Journal of Complementary and Alternative Medical Research

Volume 22, Issue 1, Page 1-10, 2023; Article no.JOCAMR.99581
ISSN: 2456-6276

GC-MS Characterization and Computational Assessment of Phytochemicals in *Heliotropium indicum* Ethanolic Leaves Extract

Bolanle Christianah Faleye [a,b*], Fisayo Abraham Bamisaye [b] and Toluwase Hezekiah Fatoki [b]

[a] Department of Chemical Science, Joseph Ayo Babalola University, Ikeji-Arakeji, Ilesa, Osun State, Nigeria.
[b] Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2023/v22i1447

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99581

Original research article

Received: 08/03/2023
Accepted: 11/05/2023
Published: 23/05/2023

ABSTRACT

Heliotropium indicum (Indian heliotrope) belongs to family *Boraginaceae*, which has wide ethnopharmacological applications such as antiulcer, antirheumatism and anti-cholera. The aim of this study was to characterize the phytochemicals in *H. indicum* ethanolic leaves extract using Gas chromatography-mass spectrometry (GC-MS), and computationally assessed its pharmacokinetics profiles, molecular targets and infer overall medicinal application. The results showed twenty-nine (29) identified constituents of *H. indicum* in its ethanolic leaves extract from GC-MS, many of which are poorly soluble and have low gastrointestinal absorption (GIA). However, some of them are soluble with high GIA and could permeate blood brain barrier (BBB). The results showed that (+)-

**Corresponding author: E-mail: bcfaleye@jabu.edu.ng;*

Isomenthol, and Ergost-22-en-3-ol are the two most active constituents of ethanolic leaves extract of *H. indicum*; targeting androgen receptor (AR) and transient receptor potential cation channel subfamily M member 8 (TRPM8), which have implication in prostate cancer. Thus, *H. indicum* plant extract is a potential anti-PCa medicinal plants. However, there is need for *in vivo* and *in vitro* studies to validate these results, hence promoting these active compounds optimization and clinical evaluations in *H. indicum*.

Keywords: Heliotropium indicum; GC-MS phytochemicals; ethanolic extract; target prediction; molecular docking; prostate cancer.

1. INTRODUCTION

Heliotropium indicum belongs to family *Boraginaceae.* The genus *Heliotropium* encompasses almost 250 species, which are distributed across warm temperate tropical, and subtropical zones of the world [1] nevertheless the species that have been systematically investigated are very few. *H. indicum* (also known as 'Indian heliotrope'), is very common in India, some parts of Africa and Bangladesh, and found in few other countries. Amusan et al. [2] found that the indigenous leafy vegetable *H. indicum* is not widely known and consumed but it is of high nutritional quality and antioxidant potential. *H. indicum* has ethnopharmacological indication for abdominal pain, dermatitis and nervous disorders such as rheumatism [3], malaria, cholera, wound-healing [4,5], intractable fever, sore throat, ulcers, abortion, venereal diseases such as dysmenorrhea (Rashed *et al*., 2018). Others include insect bites, diarrhoea, skin rashes, menstrual disorder, urticarial [3] and whooping cough [6]. Phytochemically, *H. indicum* has vast amount of pyrrolizidine alkaloids which include echinatine, 12-acetyl indicine, indicine, indicine-N-oxide, indicinine, heliotrine, heleurine, lasiocarpine, lasiocarpine-N-oxide, retronecine, supinine and trachelanthamide [7]. *H. indicum* has been extensively studied for a variety of bioactive values and screened for diverse pharmacological actions such as reproductive activity, histo-gastroprotective effect and wound healing activity [8],and analgesic activity [9].

The pharmaceutical potentials of *H. indicum* may be related to its chemical constituents, consequently responsible for their extensive bioactivities indicated in folk medicine [10]. In addition, the variant levels of toxicity and pharmacokinetics exerted by each or a combination of compounds in a natural product make it impossible to generalize the safety of such products [11]. Thus, exact scientific assessment has been necessitated for validation of health claims of medicinal plants. Therefore, it is expedient to investigate the constituents and pharmacological profile of this plant to assure therapeutic indication and safety. The aim of this study therefore, is to characterize the phytochemicals in *H. indicum* ethanolic leaves extract using GC-MS; and computationally assessed its pharmacokinetics profiles, molecular targets and infer overall medicinal application.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

H. indicum plant sample was harvested from the Federal University of Technology Akure, Ondo State, Nigeria. The raw sample was identified and authenticated at the Herbarium center, Ekiti State University, Ado-Ekiti Nigeria with Herbarium No: UHAE 2021379. Fresh, green and undamaged leaves harvested were air-dried until constant weight was obtained. After proper drying, the leaves were blended into fine powder.

