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Original Article

Headspace SPME-GC-MS Analysis and *in silico* Molecular Docking Studies of Phytochemical Compounds Present in *Houttuynia cordata* Thunb

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ABSTRACT

Aim: The study aimed to identify the phytochemical constituents present in the leaves, stems, and roots of *H. cordata* and perform *in silico* molecular docking studies of selected common compounds present in all three parts. **Materials and Methods:** The phytochemical components were investigated using the headspace solid-phase micro-extraction followed by gas chromatography-mass spectrometry and molecular docking was performed using Autodock Vina v.1.2.0. **Results:** β-pinene was found to be the major compound present in stem (73.89%), leaves (66.46%) and roots (42.88%). Four category targets were used for *in silico* molecular docking of 14 common compounds found in leaves, roots, and stems. The present study showed that the compounds caryophyllene and dihydro-cis-alpha-copaene-8-ol had antibacterial, antioxidant, anti-cancer, and anti-inflammatory activities. **Conclusion:** This work demonstrated the great utility of headspace SPME-GC-MS for the investigation of aromatic chemicals in a variety of edible and medicinal spices. With *in silico* molecular docking, we may look into the potential pharmacological activity of various volatile organic compounds present in *H. cordata*.

Keywords: *Houttuynia cordata*, Headspace SPME-GC-MS, *in silico* molecular docking, β-pinene, Caryophyllene.

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INTRODUCTION

Indigenous foods not only have a distinct flavor and taste of their own, but they also offer dietary variety and ensure the security of the household's food supply. The diets of the northeastern peoples of India are primarily composed of natural and traditional foods. *H. cordata*, locally referred to as "Toningkhok," is one such native food plant of Manipur. *H. cordata* is a perennial, aromatic herb belonging to the family Saururaceae, a fragrant medicinal plant with spreading rootstock. The Manipuri people use the herb both raw and cooked, and the local healer (Maiba) uses it to treat anaemia, gastritis, and tuberculosis as well as dysentery, muscular sprains, and stomach ulcers. Alkaloids, essential oils, flavonoids, and other chemical components with distinctive therapeutic effects are among the chemical ingredients found in *H. cordata*. In Manipur's plain and hill districts, the plant grows wild and is harvested for market sale. In Meghalaya, it is used in salads or cooked with other vegetables.[1] Leaf juices are used to cure cholera, dysentery, treat blood deficiencies, and blood purification.[2] Naga tribe of Kiphire District, Nagaland uses the whole plant to cure stomach ache, cholera, and dysentery and as diuretic. It is also applied to skin diseases.[3] Apatani Tribe in Arunachal Pradesh used the shoot of *H. cordata* for freshness, good sleep, and heart disorders.[4] In the Senapati district of Manipur, roots and leaves are used to cure measles, gonorrhea, and skin troubles.[5] In Southeast and East Asian countries, it is frequently used as an herbal anti-inflammatory, antibacterial, antiviral, and anti-cancer medicine. *H. cordata* has the potential for both antiviral activity and antiviral consequences, which is relevant given the recent COVID-19 pandemic reported by Lau *et al.* (2008) during the SARS outbreak of 2002-2003.[6] Recently, several studies have also produced scientific evidence to support and reveal its anti-inflammatory, anti-allergic, virucidal, anti-oxidative and anti-cancer activities.[7-12] Due to its quick and ease of use, Headspace SPME is useful in the identification of plant volatile profiles. In addition, it acts as a tool to distinguish the phytochemicals present in different parts of the plant of the same species or different species.

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MATERIALS AND METHODS

Sample Collection and Preparation

The fresh portions of *H. cordata* were collected in September 2022 from Takyelpat, Imphal West District, Manipur. The plant was identified and authenticated by Botanical Survey of India, Sikkim Himalayan Regional Centre with voucher number IBSD-SC/EPS/2020/IP/123. The authenticated herbarium was submitted in the IBSD Herbarium Library. The three parts of *H. cordata* viz., fresh leaves, roots, and stems were packed in a 15 mL clear vial which has a screw-top hole cap with silicone septa. All the vials were filled with one-third portions of the sample. The roots and stems were cut into identical lengths of 2 cm.

Headspace SPME-GC-MS

The SPME fiber was conditioned in the GC-MS and confirmed with no impurities through the blank run. The 15 mL glass vial containing the sample was exposed to the SPME fiber for 20 min. Each sample after the exposure was injected into the inlet injector of the GC-MS instrument for 2 min. Trace 1300 GC fitted with TG-5MS column and mass detector (TSQ DUO) with triple Quadropole was used to study the volatile organic compounds. Helium was used as the carrier gas, with a flow rate of 1 mL/min, and a split ratio of 1:20 was maintained. The initial column temperature was programmed from 40°C for 1 min to 250°C at a rate of 5°C per min, and then to 250°C for 20 min.[13,14] The relative VOC's constituents were expressed by its peak area percentage. Based on the comparison of mass spectra with those of the 2017 National Institute of Standards and Technology (NIST) GC-MS Libraries the volatile compounds were identified.

3D structure retrieval of protein targets

The Anti-Microbial (AM), Anti-Oxidant (AO), Anti-Cancer (AC), and Anti-Inflammatory (AI) targets were selected based on their prominence in the literature and their 3D structure was retrieved from Protein Data Bank (https://www.rcsb.org/).

3D ligand structure retrieval

The structure of 1,7-Octadiene, 2-methyl-6-methylene was downloaded from NIST Chemistry WebBook (https://webbook.nist.gov).[15] All the other ligand structures were downloaded from PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov/).[16]

Protein and Ligand preparation

Auto Dock MGL Tools v.1.5.7 was used for the protein preparation step.[17] The identical chains, the native co-crystallized ligands, and the water molecules were deleted at first, the protein structure was added with polar hydrogen atoms and the Gasteiger charges were introduced. For the preparation of ligand molecules Chimera v.1.1.6 software was utilized, the ligands were protonated and the charges were added.[18] For the file format conversions Openbabel tool was used.[19]

Binding site prediction

The binding sites of Antimicrobial and Antioxidant targets were comprehensively documented in previous research were used.[20] For Anticancer and Anti-inflammatory targets, the binding site grid coordinates were predicted by using BIOVIA Discovery Studio, at first the co-crystallised ligands were selected and the "Define and Edit Binding Site" option was used.[21]

Molecular docking and analysis

Autodock Vina v.1.2.0 which uses the Lamarckian algorithm was used for performing molecular docking.[22] The native co-crystallized ligands and the short-listed phytochemical compounds were docked individually using the command line. For saving the docked ligand and protein complexes Pymol software was used.[23] The images of active pocket visualization and 2D amino acid interaction between the ligand-protein complexes were obtained from BIOVIA Discovery Studio.

