Original Article

Herbal-Drug Interaction Possibility with HPTLC Standardized Aqueous Root Extract of Decalepis hamiltonii: an In-Silico Approach

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Abstract

Herbal medicine believed as a better and safe alternative to treat various ailments of humans than allopathic medicine but it is not always true. Despite accepting herbal medicine as the therapeutic agent they may possess significant herbal drug interactions when concomitantly used with allopathic drugs. Decalepis hamiltonii aqueous root extract (Nannari) has been consumed in south Asian countries widely during summer as a flavouring and cooling agent. The hypothesis of the present study is to test the possible herbal-drug interaction with cytochrome 450 enzymes in in-silico. Preliminary phytochemical analysis and HPTLC fingerprints profile of the aqueous root extract of Decalepis hamiltonii reveals the presence of glycosides, tannins, terpenoids, flavonoids, and steroids. Hence, based on the previous literature seven isolated chemical compounds of Decalepis hamiltonii were selected to test for possible herbal-drug interaction with CYP3A4 CYP2D6 and CYP2C9 enzymes by using SMARTCyp and docking analysis programmes. The results are optimistic and all the selected seven compounds possess good binding affinity and binding energy with major cytochrome enzymes which metabolizes the drugs and chemical substances selected in the study are CYP3A4 CYP2D6 and CYP2C9. CYP protein binding affinity and energy are more with CYP3A4 enzyme for all the seven compounds than CYP2D6 and CYP2C9. The findings of the present study reveals that the phyto-chemicals present in the aqueous root extract of D. hamiltonii which were confirmed by HPTLC fingerprinting are metabolized by cytochrome P450 enzymes like CYP3A4, CYP2D6, and CYP2C9. Co-administration of Nannari juice with conventional medicines that are substrates of CYP3A4, CYP2D6, and CYP2C9 may compete for the same enzymes and may precipitate herb-drug interactions. To confirm this, in-vivo and in-vitro CYP inhibition and induction studies with AREDH are warranted.

Keywords: Herbal medicine, Drug-Interactions, Cytochrome P450, Metabolism, Docking.

Introduction

Herbal medicines have been used for many centuries to treat different ailments and promote health. Traditionally herbal medicine is a common component in all indigenous traditional medicines in India including Ayurveda, Homeopathic, Siddha and Unani. The use of herbal medicine and natural therapies for various diseases has a long history all over the globe (Nelson VK et al., 2022).

World Health Organization has acknowledged the relevance of traditional medicines in developing countries and that it has an important role in providing services to a very large fraction living in rural areas. The traditional medicinal system is ubiquitous and widely practiced in India (Narasimha et al., 2018).

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Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.04.248

How to cite this article: Dhanunjaya S, Chitra V, Herbal-Drug Interaction Possibility with HPTLC Standardized Aqueous Root Extract of Decalepis hamiltonii: an In-Silico Approach, J PHARM NEGATIVE RESULTS 2022;13(4): 1806-1813.

Decalepis hamiltonii (DH), a climbing shrub belonging to the family Apocynaceae is an endemic medicinal plant that widely grows in southern parts of the Deccan Peninsula and the Western Ghats of India. The vernacular names of the roots of Decalepis hamiltonii are Swallow root in English, Telugu- Sugandha pala, and Tamil Mavilinga kizhngu. The roots are used as edible and consumed as a locally made health drink called Sharbath/Nannari which is believed to cool the body's heat in south India and other Asian countries like Sri Lanka, and Indonesia (Susila et al., 2021). The ancient literature reveals that the roots of Decalepis hamiltonii can be used as a general tonic and appetizer and to relieve flatulence, diuretic and nutrition disorders (Wealth of India, 1990). Decalepis hamiltonii possess multiple pharmacological actions both in vitro and in vivo including anti-inflammatory activity (Sengupta et al., 2013), neuroprotective activity (Srivastava and Shivanandappa. 2009) and hepatoprotective activity (Srivastava and Shivanandappa. 2009), cytoprotective and antioxidant activity (Nayaka et al., 2010) and antimicrobial activity (Susila et al., 2021). Always natural products play a significant part in combating human diseases for a long time. Constantly there is curiosity in the exploration of natural phytochemical compounds to bring into modern medicine, particularly in the last two decades.

Herbal medicine considers a better and safe alternative to treat the diseases or ailments of humans than allopathic medicine but it is not always true. Despite of widely accepted as therapeutic agents they possess significant herbal drug interactions when concomitantly used with allopathic drugs. Rather than pharmacodynamic herbal-drug interaction they produce more pharmacokinetic interactions (absorption, distribution, metabolism and elimination) by interacting with cytochrome P450 enzyme system and drug transporters proteins (Chatterjee et al., 2022). There were several families of CYP enzymes which involve in the metabolism of xenobiotics including herbal medicine. CYP3A4 was considered the important CYP enzyme which metabolizes 80% of the drugs both in the liver and in the gut and other significant enzymes including CYPD6 and CYP2C9 (Guttman et al., 2022).