2.2 Reagents and Chemicals

All the chemicals and reagents used were of analytical grade, obtained from Sigma Aldrich (USA).

2.3 Sample Treatment and Preparation

One (1) kg of the blended leaves sample was weighed into a spacious container and 2.5 L of ethanol was added. The mixture was agitated continuously for 48 hours after which it was filtered using Muslin cloth and then concentrated by rotary evaporator and the residue kept for further analyses.

2.4 Phytochemical Determination by Gas Chromatography –Mass Spectrometer (GC-MS)

The ethanolic leaves extract of *H. indicum* was analyzed at the Central Laboratory Facility of the Federal University of Technology Akure, Ondo

State Nigeria, for GC-MS bioactive constituents identification using NIST14.L database.

2.5 Ligand Preparation

The structures of identified phytochemical constituents of the ethanolic leaves extract of *H. indicum* were obtained from NCBI PubChem Compound database

(https://pubchem.ncbi.nlm.nih.gov/) in SMILES format, reconstructed and subjected to 3D structure optimization using Chem Sketch/ ACDLab software. The structures were saved in MOL format. File conversion from MOL format to PDB format was done using PyMoL v2.0.7.

2.6 *In silico* Pharmacokinetics

The ligands SMILES were used for *in silico* pharmacokinetics on SwissADME server [12] at default settings, to predicts the absorption, distribution, metabolism, and excretion (ADME) parameters.

2.7 *In-silico* Target Prediction

The ligands SMILES were used for target prediction on Swiss Target Prediction server (http://www.swisstargetprediction.ch/) with *Homo sapiens* as target organism [13].

2.8 Molecular Docking Studies

Docking simulation were conducted on molecular targets obtained from the target prediction analyses in this study, using the method of Fatoki et al. [14]. Briefly, the ligands and protein targets were prepared for docking using Auto Dock Tools (ADT) v1.5.6 [15] at default settings and the output file was saved in pdbqt format. Molecular docking simulations were done using AutoDock Vina v1.1.2 [16], and the outputs of ligand-proteins interaction poses were visualized on Pose View webserver available at https://proteins.plus/ (Stierand *et al*., 2006).

3. RESULTS

The twenty-nine (29) identified constituents of *Heliotropium indicum* ethanolic leaves extract from GC-MS are shown in Table 1 while its pharmacokinetics are shown in Table 2.

The results suggest that many of the constituents are poorly soluble and have low gastrointestinal absorption (GIA), this include ergost-22-en-3-ol, neophytadiene and 1,4-eicosadiene; some are soluble with high GIA, could pervade blood-brain barrier (BBB) and not P-glycoprotein substrate. These include (+)-isomenthol, dodecahydropyrido[1,2-b]isoquinolin-6-one and 2-(pentyloxycarbonyl)benzoic acid.

The results of target prediction for these 29 identified constituents of *H. indicum* ethanolic leaves extract showed that only three (3) compounds [(+)-isomenthol, ergost-22-en-3-ol and dodecahydropyrido[1,2-b]isoquinolin-6-one] have active molecular targets with at least 20% probability (Table 3) which include carbonic anhydrase 1/2, androgen receptor, transient receptor potential cation channel subfamily M member 8, and 11-beta-hydroxysteroid dehydrogenase 1. The docking binding energy of predicted active constituents of *Heliotropium Indicum* ethanolic leaves extract is shown in Table 4 where ergost-22-en-3-ol and (+)-isomenthol have binding scores of -8.0 (kcal.mol-1) and -5.1 (kcal.mol-1) respectively on androgen receptor. Fig. 1 showed selected molecular docking interactions.

4. DISCUSSION

Gas chromatography- mass spectrometry (GC-MS) is suitably used for identification the bioactive constituents of alkaloids, acids esters, alcohols, steroids, and long chain hydrocarbons compounds [17]. This was used to determine the phytochemical constituents, of ethanolic leaves extract of *H. indicum* in this study. The results of this work showed that androgen receptor (AR) and transient receptor potential cation channel subfamily M member 8 (TRPM8) are the best molecular targets of ethanolic leaves extract of *H. indicum*; Ergost-22-en-3-ol and (+)-Isomenthol.