RESULTS

Phytochemical analysis using Headspace SPME-GC-MS

A total of 36, 30, and 25 compounds were identified in the fresh leaves (97.9%), roots (97.55%), and stems (98.30%) of *H. cordata* respectively (Tables 1, 2, and 3). The GCMS Chromatogram of all three parts of *H. cordata* is shown in Figure 1. The major compounds present in the fresh leaves of *H. cordata* were β-pinene (66.46%), Leaf alcohol (6.05%), β-Sabinene (2.36%), Caryophyllene (2.21%), Camphene (2.21%), ç-Terpinene (1.91%), o-Cymene (1.81%), 2-Norpinene, 3,6,6-trimethyl- (1.8%), 1,7-Octadiene 2-methyl-6-methylene (1.4%), Cyclohexene, 1-methyl-4-(1-methylethylidene)-(1.36%). For fresh roots of *H. cordata*, the dominant compounds were β-pinene (42.88%), m-Mentha-6,8-diene, (R)-(+)- (12.88%), p-Mentha-1(7),3-diene (8.04%), à-Pinene (7.06%), Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(4.82%), o-Cymene (3.27%), ç-Terpinene (2.81%), Cyclohexene, 1,5,5-trimethyl-3-methylene (2.5%), 2-Thujene (2.45%), p-Mentha-1,4(8)-diene (2.14%), Camphene (1.29%), 1,3-Cyclohexadiene, 1,3,5,5-tetramethyl (1.1%) and Caryophyllene (1.07%). Other major compounds found in stem are Sabinene (3.61%), D-sylvestrene (2.47%), β-Terpinene (2.45%), Caryophyllene (1.92%), Bornyl acetate (1.91%), β-Thujene (0.95%), Camphene (0.94%) and o-Cymene (0.91%). The volatile organic compounds present in the leaves, roots, and stems were predominantly monoterpenoids. Monoterpenes have antimicrobial, anti-inflammatory, antioxidant, antipruritic, hypotensive, and analgesic pharmacological properties. Terpenoids are significant for more reasons than just their aroma; they are also linked to anti-insect action and positive aspects of human health. For *in silico* studies, 14 active compounds prevalent in all the three samples i.e. leaves, roots and stem were chosen and further used for *in silico* studies.

Molecular docking analysis

The Binding site grid coordinates used for molecular docking is listed in Table 4. The molecular docking of 14 phytochemical compounds with various targets was performed. The binding energies of all the compounds are represented as heat map as shown in Figure 2. The compounds Caryophyllene and dihydro-cis-alpha-copaene-8-ol were highlighted exclusively in this study as both of them had binding affinity to a higher number of targets compared to other compounds. Table 5 contains the binding energies and amino acid interactions of phytochemical compounds Caryophyllene and dihydro-cis-alpha-copaene-8-ol against various targets.

The compounds caryophyllene and dihydro-cis-alpha-copaene-8-ol showed high binding affinity of -7.7 and -7.8 kcal/mol against the antimicrobial target dihydrofolate reductase of S. aureus (PDB-ID: 3SRW). The enzyme Dihydrofolate Reductase (DHFR) is responsible for the NADPH-dependent conversion of dihydrofolate to tetrahydrofolate.[24] Tetrahydrofolate is essential for several biosynthetic pathways, including amino acid and nucleic acid metabolism.[25] DHFR inhibitors are effective for treating bacterial, mycobacterial, fungal, and protozoal infections hence we chose DHFR as an anti-microbial target.[25] Caryophyllene interacted with the target DHFR with 9 van der Waals interactions and 9 Pi interactions. dihydro-cis-alpha-copaene-8-ol interacted with 1 hydrogen bond, 4 van der Waals interactions, and 10 pi interactions. The amino acid interactions of the compounds to the anti-microbial target 3SRW are shown in Figure 3.

Both compounds showed high binding affinities of -7.4 and -7.1 kcal/mol against the antioxidant target cytochrome P450 CYP2C9 (PDBID: 1OG5). Cytochrome P450 enzymes produce reactive oxygen species which provide oxidative stress, inhibiting this enzyme produces an antioxidant effect.[26,27] Caryophyllene interacted with the target CYP2C9 with 4 van der Waals interactions and 10 Pi interactions. dihy-dro-cis-alpha-copaene-8-ol interacted with 1 hydrogen bond, 11 van der Waals interactions, and 10 pi interactions. The amino acid interactions of the compounds to the antioxidant target 1OG5 are shown in Figure 4.

The compounds showed higher binding affinities of -8.5 and -8 kcal/mol against the anticancer target Human Estrogen Receptor Alpha (PDBID: 3ERT) than other anticancer targets. The Estrogen Receptor alpha (ERα) is known to play an important role in cell proliferation in Breast cancer hence we used ERα as a potential anti-cancer target.[28,29] Caryophyllene inter-acted with the target 3ERT with 7 van der Waals interactions and 8 Pi interactions. dihydro-cis-alpha-copaene-8-ol interacted with 6 van der Waals interactions and 9 pi interactions. The amino acid interactions of the compounds to the anticancer target 3ERT are shown in Figure 5 (A).

