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Exploring The Anticancer Potential Of *Curcuma Caesia* Roxb. Indigenous To Manipur, India: An In-Silico Study Of GC-MS Identified Bioactives

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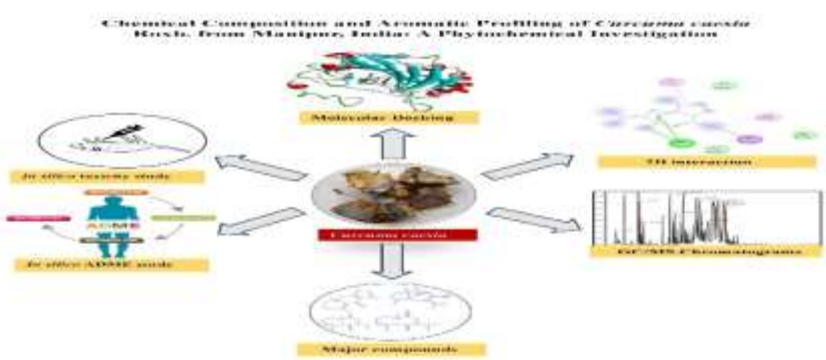
***Abstract*:**
Objective: This study explores the chemical composition and anticancer potential of Curcuma caesia (Kali Haldi), a rare medicinal herb from the Zingiberaceae family. Material & method: Using Gas Chromatography-Mass Spectrometry (GC-MS), the volatile rhizome oil from four accessions collected in Manipur was analysed and the anticancer potential of key bioactive compounds was assessed through molecular docking using PyRx against various cancer cell lines including HeLa (cervical), PA-1 (ovarian), PANC-1 & PaCa-2 (pancreatic), and HTLA-230 (neuroblastoma). Binding interactions were analysed using Biovia Discovery Studio while SwissADME and Protox 3.0 were used to evaluate pharmacokinetic and toxicity profiles. Result: Phytochemical investigation revealed the presence of 50 chemical constituents that made up 87.04%–96.99% of the essential oil. The major compounds included Epicurzerenone, Curcumenol, Eucalyptol, Camphor and (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one, with some first ever identification of essential oil in C. caesia highlighting significant geographical variations. Molecular Docking results unveiled that Curcumenol and (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one had exhibited highest binding affinity while ADMET study assessed the pharmacokinetic and safety profile of major compounds for drug development. Conclusion: These findings provide a foundation for further research into the therapeutic and anticancer potential of Curcuma caesia Roxb. compounds, opening new avenues in the field of drug discovery and herbal medicine.
***Keywords*:** *Chemical profile. Curdione. Epicurzerenone. GC/MS analysis. Hydrodistillation.*