In prostate cancer (PCa) cells, TRPM8 showed strong inward rectification and high calcium selectivity in contrast to its behavior in normal cells which is characterized by outward rectification and poor cationic selectivity. It also plays a role in prostate cancer cell migration [18]. A study has suggested that TRPM8 is possibly a vital new endoplasmic reticulum $Ca^{(2+)}$ release channel, that hypothetically involved in a number of $Ca^{(2+)}$- and store-dependent processes in prostate cancer epithelial cells, including those that are imperative for prostate carcinogenesis, such as apoptosis and proliferation [19]. Moreover, TRPM2 has been found to be a molecular target in PCa, where it maintains stability of several signaling proteins including AR, and OGX-011/custirsen is the phase 3 drug candidate for

TRPM2 [20]. TRPM8 is activated by menthol, eucalyptol, icilin, cold temperature and modulation of intracellular pH. Thus, it is possible that TRPM8 could undergo clinical trial upon discovery of acceptable drug candidates of the class of menthol and eucalyptol.

Androgen receptor (AR), a steroid receptor transcriptional factor for testosterone and dihydrotestosterone, comprises four main domains, the ligand-binding domain DNA-binding domain, N-terminal domain, and hinge region domain. AR exhibits crucial functions in prostate cancer, particularly castration-resistant prostate cancer (CRPC). AR is altered in 60% of CRPC [21,22]. The PCa and androgen insensitivity syndrome (AIS) are example of diseases that are associated with alterations in AR functions. A study has reported that androgen deprivation therapy can inhibits hormone-naïve prostate cancer, but PCa changes AR and acclimates to survive under castration levels of androgen [23]. Blocking the production of androgens with or without inhibition the action of the AR by therapeutic drug, prevented the growth of PCa [24]. AR is a validated molecular target in PCa with enzalutamide as approved standard drug [20].

Table 1. Identified constituents of *Heliotropium indicum* ethanolic leaves extract from GC-MS

SN	Identified Constituents	Retention time	Area	Reference	Quality
1	Neophytadiene	10.509	10.52	138502	99
2	2,6,6-trimethyl-bicyclo[3.1.1]heptane,	10.509	10.52	17379	91
3	1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-ylmethylphenylsulfoxide	10.553	1.54	188513	56
4	6,10,14-trimethyl 2-Pentadecanone	10.553	1.54	128827	49
5	Thunbergol	10.553	1.54	150221	46
6	(Z,E)-9,12-Tetradecadien-1-ol	10.613	2.94	74499	68
7	6-Octen-1-ol, 3,7-dimethyl-, acetate	10.613	2.94	63306	55
8	9-Octadecyne	10.694	4.59	111836	83
9	1,4-Eicosadiene	10.694	4.59	138502	66
10	(3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol	10.894	1.26	239012	55
11	3,7,11-trimethyl-, (Z,E)-2,6,10-Dodecatrien-1-ol	10.894	1.26	85762	55
12	Nonadecane	10.894	1.26	128834	53
13	Phytol	11.701	36.37	155849	94
14	(+)-Isomenthol	11.701	36.37	29169	60
15	Dodecahydropyrido[1,2-b]isoquinolin-6-one	11.849	4.45	71593	42
16	Fumaric acid, 2,4,6-trichlorophenyl tridecyl ester	11.849	4.45	262552	30
17	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	11.849	4.45	141906	25
18	1-chloro-Nonadecane	11.931	7.95	161769	93
19	Eicosane	11.931, 14.457	7.95, 3.12	142239	68, 92
20	Octadecane	11.931, 14.457	7.95, 3.12	115546	64, 92
21	n-Propyl 9,12,15-octadecatrienoate	11.968	6.86	179097	97
22	3-ethenyl-Cyclooctene	11.968	6.86	16103	50
23	(Z)-3-Heptadecen-5-yne	11.968	6.86	96839	46
24	28-Nor-17.beta.(H)-hopane	12.205	8.59	237949	89
25	(1R,2S,8R,8Ar)-8-acetoxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-trans-decalin	12.205	8.59	155648	83
26	28-Nor-17.alpha.(H)-hopane	12.205	8.59	237950	64
27	Bis(2-ethylhexyl) phthalate	14.745	11.32	233373	87
28	2-(Pentyloxycarbonyl)benzoic acid	14.745	11.32	98123	64
29	4-Methoxyanthranilic acid	14.745	11.32	37572	64

