[24] Similarly, the study of Rajalekshmy and Manimekalai[25] also concluded that the eugenol was the major compound recognized by the GCMS analysis of essential oil extracted from *Pimenta dioica* leaves. Besides all, the literature survey on eugenol described the therapeutic potentials such as analgesic activity, antioxidant effect, antimicrobial activity, anticonvulsant activity, anti-inflammatory action and anti-cancer activity.[26] Essential oils and methanol extracts from *Pimenta racemosa* (bay rum) were explored by researchers. A comprehensive phytochemical profiling was carried out, including GC-MS analysis of essential oils and HPLC-PDA-ESI-MS/MS analysis of methanol extracts. Eugenol was the primary component found in both essential oil leaves and stems.[27] Controversially, the GLC-MS analysis of essential oil made from *Pimenta racemosa* flower exposed 1,8-cineole as the leading component.[28]

The phytocomponent Phenol, 4-(2-propenyl)- is also known as Chavicol.[29] The existence of chavicol in the essential oil extracted from the stem and leaves of *Pimenta dioica* was identified by gas chromatographic analysis coupled with a mass spectrometer.[30] The work of Everton *et al*.,[31] confirmed the appearance of eugenol and chavicol in the essential oil mined from the dried leaves of *Pimenta dioica*. The existing study showed the presence of phytoconstituents such as Dodecane, 1-iodo and 2-Dodecanol, whereas, the study of Silva *et al*.,[32] disclosed that the ethanol extract of *Pimenta dioica* leaves contains 10-dodecyn-1-ol. Interestingly, this study also exposed the presence of phytocompound trans-13-octadecenoic acid methyl ester, which was found in the ethyl acetate extract of *Terminalia catappa* and it exhibited substantial activity against osmotic stress in the sickle erythrocytes.[33] Remarkably, Rajalekshmy and Manimekalai relied gas chromatography-mass spectrometry (GC-MS) to compare the main phytochemicals found in the leaves of *Syzygium aromaticum* (clove) and *Pimenta dioica*. This comparison contextualizes the chemical composition of certain spices.[25]

The current study discovered the presence of the phytocompound 1-Tridecene in the *Pimenta dioica*. The 1-Tridecene is a pheromone secreted by the insectivore invertebrates[34] and this compound is rarely found in vegetable species. But, the existence of a high concentration of 1-Tricedene was reported in the essential oil of *Senecio coincyi* and this compound fascinates the insects for the transfer of pollen grains, which ultimately contributes to the ecological function.[35] Hence, the 1-Tridecene present in the *Pimenta dioica* may also act as attractants to facilitate the entomophily.

The pharmacological action for the bioactive compounds such as DL-Valine, N-methyl-N-(3-chloropropoxycarbonyl)-, 3H-1,2-Dithiole-3-thione, 5-methyl- and Acetamide, 2-[[4-cyano-5-(2-nitriloethyl)-1Hpyrazol-3-yl] amino]-, present in the ethanolic extract of *Pimenta dioica* stem are not reported. Therefore, the results from our research aligns with further studies, emphasizing the *Pimenta dioica* compounds and their potential therapeutic effects. The occurrence of eugenol and other phytoconstituents supports the validity of our results.

CONCLUSION

The present study demonstrated that the *Pimenta dioica* stem extract possesses diverse secondary metabolites which contribute to innumerable therapeutic properties. The existence of phytochemicals was found to be significant in ethanolic extract of *Pimenta dioica* stem than the aqueous extract. The GCMS analysis displayed the presence of 21 bioactive compounds and subsequently the *Pimenta dioica* would show a vibrant role in treating numerous diseases. The GCMS-identified phytoconstituents are found to be reasonably responsible for the enormous therapeutic benefits of *Pimenta dioica* in several health ailments. Hence, these findings and results could aid in evaluating the pharmacological actions of *Pimenta dioica* in wider aspects. Moreover, this research paper may serve as valuable evidence for the development of novel phytotherapeutic drugs from the *Pimenta dioica*. But, still a greater number of research studies are in demand to confirm the medicinal properties of *Pimenta dioica*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

The authors contributed equally for concept making, data acquiring, investigating and writing the manuscript. All authors have read and approved the manuscript

ABBREVIATIONS

GCMS: Gas Chromatography-Mass Spectrometry; **NaOH:** Sodium Hydroxide; **HCl:** Hydrochloric acid; **H2SO4:** Sulphuric acid; **FeCl3:** Ferric chloride; **GAE:** Gallic Acid Equivalent; **TAE:** Tannic Acid Equivalent.

SUMMARY

The current study used standard qualitative biochemical and GCMS analysis to investigate the phytochemical substances found in different extracts of *Pimenta dioica* stem. Phytochemical analysis of *Pimenta dioica* stem extract demonstrated the presence of many phytochemicals. The ethanolic extract had a greater total phenolic and tannin content than the aqueous extract. 21 bioactive compounds were identified in the GCMS profiling. Hence, further in-vitro and in-vivo studies have to be done to determine the pharmacological efficacy of *Pimenta dioica* stem extract.

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