The phytochemical analysis of root extracts of DH reveals the presence of various phenolic compounds lupeol, α-amyrin, β-amyrin, lupeol acetate, α-amyrin acetate, β-amyrin acetate and 2-hydroxy 4-methoxy benzaldehyde has been reported as important compound (Sujith et al., 2021). 4-hydroxy isophthalic acid (DHA-I) (Zarei et a l., 2020), 14-aminotetradeconic acid (DHA-II) (Srivastava et al., 2012) 4-(1-hydroxy-1-methyl ethyl)-1-methyl-1,2-cyclohexane diol (DHA-III), 2-hydroxy methyl-3-methoxy benzaldehyde (DHA-IV), (Kamireddy et al., 2018), 2,4,8-trihydroxybicycloocatn-3-one (DHA-V) (Srivastava et al., 2012), 2-Hydroxy-4-methoxy benzaldehyde (Nagarajan and Rao. 2003) and ellagic acid (Srivastava et al. 2007). Despite of identifying these chemicals in DH root extract, still there is scope for the identification of other biologically active compounds as they are available as conjugated or esterified and insoluble-bound phenolic acids.

The key background of the present study is, Decalepis hamiltonii aqueous root extract is been consumed by the public extensively during summer as a flavouring and cooling agent. The chemical constituents present in Decalepis hamiltonii may interfere with the pharmacokinetic processes like absorption, distribution, metabolism and elimination of conventional medicine when consumed together and may precipitate herbal-drug interactions. To uncover that, as a preliminary step the cyochrome involved in metabolizing the seven chemical constituents have to be evaluated. Hence, the present study designed to investigate the seven polyphenol base compounds which were isolated previously from the aqueous root extract of Decalepis hamiltonii considered to identify the cytochrome P450 enzyme that is majorly involved in metabolizing them by using the software SMARTCyp version 2.1. Further, the protein-chemical constituent interactions with CYP3A4, CYPD6 and CYP2C9 were evaluated by using Schrödinger suit 2015 docking software. Further, in our present study, the presence of polyphenols of Decalepis hamiltonii was confirmed both qualitatively and quantitatively.

MATERIALS AND METHODS

Collection of roots of Decalepis hamiltonii

Fresh tubers of Decalepis hamiltonii were collected from the hills of Tirupati surroundings of Andhra Pradesh, India in February and were authenticated by the botanical survey of India, Hyderabad.

Preparation of aqueous root extract of Decalepis hamiltonii

The aqueous root extract of Decalepis hamiltonii (AREDH) was prepared according to Murthy et al. with some modifications. After the collection of roots of thoroughly cleaned with tap water and removed all debris and cut into small pieces and dried at the shade. 200 g of shade-dried roots were coarsely powdered and passed through sieve number 60 and extracted in distilled water at room temperature for 6 h. Then the extract was filtered through Whatman No.1 filter paper and concentrated at 60°C by using a rotary flash evaporator to obtain an aqueous extract of Decalepis hamiltonii. The extract was lyophilized by freeze dryer and stored at 4 °C for further use. All the chemicals used in the present study were of analytical grade and indigenous.

Qualitative phytochemical analysis

The qualitative phytochemical screening of the aqueous root extract of Decalepis hamiltonii was carried out according to the methods described by Khandelwal. 2002

Test for alkaloids

A 100 mg of aqueous root extract of Decalepis hamiltonii was dissolved in dilute hydrochloric acid. The solution was clarified by filtration. The filtrate was tested with

Dragendroff's and Mayer's reagents. The treated solutions were observed for any precipitation.

Test for Flavonoids

50 mg of aqueous root extract of Decalepis hamiltonii was added to 5ml of ethyl acetate and the mixture was shaken and allowed to settle. Production of yellow colour is taken as positive for flavonoids.

Test for steroids

0.5 g of aqueous root extract of Decalepis hamiltonii was dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added to the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

Test for saponins

0.5 g of AREDH was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30-minute period of time. If "honeycomb" froth above the surface of the liquid persists after 30 min. the sample is suspected to contain saponins.

Test for tannins

A portion of AREDH was dissolved in water. The solution was cleared up by the filtering process. 10% ferric chloride solution was added to the clear filtrate. Bluish to black colour change was observed.

Test for Glycosides

A pinch of AREDH dissolved in glacial acetic acid and a few drops of ferric chloride solution were added followed by the addition of Concentrated Sulphuric acid; the formation of a red ring at the junction of the two liquids indicates the presence of glycosides.