Table 2. Predicted pharmacokinetics of identified constituents of *Heliotropium indicum* ethanolic leaves extract from GC-MS

			Predicted ADME Parameters									
SN	Identified Constituents	Pubchem CID	Physicochemical properties			Lipophilicity	Water solubility		Pharmacokinetics			Drug-likeness
			MW	MR	TPSA (Å^2)	Log P	ESOL Log S	ESOL Class	GIA	BBB	P-gp	BS
1	Neophytadiene	10446	278.52	97.31	0.00	7.07	-6.77	Poorlysoluble	Low	No	Yes	0.55
2	2,6,6-trimethyl-bicyclo[3.1.1]heptane	91015300	138.25	45.7	0.00	3.38	-3.15	soluble	Low	Yes	No	0.55
3	1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-ylmethylphenylsulfoxide	565587	330.53	100.93	36.28	5.23	-5.89	Moderatelysoluble	High	No	No	0.55
4	6,10,14-trimethyl 2-Pentadecanone	10408	268.48	88.84	17.07	5.66	-5.09	Moderatelysoluble	High	No	Yes	0.55
5	Thunbergol	5363523	290.48	95.92	20.23	4.75	-4.77	Moderatelysoluble	High	No	No	0.55
6	(Z,E)-9,12-Tetradecadien-1-ol	5365760	210.36	69.63	20.23	4.16	-3.45	Soluble	High	Yes	No	0.55
7	6-Octen-1-ol, 3,7-dimethyl-, acetate	9017	198.3	60.61	26.30	3.46	-3.43	Soluble	High	Yes	No	0.55
8	9-Octadecyne	141998	250.46	86.8	0.00	6.79	-6.13	Poorlysoluble	Low	No	No	0.55
9	1,4-Eicosadiene	5365774	278.52	97.31	0.00	7.34	-6.72	Poorlysoluble	Low	No	No	0.55
10	(3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol	21159880	400.68	128.42	20.23	6.83	-7.63	Poorlysoluble	Low	No	No	0.55
11	3,7,11-trimethyl-, (Z,E)-2,6,10-Dodecatrien-1-ol	445070	222.37	73.96	20.23	4.32	-4.17	Moderatelysoluble	High	Yes	No	0.55
12	Nonadecane	12401	268.52	93.45	0.00	7.54	-6.69	Poorlysoluble	Low	No	No	0.55
13	Phytol	5280435	296.53	98.94	20.23	6.22	-5.98	Moderatelysoluble	Low	No	Yes	0.55
14	(+)-Isomenthol	45056	156.27	49.23	20.23	2.58	-2.88	Soluble	High	Yes	No	0.55
15	Dodecahydropyrido[1,2-b]isoquinolin-6-one	610048	207.31	65.27	20.31	2.48	-2.86	Soluble	High	Yes	No	0.55
16	Fumaric acid, 2,4,6-trichlorophenyl tridecyl ester	91694891	477.85	125.89	52.60	7.64	-7.99	Poorlysoluble	Low	No	No	0.55
17	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	610255	282.29	78.02	52.60	2.86	-2.41	Soluble	High	Yes	No	0.55

			Predicted ADME Parameters									
SN	Identified Constituents	Pubchem CID	Physicochemical properties			Lipophilicity	Water solubility		Pharmacokinetics			Drug-likeness
			MW	MR	TPSA (Å^2)	Log P	ESOL Log S	ESOL Class	GIA	BBB	P-gp	BS
18	1-chloro-Nonadecane	545634	302.97	98.24	0.00	7.68	-7.31	Poorly soluble	Low	No	No	0.55
19	Eicosane	8222	282.55	98.25	0.00	7.90	-7.05	Poorly soluble	Low	No	No	0.55
20	Octadecane	11635	254.49	88.64	0.00	7.18	-6.33	Poorly soluble	Low	No	No	0.55
21	n-Propyl 9,12,15-octadecatrienoate	12115559	320.51	102.92	26.3	6.26	-5.29	Moderately soluble	Low	No	No	0.55
22	3-ethenyl-Cyclooctene	5365641	136.23	47.12	0.00	3.26	-3.23	Soluble	Low	Yes	No	0.55
23	(Z)-3-Heptadecen-5-yne	5367448	234.42	81.52	0.00	6.23	-5.55	Moderately soluble	Low	No	No	0.55
24	28-Nor-17.beta.(H)-hopane	600852	384.68	125.36	0.00	7.86	-8.89	Poorly soluble	Low	No	No	0.55
25	(1R,2S,8R,8Ar)-8-acetoxy-1-(2-hydroxyethyl)- 1,2,5,5-tetramethyl-trans-decalin	6420608	296.44	86.34	46.53	3.61	-4.15	Moderately soluble	High	Yes	No	0.55
26	28-Nor-17.alpha.(H)-hopane	18625	398.71	129.65	0.00	8.28	-9.31	Poorly soluble	Low	No	No	0.55
27	Bis(2-ethylhexyl) phthalate	8343	390.56	116.3	52.60	6.17	-6.06	Poorly soluble	High	No	Yes	0.55
28	2-(Pentyloxycarbonyl)benzoic acid	90531	236.26	63.91	63.60	2.73	-3.31	Soluble	High	Yes	No	0.85
29	4-Methoxyanthranilic acid	351010	167.16	44.3	72.55	0.49	-1.33	Very soluble	High	No	No	0.85