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1.INTRODUCTION

Curcuma caesia Roxb., popularly referred to as 'Black Turmeric', is a perennial herb indigenous to North-East and Central India, with distribution in Thailand, Malaysia, Nepal, China, and Bangladesh (1). Locally referred to as 'Yaimu' in Manipuri, this species is a member of the Zingiberaceae family and reaches a height of 0.5-1.0 m. It is characterised by its huge tuberous rhizomes, wide oblong leaves, and pale yellow flowers with reddish borders (2). The species is diploid with 42 chromosomes and is considered endangered due to biopiracy (3,4). *C. caesia* has garnered considerable attention owing to its diverse array of bioactive compounds and potential therapeutic properties. Its rhizomes are known for their strong camphorous odour and bitter taste, indicating the presence of camphor, phenolics, curcuminoids, flavonoids, proteins, amino acids, alkaloids, and volatile oils (5). Conventionally, the rhizomes are used to treat various ailments, including wounds, contusions, enlargement of the spleen, snake bites, epileptic seizures, joint pain, toothache, ulcer, asthma, tumours, allergic eruptions, and gastric stress (3,6). Therapeutically, the rhizome of this plant possesses the properties of anticancer, antioxidant, antiulcer, anti-inflammation, antifungal, antibacterial, anthelmintic, and smooth muscle relaxant (6,7). Earlier analyses of *C. caesia* rhizome volatile oil from different regions have identified various compounds. For instance, the rhizomes from Madhya Pradesh showed camphor (28.3 %), ar-turmerone (12.3 %), (Z)-*β*-Ocimeme (8.2 %), ar-curcumene (6.8 %), 1,8-cineole (5.3 %), *β*-Elemene (4.8 %), borneol (4.4 %), and bornyl acetate (3.3 %) as major compounds (8). Similarly, in Calicut, thirty-five compounds were identified, with tropolone (15.86 %) as the predominant compound (4). Furthermore, forty-eight compounds were identified from the rhizomes grown in Uttar Pradesh, with cycloisolongifolene,8,9-dehydro-9-formyl (11.67%), camphor (6.05%), eucalyptol (1,8-cineole) (5.96), and β-Germacrene (5.23%) as major components (Kumar et al., 2020). Borah et al.(2020) reported androsta-1,4-dien-3-one,17-(acetyloxy)-, (17.beta.) santanol acetate (16.11%), eucalyptol (12.98%), cycloprop[e]indene-1a,2(1H) dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro- 5,5,6b-trimethyl, (1a. alpha., 3a. beta., 6a. beta., 6b. alpha) (8.96%), methyl 7,12-octadecadienoate (6.75%), and (+)-2-Bornanone (6.60%) as principal constituents from the essential oil of *C. caesia* rhizome collected from Arunachal Pradesh. However, despite these thorough investigations, the volatile content of *C. caesia* from Manipur, North-East India, remains unexplored. Thus, this study aims to analyse the chemical profile of *C. caesia* rhizomes from this region (9,10,11,12).

Figure 1. depicts representative pictures of intraspecific variation in four accessions

2. EXPERIMENTAL

2.1. Plant Material

Four genotypes of fresh *Curcuma caesia* rhizomes were procured from distinct locations in Manipur. The location details are shown in Table 1. Figure 1 depicts representative pictures of intraspecific variation in four accessions.

2.2. Extraction of Essential Oil

The volatile oil was isolated from fresh rhizomes of *C. caesia* using the hydro-distillation method employing a Clevenger apparatus for 3 hours. Approximately 250 g of fresh rhizomes were utilised for this process. The extracted volatile oil was then collected, desiccated with anhydrous sodium sulphate, and kept in an amber-coloured vial at 4°C until GC-MS analysis was conducted. The percentage of the essential oil yield was calculated using the formula given in Equation 1:

$$\text{Percentage of essential oil \%} = \frac{\text{Weight of oil(ml)}}{\text{Weight of sample (g)}} \times 100 \quad \text{----------(1)}$$

2.3. Gas chromatography–Mass spectrometry analysis

The extracted rhizome volatile oil was analysed using a TRACE 1300 Gas Chromatograph coupled with an ISQ 7000 Mass spectrometer (Thermo Fisher Scientific, USA). The GC was fitted with a capillary column (30 m x 0.25 mm diameter). Helium served as a carrier gas at a flow rate of 1 mL/min. A 1 µL of diluted essential oil was introduced into the injector, which was kept at a temperature of 290°C. The mass scan range was 50-650 amu, and the ionisation energy of 70.5 eV was maintained. The different essential oil components were identified by matching their recorded mass spectra peaks with those in the Mainlib spectral library provided by the instrument software.

2.4. Molecular Docking

The main constituents of *Curcuma caesia* Roxb. (Epicurzerenone, Curdione Curcumenol, Eucalyptol and (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]) were evaluated for their anticancer potential using various cell lines such as HeLa (cervical cancer; PDB ID: 5I9B), PA-1 (ovarian cancer; PDB ID: 2NS2), PANC-1 & PaCa-2 (pancreatic cancer; PDB ID: 4EPV & 6S6A) and HTLA-230 (neuroblastoma cancer; PDB ID: 5N9T). The docking protocol and assessment of the major compounds binding affinities with the aforementioned PDB IDs of targeted cell lines were conducted using the PyRx software tool.