HPTLC fingerprinting analysis

The aqueous root extract of Decalepis hamiltonii was filtered and applied on silica gel 60 F254 (Merck) pre-coated aluminium plate (15 x 10 cm). Using a Linomat V applicator and a Hamilton syringe, a total of 20 µL of AREDH was applied as a 10 mm band. The prepared plates were developed in a mobile phase system consisting of chloroform: ethyl acetate: formic acid in the ratio of 5:4:1 in a twin trough chamber. The plates were run by mobile phase up to 8.5cm from the bottom. The developed aluminium plates were air-dried and scanned with deuterium and tungsten lamps at wavelengths between 254 and 365 nm, and photo documented with Reprostar.

In Silico

SMARTCyp analysis

SMARTCyp version 2.1 was used, which is freely available as a web service or a downloadable Java program from the University of Copenhagen (Denmark). It is a method for prediction of which sites in a molecule that is most liable to metabolise by Cytochrome P450 enzymes. It is applicable to metabolism by the cytochrome P450 isoforms 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 (Rajashekar et al., 2012).

Protein preparation

The three-dimensional structure of targeted proteins including CYP3A4 CYP2D6 and CYP2C9 was retrieved from the RCSB protein data bank. The hetero atoms of the three-dimensional structure of CYP 450 proteins were removed and hydrogen atoms were added.

Preparation of Ligands

After confirming the presence of polyphenols both qualitatively and quantitatively in the aqueous root extract of Decalepis hamiltonii, seven previously isolated compounds including 4-hydroxy isophthalic acid (DHA-I) (Zarei et al., 2020), 14-aminotetradeconic acid (DHA-II) (Srivastava et al., 2012) 4-(1-hydroxy-1-methyl ethyl)-1-methyl-1,2-cyclohexane diol (DHA-III), 2-hydroxy methyl-3-methoxy benzaldehyde (DHA-IV), (Kamireddy et al., 2018), 2,4,8-trihydroxybicycloocatn-3-one (DHA-V) (Srivastava et al., 2012), 2-Hydroxy-4-methoxy benzaldehyde (Nagarajan and Rao. 2003) and ellagic acid (Srivastava et al. 2007) were selected and smile strings were prepared to use for further evaluation with SMARTCyp. The two-dimensional structures of the above-mentioned bio-molecules from the AREDH were obtained from the PUB Chem site and their 3D structures were drawn using a Corina 3D converter.

Docking analysis

Molecular docking was performed using Schrödinger suit 2015 to predict the binding orientation of the seven molecules to the protein CYP3A4 CYP2D6 and CYP2C9 to calculate the affinity and activity of the molecules. The docking score with the highest negative value indicates the best binding affinity and accurate fit with the best pose of binding. Binding energy is another important parameter in docking, which predicts the stability of a protein-ligand complex. The binding energy with a higher negative value indicates the best stable complex of a molecule-target protein. Hydrogen bonding with amino acid residues of target protein might further strengthen the complex stability or binding affinity.

RESULTS

Phytochemical analysis

The qualitative phytochemical analysis of the aqueous root extract of Decalepis hamiltonii reveals the presence of plant-based secondary metabolites like glycosides, tannins, terpenoids, flavonoids, and steroids.

HPTLC analysis

HPTLC analysis of the aqueous root extract of Decalepis hamiltonii was done under 254 nm, 366 nm and 575 nm using the solvent system Toluene–ethyl acetate–glacial acetic acid 7:2:1 (v/v). At 254 nm there were four spots

green coloured appeared with different Rf values similar at 366 with blue, violet and purple colour and at 575 nm with brick red and blue colours (represented in Fig 1 and Table 1).

SMARTCyp Prediction

Ligand based interactions are useful since there is no involvement of protein and are computationally efficient. The selected seven compounds of D. hamiltonii were used to predict the possible drug-herbal interactions with CYP 450 enzymes by using one of the broadly used reactivity bases software SMARTCyp. In the present study seven phytochemical compounds (ligands) of D. hamiltonii the possible the carbon atoms of each ligand were graded according to the assigned SMARTCyp reactivity score (S score) and are tabulated in Table 2. All the seven ligand compounds were well interacted with CYP3A4 CYP2D6 and CYP2C9 enzymes. The obtained data reveals that seven ligand of D. hamiltonii may possible to induce or inhibit the CYP3A4 CYP2D6 and CYP2C9 enzymes.