Legend: Molecular weight (MW), Molar Refractivity (MR), Total polar surface area (TPSA). Consensus Log P, ESOL Log S, ESOL Class, Gastrointestinal absorption (GIA), Blood-brain barrier (BBB), P-glycoprotein (P-gp) substrate, Bioavailability Score (BS)

Table 3. Predicted Molecular targets of active constituents of *Heliotropium Indicum* ethanolic leaves extract

S.N	Target Proteins	Gene Code	UniProt ID	% Probability of Target		
				L1	L2	L3
1	Androgen receptor (AF-P10275-F1)	AR	P10275	65	20	
2	Aromatase (AF-P11511-F1)	CYP19A1	P11511	30		
3	Sodium-dependent noradrenaline transporter (AF-P23975-F1)	SLC6A2	P23975	25		
4	Cytochrome P450 2C19 (AF-P33261-F1)	CYP2C19	P33261	30		
5	Carbonic anhydrase 1 (AF-P00915-F1)	CA1	P00915		50	
6	Carbonic anhydrase 2 (AF-P00918-F1)	CA2	P00918		50	
7	Transient receptor potential cation channelsubfamily M member 8 (AF-Q7Z2W7-F1)	TRPM8	Q7Z2W7		95	
8	11-beta-hydroxysteroid dehydrogenase 1 (AF-P28845-F1)	HSD11B1	P28845			40
9	Metabotropic glutamate receptor 5 (AF-P41594-F1)	GRM5	P41594			30
10	Steroid 5-alpha-reductase 1 (AF-P18405-F1)	SRD5A1, SRD5A2	P18405, P31213			20

L1 = (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol. L2 = (+)-Isomenthol. L3 = Dodecahydropyrido[1,2-b]isoquinolin-6-one

Table 4. Docking parameters and binding energy of predicted active constituents of *Heliotropium Indicum* ethanolic leaves extract

S.N	Target Proteins	Docking parameters	Binding Energy ΔG (kcal.mol^{-1})		
			L1	L2	L3
1	Androgen receptor(AF-P10275-F1)	Spacing: 1.000 Npts: 126 × 126 × 126 Center: -7.562 × 4.898 × -1.829	-8.0	-5.1	
2	Aromatase (AF-P11511-F1)	Spacing: 0.550 Npts: 126 × 126 × 126 Center: -2.524 × 4.809 × -4.567	-8.0		
3	Sodium-dependent noradrenaline transporter (AF-P23975-F1)	Spacing: 0.550 Npts: 126 × 126 × 126 Center: -2.641 × -1.605 × 3.603	-8.5		
4	Cytochrome P450 2C19(AF-P33261-F1)	Spacing: 0.550 Npts: 126 × 126 × 126 Center: 1.631 × 0.093 × -0.267	-6.9		
5	Carbonic anhydrase 1(AF-P00915-F1)	Spacing: 0.375 Npts: 126 × 126 × 126 Center: -1.071 × 2.774 × 0.190		-4.1	
6	Carbonic anhydrase 2(AF-P00918-F1)	Spacing: 0.375 Npts: 126 × 126 × 126 Center: 0.596 × 1.998 × -3.094		-4.0	