2.5.2D interaction

The software Biovia Discovery Studio was used to execute 2D interactions of compounds with the interacting amino acid residues of the target receptors' active region which provides a clear visual representation of the interacting binding residues of targets with the major compounds of *Curcuma caesia* Roxb.

2.6. ADME Study

The pharmacokinetic parameters of the major components of *Curcuma caesia* Roxb were predicted using the web tool SwissADME which accepts chemical structures via SMILES notation, SDF/MOL file upload or interactive molecule drawing and aims to assess the ADME parameters like solubility, permeability (such as blood-brain barrier penetration), metabolic stability and interactions with cytochrome P450 enzymes. Physical data such as molecular weight, logP and pharmacokinetic simulations are also provided by the software. Using the Brain and Intestinal Estimated Permeation (BOILED-Egg) model, the software provides data about brain access and gastrointestinal absorption. It evaluates the likelihood and probability of compounds being orally active using parameters such as Lipinski's Rule of Five, Ghose, Veber and Egan and identifies infractions to direct optimisation.

2.7. Toxicity study

The toxicity profiles of the compounds are predicted using the software Protox 3.0 which assesses the probable toxicities of the compounds prior to their screening in laboratory which helps in prioritizing safer compounds for the drug discovery process.

3. RESULT AND DISCUSSION

3.1. Phytochemical investigation

The hydro-distillation of *C. caesia* rhizomes obtained a viscous volatile oil with a yellowish to light purplish hue. Accession CMP005 yielded the highest oil at 0.6%, followed by CMP025 at 0.4%, CMP009 at 0.32%, and CMP006 at 0.18%. The results closely align with previous reports on the volatile oil yields from *C. caesia* (11-13). The maximum yield of *C. caesia* has been reported as up to 1.5% (8).

The chemical composition, molecular structure of some major compounds, and chromatograms of the essential oil are presented in Table 2, Figure 2, and Figure 3. A total of fifty chemical constituents were identified, comprising 87.04%-96.99% of the total oil content. Among the studied genotypes, CMP005 exhibited the highest total area percentage of identified compounds (96.99%), while CMP006 had the lowest (87.04%). Among the identified constituents, epicurzerenone was the predominant compound, with concentrations ranging from 11.22% in genotype CMP005 to 41.44% in CMP025. This was followed by 5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone, which ranged from 2.68% in CMP005 to 16.14% in CMP009. Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]- content varied from 4.48% in CMP009 to 10.85% in CMP006. Curcumenol ranged from 6.60% in CMP009 to 9.84% in CMP025, benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans- from 6.58% in CMP005 to 9.84% in CMP025, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one from 7.69% in CMP009 to 9.74% in CMP005, β-Cyclocostunolide from 4.99% in CMP005 to 9.46% in CMP009, eucalyptol from 3.38% in CMP006 to 8.78% in CMP009, camphor from 2.43% in CMP025 to 7.97% in CMP009, and γ –Elemene from 2.93% in CMP009 to 6.65% in CMP006. The component 4,4´-Dimethyl-2,2´-dimethylenebicyclohexyl-3,3´-diene was found only in genotype CMP005. Curdione (12.7%), tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene,trans-2,9-anti-9,10-trans-1,10- (10.39%), humulene (5%), caryophyllene (4.4%), and cyclohexane,1,2,3-trimethyl- (4.07%) were observed only in genotype CMP006. 3,5,8a-Trimethyl-4,4a,8a,9-tetrahydronaphtho[2,3-b]furan (5.07%) and (4aR,5S)-1-Hydroxy-4a,5-dimethyl-3-(propan-2-ylidene)-4,4a,5, 6-tetrahydronaphthalen-2(3H)-one (4.47%) were recorded only in CMP009. Reynosin (8.7%) was observed in CMP025. The volatile oil composition of *C. caesia* rhizome differed greatly from earlier reported studies, possibly attributable to geographical variations. Notably, the analysed rhizome oil lacks cis-β-ocimene as compared to studies reported by Sakuntala (2000). Furthermore, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one, curcumenol, benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans-, cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-, curdione, and reynosin were newly identified as major components in the essential oil of this species. Nonetheless, curdione was reported as a major component in *Curcuma aromatica* (11) and *Curcuna wenyujin* (14). While previous investigations by Pandey and Chowdhury (2003) (8) and Paw et al. (2019) (15) reported camphor, 1,8-Cineole, and epicurzerenone as predominant components. Lately, similar researches have been conducted on the essential oil of C. *caesia* and highlighted camphor, 1,8-cineole, curzerenone, and α-bulnesene as major compounds (16-18). Our findings align with these investigations to a certain extent, yet curzerenone and α-bulnesene were absent in our studies. However, there is considerable quantitative variation in the percentage of the compounds