Docking analysis

Molecular docking of seven selected compounds of D. hamiltonii were done by using Schrodinger suit 2015 software to predict the binding orientation of the seven molecules to the protein CYP3A4 CYP2D6 and CYP2C9 to calculate the affinity and activity of the molecules. The compounds showing with the highest negative value indicates the best binding affinity and accurate fit with the best pose of protein binding. The results of the present study reveals that Ellagic acid and 2, 4, 8-trihydroxybicycloocatn-3-one possess the highest docking score and bonding energy than other compounds. The results of all the seven compounds of D. hamiltonii were depicted in 2D and 3D images in Table 3.

Table 1: Rf values and colour spots of aqueous root extract of D. hamiltonii

□ = 254 nm		□ = 366 nm		□ = 575 nm (Derivatized)	
Color	**Rf value (s)**	**Color**	**Rf value(s)**	**Color**	**Rf value(s)**
Green	0.23	Blue	0.10	Brick Red	0.07
Green	0.32	Blue	0.28	Brick Red	0.21
Green	0.49	Black	0.32	Brick Red	0.30
Green	0.58	Light Green	0.57	Brick Red	0.41
		Sky Blue	0.59	Violet	0.59
		Black	0.63	Blue	0.63
		Sky Blue	0.74	Blue	0.70
		Blue	0.82	Blue	0.81
		Sky Blue	0.85	Blue	0.88
		Sky Blue	0.91	Blue	0.99

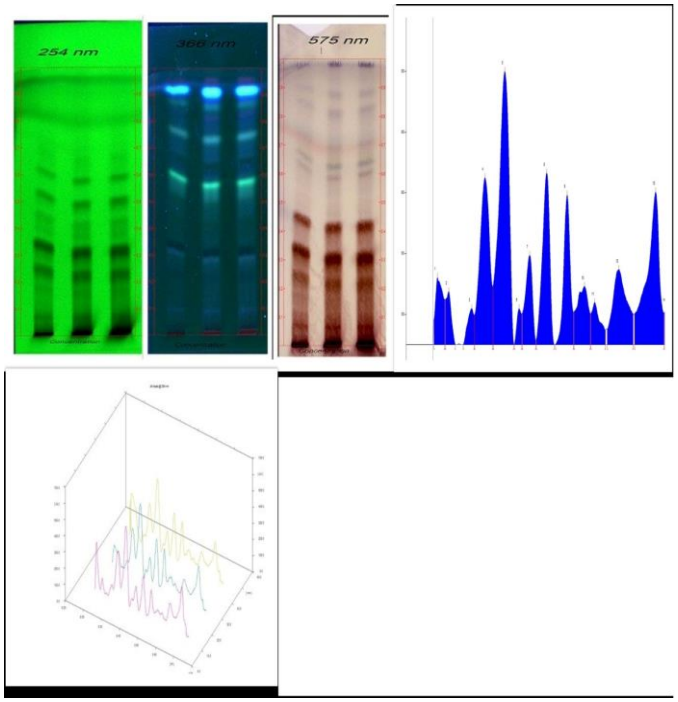
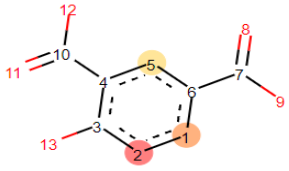
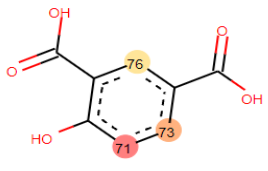


Figure 1: HPTLC fingerprinting profile of aqueous root extract of Decalipis hamiltonii

Table 2: Selected phyto compounds and structure of Decalipis. hamiltonii and S score value on individual selected CYP enzyme

Name of the compound	**Chemical structure**	**S score value**	**S score value on individual CYP 450 enzyme**		
			CYP3A4	**CYP2D6**	**CYP2C9**
DHA-I			70.8	86.4	103.3
			73.1	89.5	111.5
			76	100.1	127.2