Though the compounds were having binding affinity to other anti-inflammatory targets, TNF-alpha was particularly chosen due to its extensive usage in the literature as an anti-inflammatory

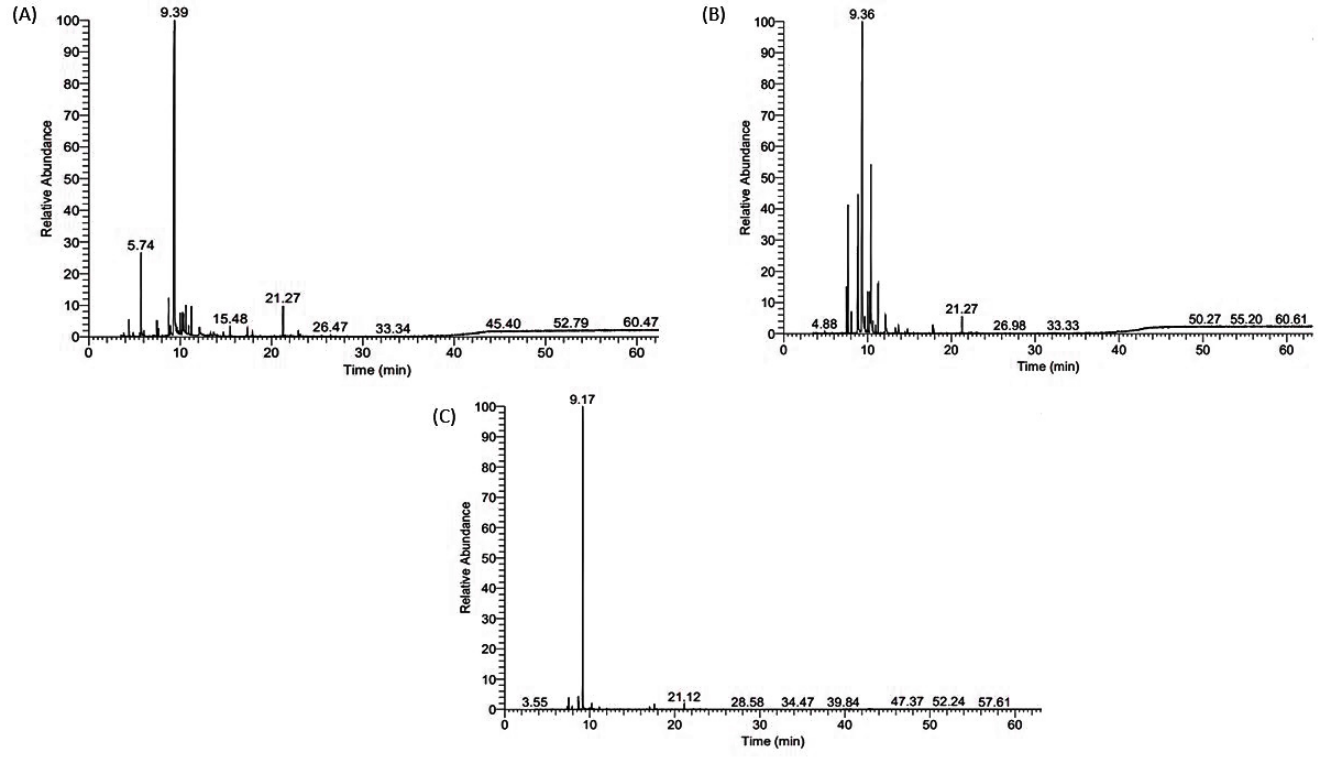

Figure 1: (A) Chromatogram of Headspace SPME-GC-MS *H. cordata* leaves, (B) Chromatogram of Headspace SPME-GC-MS *H. cordata* roots. (C) Chromatogram of Headspace SPME-GC-MS *H. cordata* stem.

Table 1: Major volatile organic compounds present in *Houttuynia cordata* leaves.

Sl. No.	RT[a]	Compound	RSI[b]	RA[c] %	Mol. Wt
1	9.38	β-pinene	894	66.46	136
2	5.73	Leaf alcohol	911	6.05	100
3	8.76	β-Sabinene	944	2.36	136
4	21.27	Caryophyllene	941	2.21	204
5	10.36	Camphene	872	2.21	136
6	11.25	ç-Terpinene	905	1.91	136
7	10.25	o-Cymene	920	1.81	134
8	10.64	2-Norpinene, 3,6,6-trimethyl-	897	1.80	136
9	8.93	1,7-Octadiene, 2-methyl-6-methylene	885	1.40	136
10	10.01	p-Menth-4(8)-ene	926	1.36	136
11	12.12	(+)-4-Carene	885	1.00	136
12	7.46	β-Thujene	931	0.94	136
13	15.48	Decanal	878	0.75	156
14	10.94	á-Ocimene	908	0.72	136
15	13.35	2,6-Dimethyl-1,3,5,7-octatetrae ne, E,E	909	0.67	134
16	13.68	Alloocimene	947	0.65	136
17	17.36	1-Decanol	928	0.63	158
18	22.94	[1,4]Dioxino[2,3-b]-1,4-dioxin, hexahydro-2,2,3,3-tetramethyl	826	0.52	202
19	7.64	3-Carene	922	0.49	136
20	17.92	gamma-Terpinene diepoxide	928	0.47	168
21	8.84	3-p-Menthene	931	0.41	136
22	6.04	Cyclopropane, propyl-	879	0.36	84
23	9.67	α-Thujene	913	0.34	136
24	23.15	1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1aà,3aà,7aá,7bà)]-	892	0.32	204
25	14.74	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	895	0.30	154
26	5.62	2-Hexenal	909	0.28	98
27	9.80	(Z),(Z)-2,4-Hexadiene	861	0.26	82
28	10.09	Cyclopropane, 1-ethenyl-2-hexenyl-, [1à,2á(E)]-(ñ)-	849	0.25	150
29	22.12	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexad ien-1-yl]-1-methyl	893	0.15	204
30	7.10	Furan, 2-ethyl-	882	0.15	96
31	26.47	2-Hydroxy-1-(1'-pyrrolidiyl)-1- buten-3-one	799	0.14	155
32	8.48	3,5-Dimethylamphetamine	889	0.13	163
33	13.10	2,6-Dimethyl-1,3,5,7-octatetrae ne, E,E	902	0.11	134
34	14.04	2-Hydroxymandelic acid, ethyl ester, di-TMS	880	0.10	340
35	25.49	2,2,4-Trimethyl-1,3-pentanedio l diisobutyrate	862	0.10	286
36	21.10	Dihydro-cis-à-copaene-8-ol	905	0.09	222
		Total area %		97.9	

RT[a]: Retention time; RSI[b]: Reversed search index on TG-5MS capillary column, RA[c]%: Relative area (peak area relative to the total peak area).