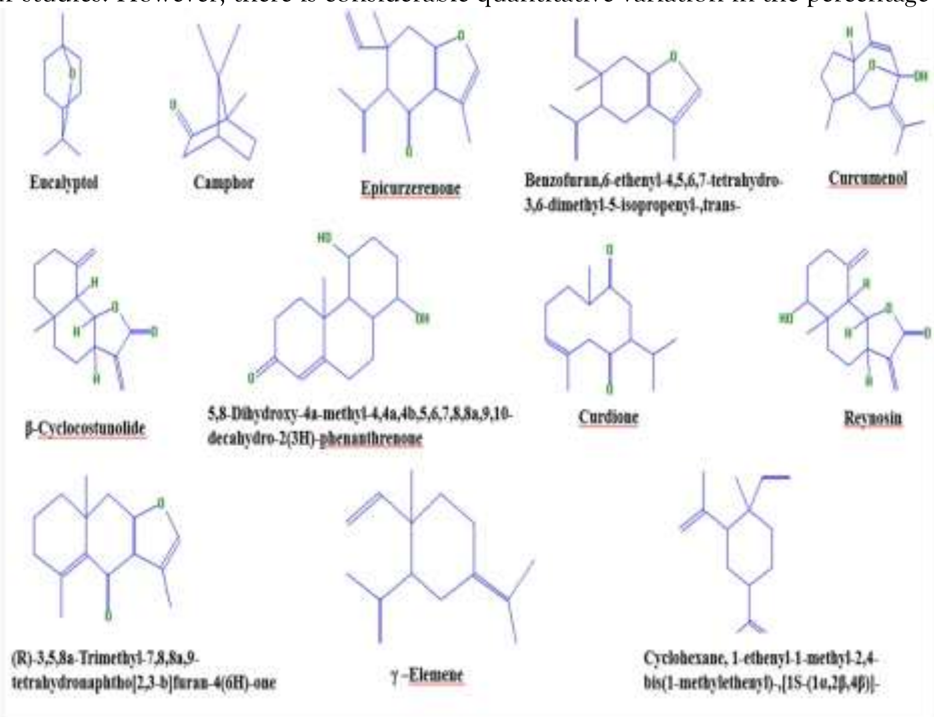

Figure 2. Molecular structures of major components of C. caesia

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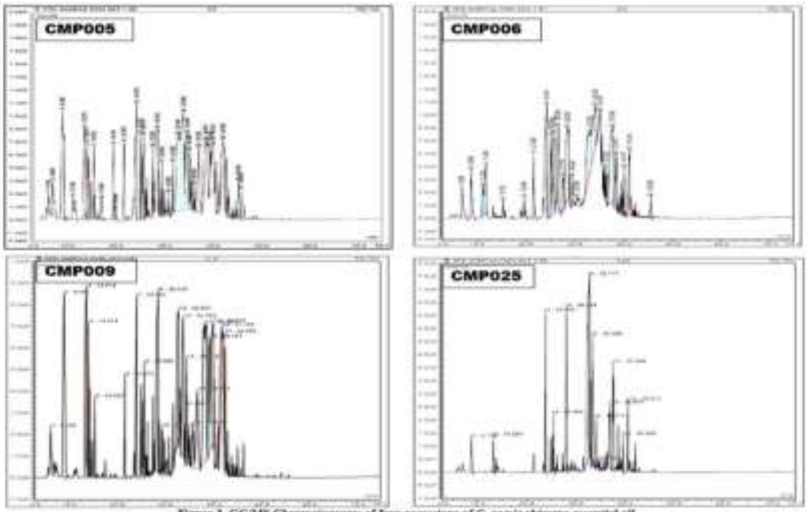

Figure 3. GC/MS Chromatograms of four accessions of C. caesia rhizome essential oil

Geographical variations in the chemical profiles were further underscored by comparisons with studies from other regions. Tropolone was noted to be the predominant component in the essential oil of this species from South India (4), while Cycloisolongifolene,8,9-dehydro-9-formyl was predominant in North India (10). Similarly, androsta-1,4-dien-3-one,17- (acetyloxy)-, (17.beta.) santanol acetate, eucalyptol, and cycloprop[e]indene-1a,2(1H) dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro- 5,5,6b-trimethyl, (1a. alpha., 3a. beta., 6a. beta., 6b. alpha) were reported as major constituents from Arunachal Pradesh (9). Interestingly, the present findings differed significantly from these studies, except for eucalyptol, epicurzerenone, and camphor. Additionally, the findings of the species from Thailand were compared with our findings, where 1,8-cineole, camphor, curzerene, and curzerenone were reported as major compounds (19,20). 1,8-cineole and camphor are in congruence with their findings; however, curzerene and curzerenone were not identified in the study. Hence, the species grown in Manipur is deficient in curzerene and curzerenone.

Table 1. Location details of *Curcuma caesia* Roxb. accessions collected from Manipur, India