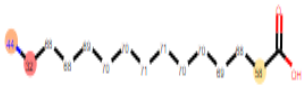
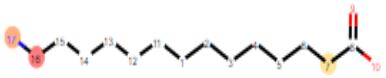
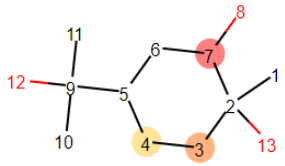
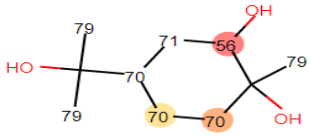
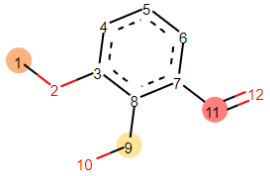
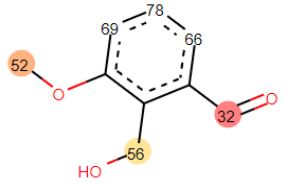
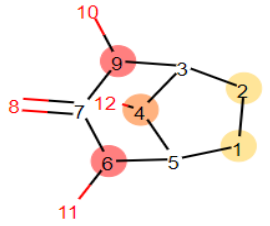
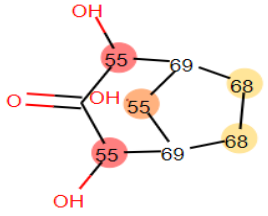
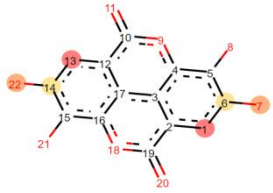
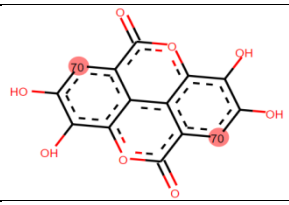
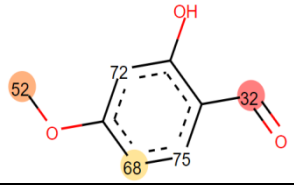
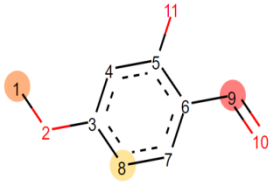
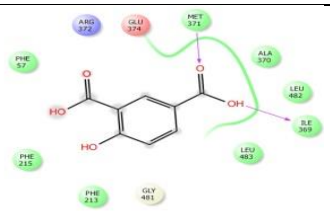
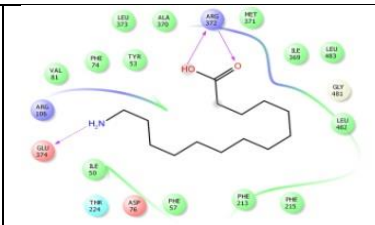
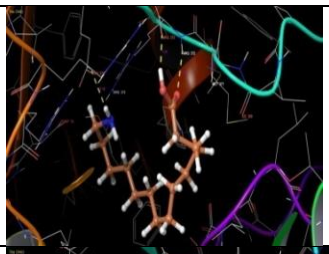
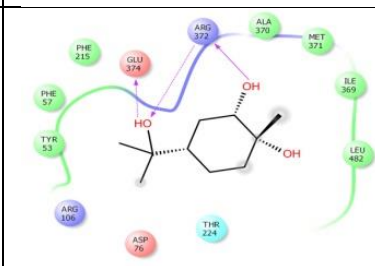
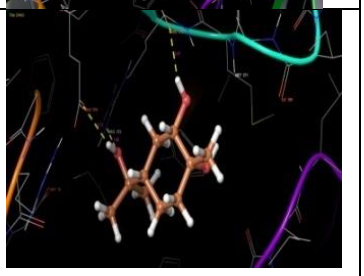
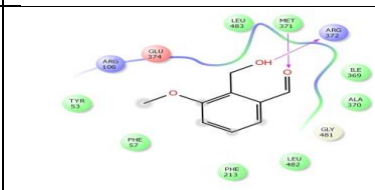
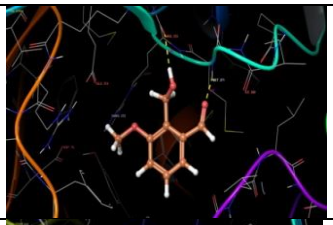
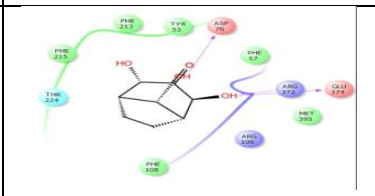
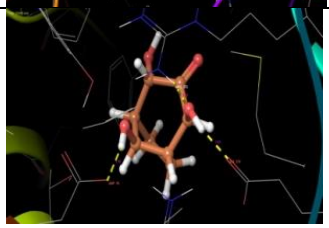
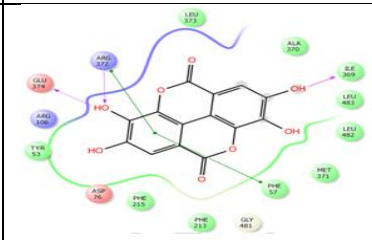
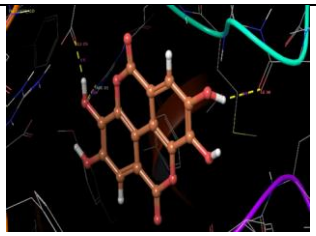
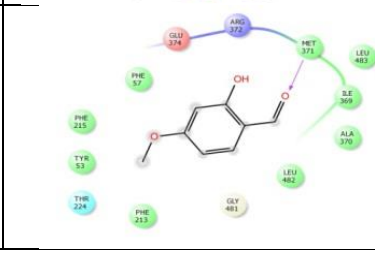
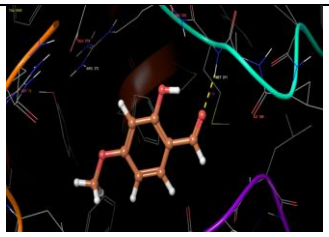
DHA-II			32.2	78.7	45.6
			43.8	93.3	51.8
			58.3	95.0	86.5
DHA-III			56.5	75.2	73.6
			69.6	87.3	86.7
			69.6	87.4	86.7
DHA-IV			32.4	45.7	44.9
			51.6	59.6	59.6
			55.7	74.4	72.8
DHA-V			54.7	68.4	67.6
			54.7	72.9	71.3
			55.3	72.9	71.3
Ellagic acid			77.2	77.2	77.2
			77.2	77.2	77.2
HMB			62.2	40.2	45
			62.2	40.2	45
			74.1	74.1	43