Table 2: Major volatile organic compounds present in *Houttuynia cordata* roots.

Sl. No.	RT[a]	Compound	RSI[b]	RA[c] %	Mol.wt
1	9.36	β-pinene	884	42.88	136
2	10.39	D-sylvestrene	880	12.88	136
3	8.87	p-Mentha-1(7),3-diene	925	8.04	136
4	7.66	alpha-Pinene	929	7.06	136
5	8.78	Sabinene	945	4.82	136
6	10.27	o-Cymene	919	3.27	134
7	11.26	ç-Terpinene	900	2.81	136
8	10.02	Cyclohexene, 1,5,5-trimethyl-3-methylene	894	2.5	136
9	7.48	2-Thujene	921	2.45	136
10	12.13	p-Mentha-1,4(8)-diene	937	2.14	136
11	8.06	Camphene	944	1.29	136
12	13.67	1,3-Cyclohexadiene, 1,3,5,5-tetramethyl	921	1.1	136
13	21.27	Caryophyllene	942	1.07	204
14	13.31	Alloocimene	931	0.86	136
15	9.67	3-Thujene	900	0.79	136
16	8.94	1,7-Octadiene, 2-methyl-6-methylene	874	0.75	136
17	10.65	2-Norpinene, 3,6,6-trimethyl	900	0.67	136
18	11.26	á-Ocimene	935	0.64	136
19	17.78	Bornyl acetate	932	0.51	196
20	14.75	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	897	0.32	154
21	17.91	4,8-Dioxatricyclo[5.1.0.0(3,5)] octane, 1-methyl-5-(1-methylethyl)-, (1à,3á,5á,7à)-	906	0.27	168
22	9.59	2,6-Dimethyl-2-trans-6-octadiene	844	0.15	138
23	11.58	2-Cyclohexen-1-ol, 4-ethyl-1,4-dimethyl	854	0.15	154
24	14.44	Borneol	917	0.13	154
25	9.84	1-Propanone, 1-(1-cyclohexen-1-yl)-	905	0.11	138
		Total Area %		97.55	

RT[a]: Retention time; RSI[b]: Reversed search index on TG-5MS capillary column, RA[c]%: Relative area (peak area relative to the total peak area).

List of shortlisted compounds	Anti-microbial targets					Anti-oxidant targets				Anticancer targets					Anti-inflammatory targets				
	1JZQ	1KZN	2ZDQ	3SRW	3TTZ	1N8Q	1OG5	2CDU	3NRZ	3ERT	1DI8	1M17	3OG7	6JOK	4O1Z	5KIT	3w5e	5KX7	2AZ5
Co-crystalised ligands	-8.9	-8.5	-7	-9.7	-7.7	-5.6	-9.4	-8	-6.1	-10	-8.8	-6.3	-10.1	-7.4	-8.2	-8.8	-11.3	-7	-9.2
1,7-Octadiene, 2-methyl-6-methylene	-5.3	-5.1	-5.1	-2.3	-5.1	-5.6	-5.3	-4.4	-6	-5	-5.5	-4.2	-5.3	-5.7	-5.5	-5.7	-6.2	-5.8	-4.8
á-Ocimene	-5.2	-5.2	-5.3	-5	-5.2	-5.3	-5.3	-4.5	-6.2	-5.3	-6	-4.6	-5.6	-5.9	-5.8	-5.9	-6.5	-6	-5
Alloocimene	-5.5	-5.5	-5.3	-5.3	-5.6	-5.2	-5.7	-4.7	-6.3	-5.4	-6.3	-4.9	-5.8	-5.8	-5.8	-5.9	-6.8	-5.8	-5.2
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	-5.9	-6.1	-6	-5.9	-5.4	-5.8	-5.9	-4.4	-4.4	-6	-6.5	-5.2	-6.1	-5.7	-6	-5.8	-6.4	-6.4	-5.6
β-pinene	-5.3	-4.6	-4.9	-5.5	-4.9	-4.9	-5.6	-3.4	-1.1	-6.6	-5.9	-5.2	-5.7	-5.6	-6.1	-5.4	-6	-5.8	-5.6
Caryophyllene	-6.5	-5.6	-5.9	-7.7	-6	-2.1	-7.4	-4.3	2	-8.5	-7.7	-6.9	-7.4	-5.9	-7.9	-5	-7.8	-6.6	-7
Camphene	-5.2	-4.7	-4.7	-5.5	-4.7	-3.7	-5.6	-3.3	0.3	-6.4	-5.9	-5.1	-5.8	-5.4	-6.8	-5.4	-6.5	-5.6	-5.5
ç-Terpinene	-5.5	-5.8	-5.5	-5.6	-5.7	-5.6	-6.1	-3.9	-6.8	-5.8	-6.7	-5.3	-6.2	-6.3	-6.2	-6.5	-7.1	-6.5	-5.5
o-Cymene	-5.1	-5.5	-5.3	-5.5	-5.3	-5.9	-5.8	-3.4	-7	-5.8	-6.3	-5.3	-6.2	-6.4	-6.4	-6.5	-7	-6.1	-5.4
β-Thujene	-5	-5	-5.1	-5.4	-5	-5.1	-5.4	-5	-3.5	-5.8	-6.1	-5.1	-6.2	-6.5	-6	-6	-6.3	-6.1	-5.5
2,6-Dimethyl-1,3,5,7-octatetrae ne, E,E	-5.5	-5.5	-5.3	-5.2	-5.7	-5.5	-5.8	-4.6	-6.3	-5.5	-6.4	-4.9	-5.8	-6.1	-5.7	-6	-6.8	-5.7	-5.3
Dihydro-cis-à-copaene-8-ol	-6.7	-6.2	-4.6	-7.8	-6.8	-0.1	-7.1	-4.2	2	-8	-7.7	-6.8	-7.6	-5.2	-7.2	-2.3	-7.5	-6.9	-7.8
D-sylvestrene	-5.2	-5.9	-5.4	-5.7	-5.6	-4.8	-6	-4.2	-6.7	-5.9	-6.5	-5.3	-6.2	-6.5	-6.3	-6.7	-7.3	-6	-5.7
Bornyl acetate	-5.8	-5.2	-5.5	-6.5	-5.6	-1	-6.1	-3.9	0.8	-6.7	-6.2	-5.3	-5.5	-4.9	-6.8	-3.5	-5.8	-4.7	-6.5

Figure 2: Heat-map representation of binding energies (in kcal/mol) of Phytochemical compounds against various targets.