Major compounds	Binding Affinity(kcal/mol) score of PDB IDs of cancer cell lines				
	5N9T	5I9B	4EPV	6S6A	2NS2
(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one	-9.6	-6.8	-6.9	-6.5	-6.9
Epicurzerenone	-5.1	-6.0	-6.0	-5.7	-6.4
Eucalyptol	-4.6	-5.1	-5.1	-4.6	-5.2
Curdione	-8.5	-6.1	-6.8	-5.6	-5.7
Curcumenol	-6.8	-6.8	-7.2	-6.9	-6.0

Sl. No.	Accession	Place of Collection	Latitude (N)	Longitude (E)	Altitude (m)
1	CMP005	Chothe village, Bishnupur	24°36’28”	93°44’44”	833
2	CMP006	Kakching	24°32’32”	93°57’55”	794
3	CMP009	Litan, Chandel	24°26’49”	93°57’34”	791
4	CMP025	Senapati	25°15’38”	94°0’49”	1103

Table 3. 2D interaction of the most active compounds with target receptors

Compounds	Interacting amino acid residues of target receptor				
	5N9T	5I9B	4EPV	6S6A	2NS2
(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one	TYR B:514, TYR B:224 (Alkyl interaction), VAL B:296 (Pi-alkyl interaction)	TYR A:276 (Van der Waals interaction)	-	-	ILE B: 108, ALA B: 132 (Alkyl interaction)
Curcumenol	-	CYS A:163 (Alkyl interaction), TYR A:204 (Pi-alkyl interaction), ARG A: 207 (Convention	ASP A: 119 (Conventional Hydrogen bond interaction), PHE A:28 (Pi-alkyl interaction),	VAL D: 366 (Conventional Hydrogen bond interaction), PHE D:367 (Carbon-Hydrogen	-

		al Hydrogen bond interaction), TRP A: 206 (Pi-Sigma interaction)	LYS A:147 (Alkyl interaction)	bond interactions), LEU D: 335, LEU D: 243 (Alkyl interaction), PHE D: 109 (Pi-alkyl interaction)	