Table 3: Docking score and binding energy of selected phyto compounds of Decalipis. Hamiltonii

Compound	Docking Score	No of H-bonds	Interacting amino acids	H-bond distance (Å)	Binding energy	2D Docking pose	3D Docking pose
DHA-I	-5.914	2	MET 371 ILE 369	1.93 2.03	-26.452		

DHA-II	-3.689	3	Arg 372 Glu 374	1.75, 2.19 2.16	-27.77		
DHA-III	-4.884	3	Arg 372 Glu 374	2.07, 1.92 2.13	-24.004		
DHA-IV	-5.238	2	ARG 372 MET 371	2.06 1.91	-24.113		
DHA-V	-5.779	3	Arg 372 Glu 374 ASP 76	1.75 1.63 1.95	-28.459		
Ellagic acid	-6.993	3	Arg 372 Glu 374 Ile 369	1.99 1.70 2.46	-34.104		
HMB	-4.781	1	MET 371	2.10	-19.35		

Discussion

Herbal products contain several active constituents exhibiting multiple pharmacological effects (Pullaiah et al., 2021). Co-administration of herbal products can potentially interfere with prescription drugs by activation of drug transporters or metabolizing enzymes, and such patients are at the edge of risk of serious drug-herbal interactions. Patients must enquire about the possible interactions when intake of herbal medicines or natural products concurrently used with prescription medication (Rombolà et al., 2020). The combination of drugs and phytochemicals exerts additive and synergistic effects when used in the right combination. Alike, a wrong combination can exert an inhibitive or negative effect including drug-herbal interactions (Susila et al., 2021). The metabolism of xenobiotics (drug molecules, bio-molecules in herbs, food ingredients, and chemicals) in the environment is an essential part of the human organism's defense system. Numerous enzymes especially liver CYP 450 involved in

the conversion of foreign compounds to more soluble metabolites to facilitate excretion or pharmacological agents. Some of the metabolites are harmless, whereas others are reactive and potentially dangerous for the human organism (Thomson et al, 2016). Prediction of the possibility of drug-herbal interactions particularly with cytochrome P450 enzymes is quintessential as a part of preliminary research to narrow down the extensive research.

Roots of Decalipis hamiltonii is used in various Ayurveda preparations and traditionally used as pickles and to store food grains (as bio-insecticide) by tribal people. Aqueous root extract of Decalipis hamiltonii is the major component of the popularly used herbal drink called 'Nannari' consumed during summer in rural areas of South India. The previous works reveal the presence of various phytoconstituents in D. hamiltonii roots including aldehydes, amyrins, lupeol, and volatile flavor compounds such as 2-hydroxy-4methoxybenzaldehyde, vanillin, etc, and an essential oil like 4- methylresorcylaldehyde, atlantone, terpinene, geraniol (Thangadurai et al., 2002). The present study is in agreement with previous studies that, similar compounds were found in preliminary phytochemical and HPTLC fingerprinting analysis of the aqueous root extract of Decalepis hamiltonii.

The HPTLC fingerprinting of the aqueous root extract of Decalepis hamiltonii reveals multiple peaks corresponding to both polar and non-polar substances. The presence of blue dark bands under 254 nm may denote polyphenolic compounds whereas bluish-purple bands signify terpenoids or volatile oil substances (Srinivasan et al., 2016). Volatile oils and polyphenols are biologically active compounds in Decalepis hamiltonii and these compounds hold responsible for drug-herbal interactions (Harish et al., 2005).

Metabolism of drugs and other chemical substances was done by several isoforms of the cytochrome P450 super family enzymes including CYP3A4, CYP2D6, and CYP2C9. Induction or inhibition of these enzymes by drugs or herbal molecules may cause drug-drug or drug-herbal interactions (Wanwimolruk et al., 2014). However, these drug-herbal interactions were underreported and the present research did not much focus on why the drug therapies failed. Hence the present study attempted to identify the possible drug-herbal interaction with the aqueous root extract of Decalipis hamiltonii.