Table 3: Major volatile organic compounds present in *Houttuynia cordata* stem.

Sl. No.	RT[a]	Compound name	RSI[b]	RA[c] %	Mol. Wt
1	9.17	β-pinene	905	73.89	136
2	8.64	β-Terpinene	930	3.61	136
3	7.52	alpha-pinene	933	3.48	136
4	10.23	D-sylvestrene	882	2.47	136
5	8.72	β-Terpinene	939	2.45	136
6	21.12	Caryophyllene	919	1.92	204
7	17.60	Bornyl acetate	912	1.91	196
8	7.35	β-Thujene	915	0.95	136
9	7.93	Camphene	947	0.94	136
10	10.12	o-Cymene	931	0.91	134
11	11.12	ç-Terpinene	892	0.88	136
12	17.04	6-(3-Methyl-3-cyclohexenyl)- 2-methyl-2,6-heptadienol	877	0.88	220
13	9.88	α-Terpinolene	897	0.48	136
14	11.99	α-Terpinolene	889	0.39	136
15	17.87	Heptane, 3,3-dimethyl-	904	0.32	128
16	9.96	Diazoadamantane	890	0.29	162
17	22.99	10-epi-ç-Eudesmol	885	0.29	222
18	16.70	Phenylacetaldehyde N-methyl-N-formylhydrazone	829	0.28	176
19	22.22	Cyclohexene, 1,5,5-trimethyl-6-(2-propenylidene)-	936	0.26	162
20	13.54	2,4,6-Octatriene, 2,6-dimethyl-	867	0.25	136
21	13.20	2,6-Dimethyl-1,3,5,7-octatetrae ne, E,E	825	0.23	134
22	10.51	4-Carene, (1S,3R,6R)-(-)-	884	0.20	136
23	22.81	Dihydro-cis-à-copaene-8-ol	938	0.17	222
24	21.97	Camphene	850	0.16	136
25	10.81	Tricyclo[3.2.1.0(2,4)]octane, 8-methylene-, (1à,2à,4à,5à)-	925	0.14	120
26	14.58	2-Cyclopenten-1-one, 2,3,5-trimethyl-4-methylene	889	0.13	136
27	9.52	β-Thujene	878	0.12	136
28	12.09	Tricyclo[3.2.1.0(2,,4)]oct-6-en e, 8-methylene-, (1à,2à,4à,5à)-	891	0.10	118
29	14.27	2,8-Bornanediol	925	0.10	170
30	17.75	2-Nonanone, 3-(hydroxymethyl)-	888	0.10	172
		Total Area %		98.30	

RT[a]: Retention time; RSI[b]: Reversed search index on TG-5MS capillary column, RA[c]%: Relative area (peak area relative to the total peak area).

target, making it an ideal selection for this study. The compounds showed high binding affinities of -7 and -7.8 kcal/mol against the anti-inflammatory Target Tumor Necrosis factor (TNF-alpha) (PDBID: 2AZ5). TNF-alpha is a pro-inflammatory cytokine that plays a major role in the pathogenesis of several inflammatory diseases. We chose TNF-alpha as a potential anti-inflammatory target because TNF-alpha inhibitors are used in the treatment of Rheumatoid arthritis, Inflammatory Bowel Disease, Psoriasis, and other inflammatory diseases.[30-32] Caryophyllene interacted with the target TNF-alpha with 10 van der Waals interactions and 7 Pi interactions. dihydro-cis-alpha-copaene-8-ol interacted with the target 3SRW with 10 van der Waals interactions and 5 pi interactions. The amino acid interactions of the compounds to the anti-inflammatory target 2AZ5 are shown in Figure 5 (B).

Table 4: Binding site grid coordinates of targets used for molecular docking.

Target Type	PDB ID	Target Name	Grid centre coordinates
Anti-microbial targets.	1JZQ	Isoleucyl-tRNA synthetase.	-26.73582776x
			6.926711078y
			-27.82592825z
	1KZN	DNA GYRASE	19.46390268x
			31.38737131y
			36.35869076z
	2ZDQ	D-alanine--D-alanine ligase.	48.35624583x
			18.85051502y
			-1.467031607z
	3SRW	Dihydrofolate reductase.	-5.437161837x
			-31.03416816y
			5.382902144z
	3TTZ	DNA gyrase subunit B.	15.59966623x
			-18.15613991y
			7.092968912z
Anti-oxidant targets.	1N8Q	Lipoxygenase	22.36296039x
			1.272871124y
			20.2650223z
	1OG5	CYP2C9	-19.82366963x
			86.69793369y
			38.27579945z
	2CDU	NADPH-oxidase	18.99749909x
			-5.670402997y
			-1.718618562z
	3NRZ	Xanthine oxidase	37.47367438x
			19.30785549y
			18.15215059
Anticancer targets	3ERT	Estrogen receptor alpha.	31.574552x
			-1.590379y
			25.599483z
	1DI8	Cyclin-dependent kinase 2.	-8.773273x
			50.057273y
			12.795045z
	1M17	Epidermal growth factor receptor.	22.01369x
			0.252828y
			52.794034z
	3OG7	AKAP9-BRAF fusion protein.	1.868515x
			-2.637667y
			-19.917727z
	6JOK	Platelet-derived growth factor receptor alpha.	17.588379x
			132.559517y
			-6.030276z

Target Type	PDB ID	Target Name	Grid centre coordinates
Anti-inflammatory targets.	4O1Z	Cyclooxygenase-1	252.107414x
			106.979717y
			4.707935z
	5KIT	Nicotinamide phosphoribosyltransferase.	165.421241x
			185.734552y
			192.381517z
	3w5e	Phosphodiesterase 4B	24.701364x
			18.137182y
			-18.425333z
	5KX7	Interleukin-1 receptor-associated kinase 4.	33.833333x
			40.25825y
			59.695417z
	2AZ5	TNF-alpha	-19.40960x
			74.650750y
			33.849550z