Table 4: ADME parameters of the major components of *Curcuma caesia* Roxb

Compounds	Lipophilicity	Water solubility	GI absorption	BBB permeant	Lipinski violation	Bioavailability score	Log K_p (skin permeation)	Cytochrome P450 enzyme inhibitor
(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one	2.94	Soluble	High	Yes	0	0.55	-5.89 cm/s	No
Curcumenol	2.85	Soluble	High	Yes	0	0.55	-6.14 cm/s	No
Epicurzerenone	3.05	Soluble	High	Yes	0	0.55	-5.50	No
Curdione	3.01	Soluble	High	Yes	0	0.55	-5.86	No
Eucalyptol	2.67	Soluble	High	Yes	0	0.55	-5.30	No

Table 5. Toxicity profiles of the major components of *Curcuma caesia* Roxb

Compounds	LD_{50} value(mg/kg)	Toxicity class
Eucalyptol	2480	5
Epicurzerenone	4920	5
Curcumenol	6000	6
Curdione	5000	5
(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one	63	3

3.2. Molecular Docking:

Molecular docking study of major components on the HeLa, PA-1, PANC-1, PaCa-2 and HTLA-230 cell lines revealed that the compound (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one had exhibited a very excellent binding affinity score in the neuroblastoma HTLA-230 cell lines with a docking score of -9.6 kcal/mol. Moreover, this compound had also displayed good activity in the cervical and ovarian cancer cell lines with a binding affinity score of -6.8 & 6.9 kcal/mol respectively. Another compound namely Curcumenol had displayed promising scores in cervical and pancreatic cancer cell lines with a docking score of -6.8, -7.2 & -6.9 kcal/mol respectively. Therefore, the compounds (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one and Curcumenol can be further evaluated for in vitro anticancer studies in order to validate their biological efficacy Table 3 represents the binding affinity score of the compounds.

3.3. 2D interaction:

It has been found that (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one interacts with 5N9T by TYR B:514, TYR B:224 (Alkyl interaction), VAL B:296 (Pi-alkyl interaction) residues and with 5I9B and 2NS2 via TYR A:276(Van der Waals interaction) and ILE B: 108, ALA B: 132 (Alkyl interaction) amino acid residues respectively. Curcumenol interacts with 5I9B using CYS A:163(Alkyl interaction), TYR A:204 (Pi-alkyl interaction), ARG A: 207 (Conventional Hydrogen bond interaction), TRP A: 206 (Pi-Sigma interaction) and with 4EPV & 6S6A using ASP A: 119 (Conventional Hydrogen bond interaction), PHE A:28 (Pi-alkyl interaction), LYS A:147 (Alkyl interaction) and VAL D: 366 (Conventional Hydrogen bond interaction), PHE D:367 (Carbon-Hydrogen bond interactions), LEU D: 335, LEU D: 243 (Alkyl interaction), PHE D: 109 (Pi-alkyl interaction) respectively. 2D interactions of the most active compounds with the target receptors are given below in table 4 and their visual illustration is represented in figure 4.

3.4. ADME profile

ADME study using the software SwissADME had demonstrated that all the compounds had exhibited the lipophilicity within the ideal range i.e. <5; compounds have water solubility; Blood-Brain-Barrier permeability; 0 Lipinski violation; Bioavailability score 0.55; Skin permeation value > -5 which indicates that the compounds are poor candidates for topical administration and the compounds doesn't inhibit any of the cytochrome P450 metabolic enzymes. Table 5 illustrates the ADME parameters of the compounds.