Considering the above statement, the present study attempted to evaluate the possible cytochrome P450 enzyme-associated drug-herbal interactions by using in silico models. The identified seven biologically active chemical substances from AREDH were considered to dock with three prominent CYP enzymes CYP3A4, CYP2D6, and CYP2C9. The prediction of the metabolism sites by the SMARTcyp software for CYP3A4, CYP2D6, and CYP2C9 occurred by use of algorithms that were used for the activation energy of cytochrome P450 for reaction with a molecule. All the seven molecules selected in the present study have shown at least three atoms as metabolic sites for the three enzymes and the most probable CYP involved as CYP3A4.

Further, the binding affinity and the binding energies were calculated for all the seven molecules with CYP3A4, CYP2D6, and CYP2C9 found more selectivity to CYP3A4. Among the seven compounds chosen, ellagic acid has shown the highest value of docking score (-6.993) followed by DHA-I (-5.94), V (-5.779), IV (-5.238), DHA-III (-4.884), HMB (-4.781), and DHA-II (-3.689). All seven molecules have shown the best binding affinity and accurate fit with the best pose of binding. In addition, the most stable complex with the highest binding energy was shown by ellagic acid (-34.104) followed by 2,4,8-trihydroxybicycloocatn-3-one (-28.459), 14-aminotetradeconic acid (-27.77), 4-hydroxy isophthalic acid (-26.452), 2-hydroxy methyl-3-methoxy benzaldehyde (-24.113), 4-(1-hydroxy-1-methyl ethyl)-1-methyl-1,2-cyclohexane diol (-24.004), and 2-Hydroxy-4-methoxy benzaldehyde (-19.35). The results of SMARTCyp and docking studies show that all the seven biologically active molecules of AREDH have the highest possibility to undergo metabolism with CYP enzymes, particularly with CYP3A4, CYP2D6, and CYP2C9 isoenzymes by which most of the drugs and chemicals were metabolized with good binding affinity and binding energy.

CONCLUSION

In conclusion, the finding of the present study reveals that the phyto-chemicals present in the aqueous root extract of D. hamiltonii which were confirmed by HPTLC fingerprinting are metabolized by cytochrome P450 enzymes like CYP3A4, CYP2D6, and CYP2C9. Further, the study identified the major binding sites of chemical substances where the above mentioned CYP enzymes may attack during metabolism of the respective compounds. Co-administration of Nannari juice with conventional medicines that are substrates of CYP3A4, CYP2D6, and CYP2C9 may compete for the same enzymes and may precipitate drug interactions. Further, in-vivo and in-vitro CYP inhibition and induction studies with AREDH are warranted to confirm its involvement in herb-drug interactions.

REFERENCES

1. Nelson VK, Pullaiah CP, Saleem Ts M, Roychoudhury S, Chinnappan S, Vishnusai B, et al. Natural Products as the Modulators of Oxidative Stress: An Herbal Approach in the Management of Prostate Cancer. Adv Exp Med Biol. 2022; 1391: 161-179.
2. Narasimha GVK, Dayanand RG and Pullaiah CP. Traditional Medicine: Safety Evaluation-Need of the Hour. Nat Ayurvedic Med. 2018, 2(7): 000-152.
3. Susila AV, Sangeeta S. Decalepis hamiltonii (Swallow Root) as a Potential Antimicrobial Agent against Endodontic Pathogens: An In Vitro Study. J Oper Dent Endod. 2021; 6 (1):1–5.
4. Wealth of India (1990). A dictionary of Raw Materials. CSIR, New