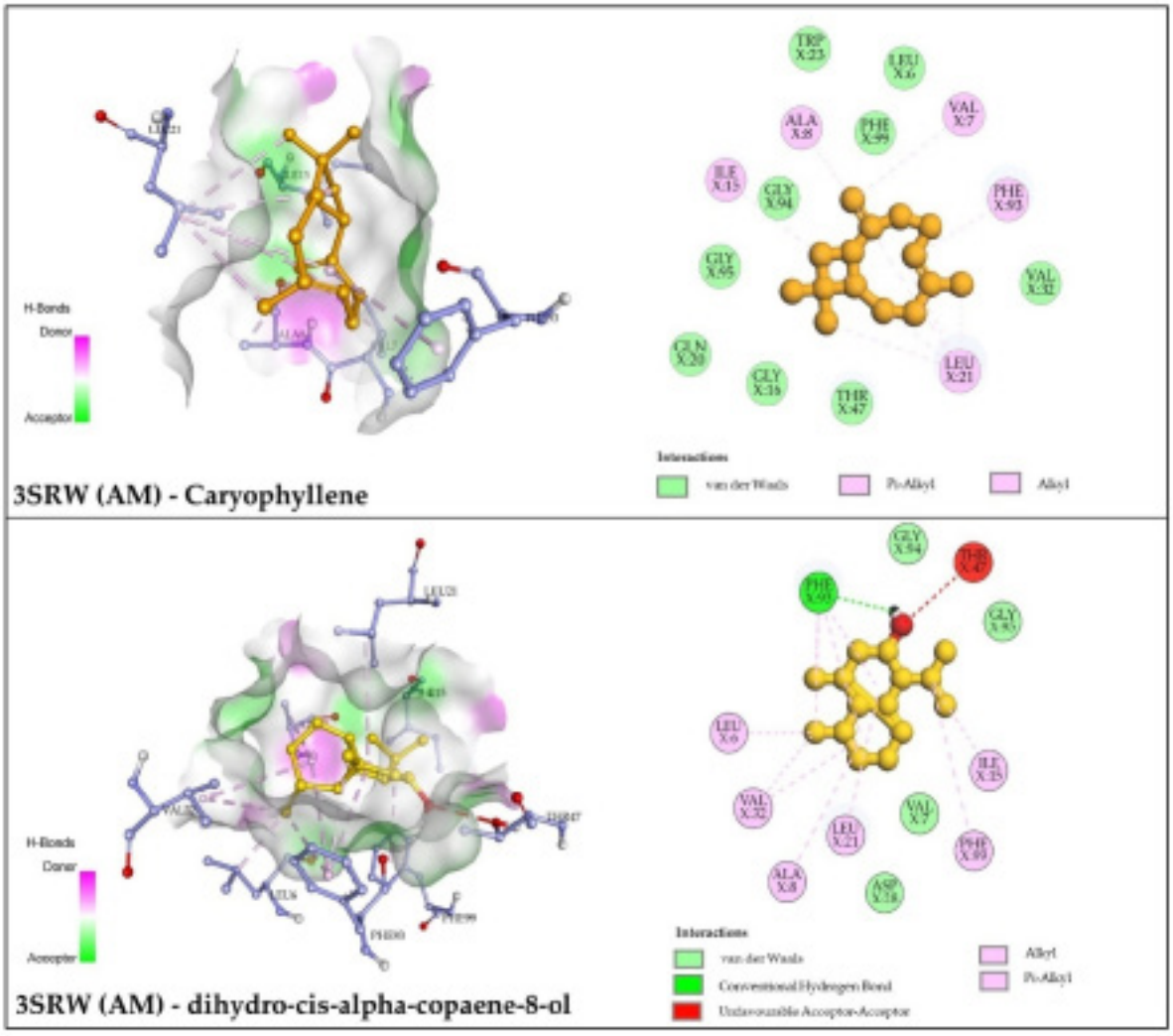

Figure 3: Docking results depiction of the key amino acid interactions in the binding site of anti-microbial target DHFR (PDBID: 3SRW) with Caryophyllene and dihydro-cis-alpha-copaene-8-ol.