3.5. Toxicity study

Toxicity study using Protox 3.0 software had unveiled that among all the compounds Curcumenol is the safest compound which falls in the toxicity class 6 with LD_{50} value of 6000 mg/kg; Eucalyptol, Epicurzerenone and Curdione falls in the toxicity class 5 with LD_{50} value of 2480, 4920 and 5000 mg/kg respectively which can also be considered as safe whereas (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one is the most toxic of all the compounds which falls in the toxicity class 3 with LD_{50} value of 63 mg/kg. The toxicity profile of all the components is demonstrated below in table 6.

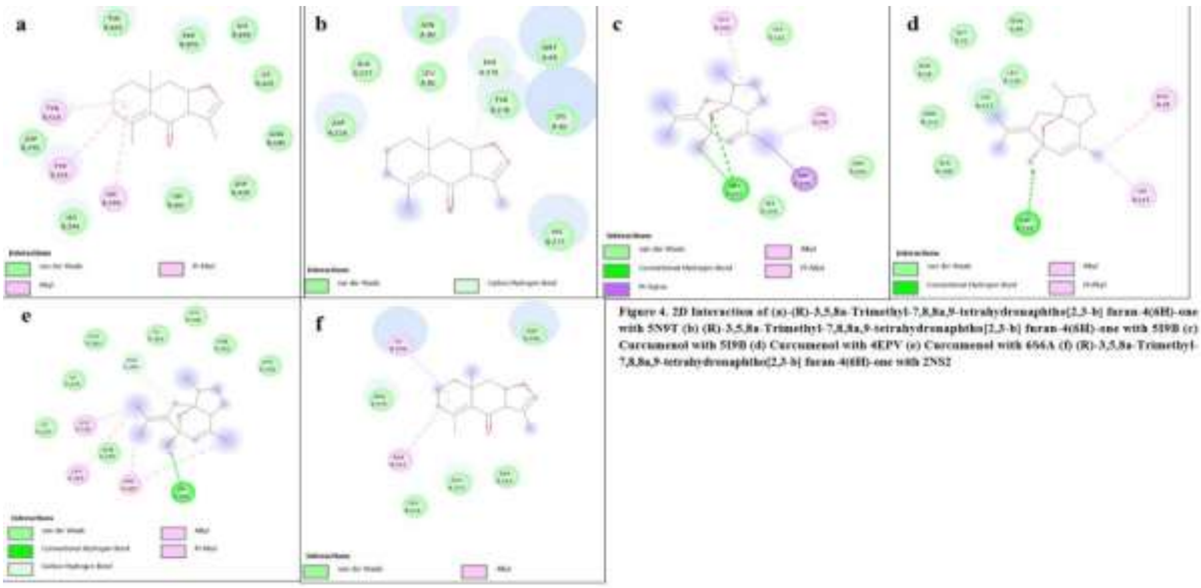

Figure 4. 2D Interaction of (a)-(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one with 5N9T (b) (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one with 5I9B (c) Curcumenol with 5I9B (d) Curcumenol with 4EPV (e) Curcumenol with 6S6A (f) (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b] furan-4(6H)-one with 2NS2

4. CONCLUSION

The present investigation highlights the variability in the chemical components of the rhizome essential oil, with major compounds including epicurzerenone, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one, eucalyptol, curdione, and curcumenol. An *in-silico* study, particularly molecular docking, targeting various anticancer cell lines for pancreatic, cervical, ovarian, and neuroblastoma cancers, reveals the potential of (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one and Curcumenol as effective anticancer agents. Furthermore, the *in-silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) evaluation demonstrated good lipophilicity, water solubility and blood-brain barrier permeability for all major compounds. These compounds showed zero Lipinski rule violations and were classified under toxicity classes 5 and 6, indicating safety. However, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one falls under toxicity class 3, suggesting a moderate level of toxicity. These findings offer valuable insights into the physicochemical profile of the volatile oils of *C. caesia*, which will pave the way for novel drug development and herbal remedies utilising this indigenous species.

Credit authorship contribution statement

All authors have contributed equally.

Declaration of Competing Interest

The authors declare no conflict of interest.

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