Delhi, India 1:161.
5. Sengupta M, Ghosh AK, Dalal I, Paul S. Anti-inflammatory activity of root of Decalepis hamiltonii, J Drug Delivery Ther. 2013; 3(1):70-73.
6. Srivastava A, Shivanandappa T. Neuroprotective effect of Decalepis hamiltonii roots against ethanol-induced oxidative stress. Food Chem. 2009; 119: 626-29.
7. Srivastava A, Shivanandappa T. Hepatoprotective effect of the root extract of Decalepis hamiltonii against carbon tetrachlorideinduced oxidative stress in rats. Food Chem. 2009; 118: 411-17.
8. Nayaka, M.H., Sathisha, U.V. and Dharmesh, S.M., Cytoprotective and antioxidant activity of free, conjugated and insoluble-bound phenolic acids from swallow root (Decalepis hamiltonii). Food chemistry. 2010; 119(4): 1307-1312.
9. Sujith, T., Susikumar, S., Sunilkumar, K.N., Radha, P., Shakila, R. and Gopinath, P. Detection of adulteration of Decalepis hamiltonii Wight & Arn. with Hemidesmus indicus (L.) R. Br. by pharmacognostic, molecular DNA fingerprinting by RAPD, chemical and HPTLC studies. Plant Science Today. 2021; 8(3): 610-620.
10. Kamireddy K, Chinnu S, Priyanka PS, Rajini PS, Giridhar P. Neuroprotective effect of Decalepis hamiltonii aqueous root extract and purified 2-hydroxy-4-methoxy benzaldehyde on 6-OHDA induced neurotoxicity in Caenorhabditis elegans. Biomed Pharmacother. 2018 Sep ; 105: 997-1005.
11. Murthy KN, Rajasekaran T, Giridhar P, Ravishankar GA. Antioxidant property of Decalepis hamiltonii Wight & Arn. Indian J Exp Biol. 2006 Oct; 44(10): 832-7.
12. Khandelwal K R. Practical Pharmacognosy techniques & experiments. New Delhi. Nirali Prakashan. 2002. P. 156–161.
13. Zarei M, Shivanandappa T, Zarei M. Natural bioactive 4-Hydroxyisophthalic acid (4-HIPA) exhibited antiproliferative potential by upregulating apoptotic markers in in vitro and in vivo cancer models. Mol Biol Rep. 2020 Jul; 47(7): 5343-5353
14. Srivastava A, Rao LJ, Shivanandappa T. 14-aminotetradecanoic acid exhibits antioxidant activity and ameliorates xenobiotics-induced cytotoxicity. Mol Cell Biochem. 2012 May; 364(1-2):1-9.
15. Srivastava A, Jagan Mohan Rao L, Shivanandappa T. 2, 4, 8-trihydroxybicyclo [3.2.1]octan-3-one scavenges free radicals and protects against xenobiotic-induced cytotoxicity. Free Radic Res. 2012 Mar; 46(3): 320-8.
16. Nagarajan S, Rao LJ. Determination of 2-hydroxy-4-methoxybenzaldehyde in roots of Decalepis hamiltonii (Wight & Arn.) and Hemidesmus indicus R.Br. J AOAC Int. 2003 May-Jun; 86(3): 564-7.
17. Srivastava, A., Rao, L.J.M. and Shivanandappa, T. Isolation of ellagic acid from the aqueous extract of the roots of Decalepis hamiltonii: Antioxidant activity and cytoprotective effect. Food chemistry. 2007; 103(1): 224-233.
18. Chatterjee, S., Jain, S., Jangid, R. and Sharma, M.K. Cytochrome P450 and P-gp mediated herb–drug interactions of some common Indian herbs. Studies in Natural Products Chemistry. 2022; 72, 225-258.
19. Guttman, Yelena, and Zohar Kerem. Computer-Aided (In Silico) Modeling of Cytochrome P450-Mediated Food–Drug Interactions (FDI). International Journal of Molecular Sciences. 2022; 23(15): 8498.
20. Rajashekar, Y., Rao, L.J. and Shivanandappa, T. Decaleside: a new class of natural insecticide targeting tarsal gustatory sites. Naturwissenschaften, 2012; 99(10): 843-852.
21. Pullaiah CP, Nelson VK, Rayapu S, G V NK, Kedam T. Exploring cardioprotective potential of esculetin against isoproterenol induced myocardial toxicity in rats: in vivo and in vitro evidence. BMC Pharmacol Toxicol. 2021 Jul 15; 22(1): 43.
22. Rombolà L, Scuteri D, Marilisa S, Watanabe C, Morrone LA, Bagetta G, Corasaniti MT. Pharmacokinetic Interactions between Herbal Medicines and Drugs: Their Mechanisms and Clinical Relevance. Life (Basel). 2020 Jul 4; 10(7): 106.
23. Thangadurai D, Anitha S, Pullaiah T, Reddy PN, Ramachandraiah OS. Essential oil constituents and in vitro antimicrobial activity of Decalepis hamiltonii roots against foodborne pathogens. J Agric Food Chem. 2002 May 22; 50(11): 3147-9.
24. Srinivasan S, Wankhar W, Rathinasamy S, Rajan R. Free radical scavenging potential and HPTLC analysis of Indigofera tinctoria linn (Fabaceae). J Pharm Anal. 2016 Apr; 6(2):125-131.
25. Harish R, Divakar S, Srivastava A, Shivanandappa T. Isolation of antioxidant compounds from the methanolic extract of the roots of Decalepis hamiltonii (Wight and Arn.). J Agric Food Chem. 2005 Oct 5; 53(20): 7709-14.
26. Wanwimolruk S, Phopin K, Prachayasittikul V. Cytochrome P450 enzyme mediated herbal drug interactions (Part 2). EXCLI J. 2014 Aug 20; 13: 869-96.