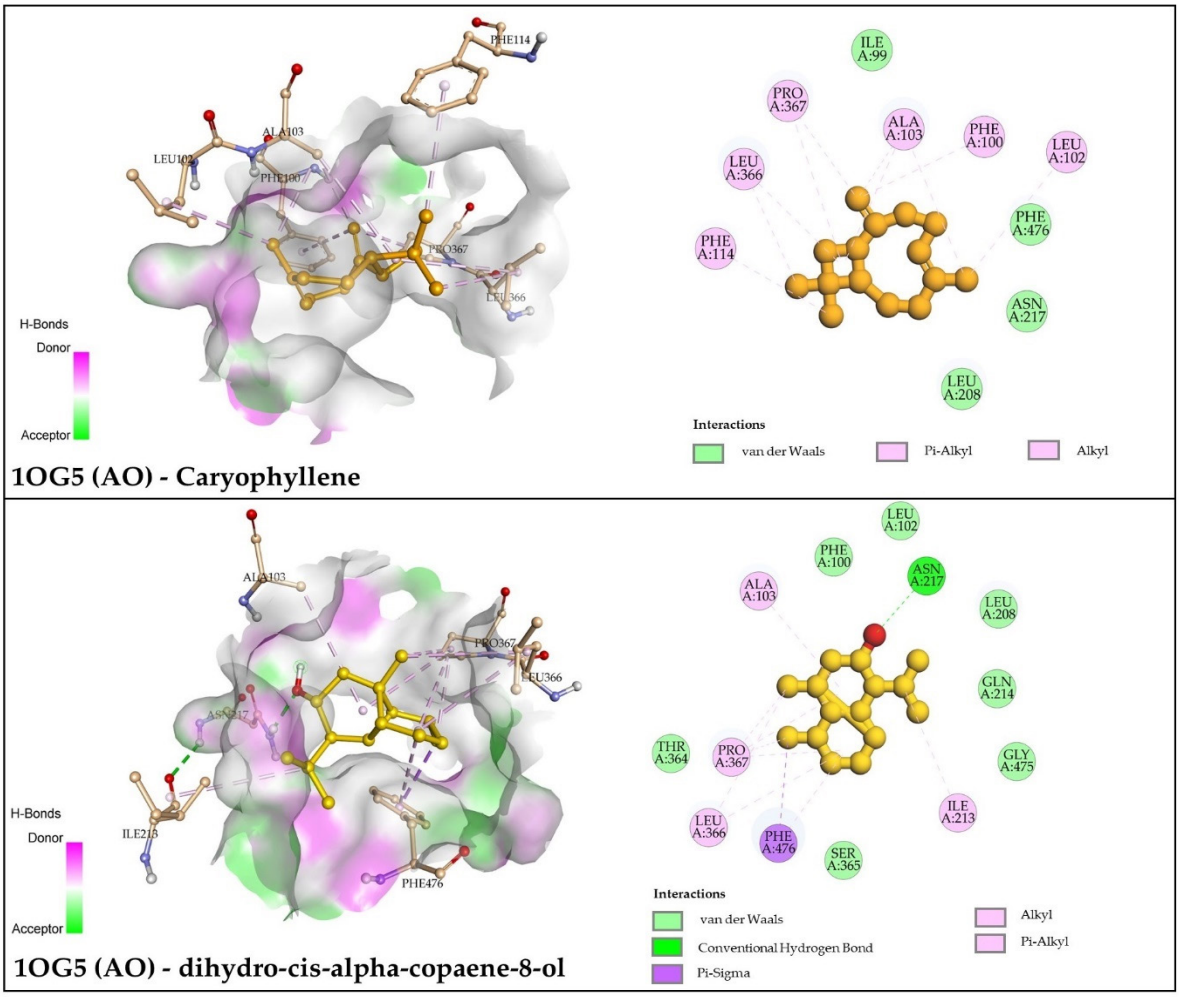

Figure 4: Docking results depiction of the key amino acid interactions in the binding site of anti-oxidant target CYP2C9 (PDBID: 1OG5) with Caryophyllene and dihydro-cis-alpha-copaene-8-ol.

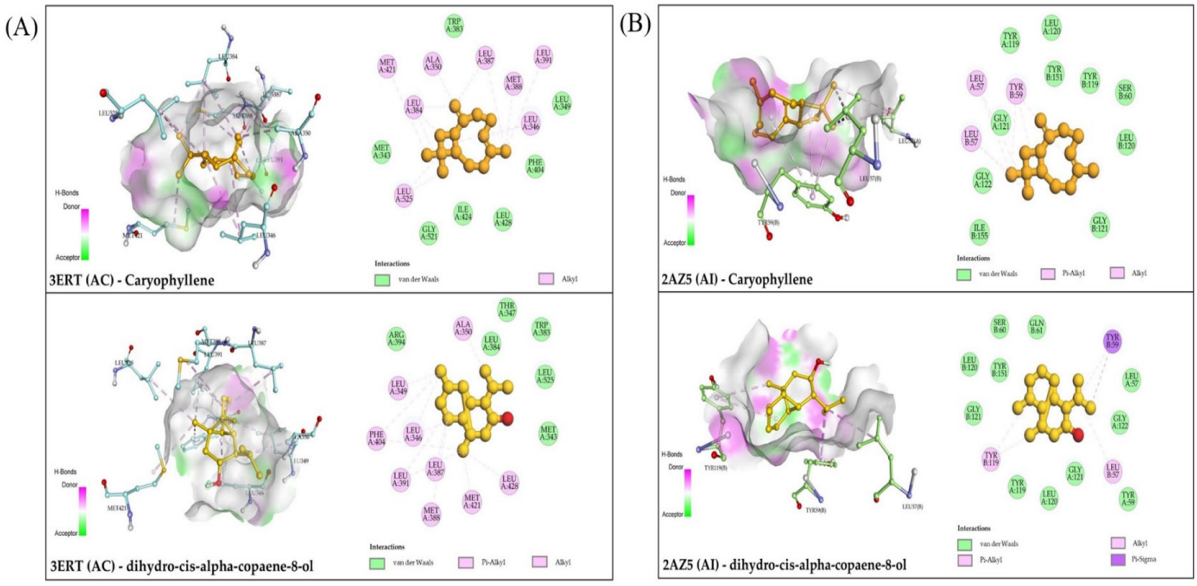

Figure 5: (A) Docking results depiction of the key amino acid interactions in the binding site of anti-cancer target ERα (PDBID: 3ERT) with Caryophyllene and dihydro-cis-alpha-copaene-8-ol. (B) Docking results depiction of the key amino acid interactions in the binding site of anti-inflammatory target TNF-alpha (PDBID: 2AZ5) with Caryophyllene and dihy-dro-cis-alpha-copaene-8-ol.

Table 5: Binding energies and amino acid interactions of phytochemical compounds Caryophyllene and dihydro-cis-alpha-copaene-8-ol against various targets.

Targets	PDB-ID	Compounds	Binding energy (kcal/mol)	H-bonds	Vander Valls interactions	Pi interactions
Antimicrobial	3SRW	Native ligand - CID 53346511	-9.7	LEU-6, ALA-8, ASP-48, PHE-93	VAL-7, GLN-20, HIS-31, THR-47, SER-50, GLY-94, PHE-99, THR-112.	LEU-6, ALA-8, LEU-21(2), LEU-29(2), VAL-32(2), ILE-51(2), LEU-55, PHE-93(3)
		Caryophyllene	-7.7	-	LEU-6, GLY-16, GLN-20, TRP-23, VAL-32, THR-47, GLY-94, GLY-95, PHE-99.	VAL-7, ALA-8, ILE-15, LEU-21(5), PHE-93
		dihydro-cis-alpha-copaene-8-ol	-7.8	PHE-93	VAL-7, ASP-28, GLY-94, GLY-95.	LEU-6, ALA-8, ILE-15, LEU-21, VAL-32(2), PHE-93(3), PHE-99.
Antioxidant	1OG5	Native ligand - CID 54688261	-9.4	ASN-217	GLY-98, LEU-102, VAL-113, ILE-213, GLN-214, THR-364, SER-365, LEU-388.	ARG-97, ILE-99, PHE-100, ALA-103(2), PHE-114(2), LEU-366, PRO-367(2), PHE-476.
		Caryophyllene	-7.4	-	ILE-99, LEU-208, ASN-217, PHE-476.	PHE-100, LEU-102, ALA-103(3), PHE-114, LEU-366(2), PRO-367(2).
		dihydro-cis-alpha-copaene-8-ol	-7.1	ASN-217	PHE-100, LEU-102, LEU-208, GLN-214, THR-364, SER-365, GLY-475.	ALA-103, ILE-213, LEU-366(2), PRO-367(5), PHE-476(2).
Anticancer	3ERT	Native ligand - 4-Hydroxytamoxifen	-10	-	MET-343, THR-347, LEU-349, GLU-353, LEU-354, LEU-384, MET-388, LEU-391, ARG-394, PHE-404, GLU-419, GLY-420, ILE-424, LEU-428, GLY-521, HIS-524.	LEU-346(2), ALA-350(2), ASP-351, TRP-383, LEU-386, LEU-387, MET-421(2), LEU-525(2).
		Caryophyllene	-8.5	-	MET-343, LEU-349, TRP-383, PHE-404, ILE-424, LEU-428, GLY-521.	LEU-346, ALA-350, LEU-384, LEU-387, MET-388, LEU-391, MET-421, LEU-525.
		dihydro-cis-alpha-copaene-8-ol	-8	-	MET-343, THR-347, TRP-383, LEU-384, ARG-394, LEU-525.	LEU-346, ALA-350, LEU-386, LEU-387, MET-388, LEU-391, PHE-404, MET-421, LEU-428.
Anti inflammatory	2AZ5	Native ligand - CID 5327044	-9.2	GLY-121(A)	LEU-57(A), GLY-121(A), GLY-122(A), TYR-151(A), ILE-155(A), SER-60(B), GLN-61(B), GLY-121(B), GLY-122(B).	TYR-59(A)(2), TYR-119(A)(2), LEU-57(B), TYR-59(B)(2), TYR-119(B)(2), TYR-151(B).

Targets	PDB-ID	Compounds	Binding energy (kcal/mol)	H-bonds	Vander Valls interactions	Pi interactions
		Caryophyllene	-7	-	TYR-119(A), LEU-120(A), GLY-121(A), GLY-122(A), SER-60(B), TYR-119(B), LEU-120(B), GLY-121(B), TYR-151(B), ILE-155(B).	LEU-57(A)(2), LEU-57(B)(2), TYR-59(B)(3).
		dihydro-cis-alpha-copaene-8-ol	-7.8	-	TYR-59(A), TYR-119(A), LEU-120(A), GLY-121(A), GLY-122(A), SER-60(B), GLN-61(B), LEU-120(B), GLY-121(B), TYR-151(B).	LEU-57(B), TYR-59(B)(2), TYR-119(B)(2).

Bolded amino acid residues are the residues that matched with active residues of target from PDB.

DISCUSSION

β-pinene which was present in the leaves, roots, and stem of *H. cordata* is a well-known representative of the monoterpenes group, and is found in many plants' essential oils. There have been reports of a wide range of pharmacological activity, including the modulation of antibiotic resistance and analgesic, anticoagulant, anticancer, and antibacterial, effects that are anti-leishmanial, anti-inflammatory, anti-malarial, and antioxidants. β-pinene is used as an antibacterial due to its toxic effects on membranes and has been found to have inhibitory effects on leukemia and breast cancer. Leaf alcohol and leaf aldehyde are responsible for the green odor in leaves and fruits. Because of its pleasant scent and its anti-fungal and anti-inflammatory properties, Sabinene is utilized in the fragrance and flavoring industry as well as in the medicinal industry.[30-32] α-Pinene is a monoterpene that is known to possess antimicrobial, apoptotic, anti-metastatic, and antibiotic properties. α-pinene is one promising agent for the treatment of various inflammatory diseases as it has been found to suppress MAPKs and the NF-κB pathway.[33] Caryophyllene is sesquiterpenes which have anti-cancer, local anaesthetic, antimicrobial, antibacterial, anti-inflammatory, anticonvulsant, and analgesic properties.[34-38] Camphene is a cyclic monoterpene that has anti-viral, insecticidal, antinociceptive, and antioxidant properties.[39,40] o-Cymene is an insecticidal and repellent which also has antifungal and antimicrobial properties.[41-43] 2-Norpinene, 3,6,6-trimethyl, a chemical compound present in the leaves of *H. cordata* have antifungal properties. Due to its many benefits and uses, including its popularity in Northeast India traditional cuisine, this plant offers positive health benefits.

CONCLUSION

This study showed that headspace SPME-GC-MS is a very useful tool for the analysis of aromatic compounds of several edible and medicinally useful spices. *In silico* studies further supported the antimicrobial, antioxidant, anti-cancer, and anti-inflammatory properties of caryophyllene and dihydro-cis-alpha-copaene-8-ol found in *H. cordata*. Monoterpenes as the major components found in the leaves, roots, and stem of *H. cordata*, are in agreement with the *in silico* results that include antimicrobial, antioxidant, anti-inflammatories, and anti-cancer properties. Given all the beneficial active components, *H. cordata* acts as an excellent edible genetic resource and its consumption as a diet can benefit greatly in human health and promotes natural product research. Despite the potential and presence of numerous significant compounds, more research on the therapeutic applications of *H. cordata* can be performed.

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CONFLICT OF INTEREST

The authors declared that they have no competing interests.

ABBREVIATIONS

VOC's: Volatile organic compounds; **GC-MS:** Gas chromatography mass spectrometry; **SPME:** Solid phase microextraction; **NIST:** National Institute of Standards and Technology; **AM:** Anti-microbial; **AO:** Anti-oxidant; **AC:** Anti-cancer; **AI:** Anti-inflammatory; **DHFR:** Dihydrofolate reductase; **Erα:** Estrogen Receptor Alpha.

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