S.N	Target Proteins	Docking parameters	Binding Energy ΔG (kcal.mol^{-1})		
			L1	L2	L3
7	Transient receptor potential cation channelsubfamily M member 8 (AF-Q7Z2W7-F1)	Spacing: 1.000 Npts: 126 × 126 × 126 Center: -3.765 × 0.769 × 1.248		-5.1	
8	11-beta-hydroxysteroid dehydrogenase 1(AF-P28845-F1)	Spacing: 0.400 Npts: 126 × 126 × 126 Center: -6.057 × 5.268 × -3.477			-5.1
9	Metabotropic glutamate receptor 5(AF-P41594-F1)	Spacing: 1.000 Npts: 126 × 126 × 126 Center: -13.333 × -3.795 × 8.435			-6.3
10	Steroid 5-alpha-reductase 1 (AF-P18405-F1)	Spacing: 0.472 Npts: 126 × 126 × 126 Center: -6.617 × 2.581 × -0.903			-4.8

L1 = (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol. L2 = (+)-Isomenthol. L3 = Dodecahydropyrido[1,2-b]isoquinolin-6-one

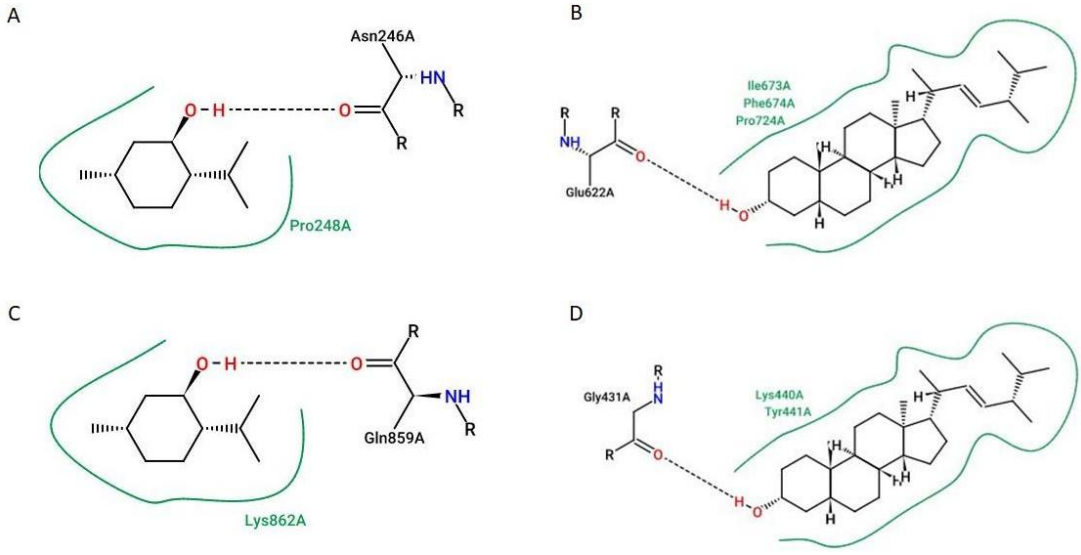

Fig. 1. Selected molecular docking interactions, (A) Carbonic anhydrase 1 (AF-P00915-F1) and (+)-Isomenthol. (B) Androgen receptor (AF-P10275-F1) and (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol. (C) Androgen receptor (AF-P10275-F1) and (+)-Isomenthol. (D) Aromatase (AF-P11511-F1) and (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol

5. CONCLUSION

This work showed that (+)-Isomenthol, and (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol are the two most active constituents of ethanolic leaves extract of *H. indicum*; targeting transient receptor potential cation channel subfamily M member 8 (TRPM8) and androgen receptor (AR), which have implication in prostate cancer. Thus, *H. indicum* plant extract is a potential anti-PCa medicinal plant, and further *in vivo* and *in vitro* studies are required to validate the results of the study; and also promote *H. indicum* active compounds optimization and its clinical evaluations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gurib-Fakim A, Schmelzer GH. Medicinal plants. PROTA Foundation, Netherlands. 2008;1:790.
ISBN: 9789057822049
2. Amusan T, Bello M, Oladeji O. Proximate, vitamin assays and anti-oxidant properties of an underutilised indigenous vegetable *Heliotropium indicum* L. (Lamiales:

Boraginaceae) in West Africa in enhancing diet diversification. Revista Brasileira de Gestão Ambiental e Sustentabilidade. 2016;3(6):337-353.
ISSN: 2359-1412
Available:http://dx.doi.org/10.21438/rbgas.030608

3. Muthu C, Ayyanar M, Raja N, Ignacimuttu S. Medicinal plants used by traditional healers in Kancheepuran district of Tamil Nadu, India. Journal of Ethnobiol. Ethnomed. 2016;2:48.
4. Togola A, Diallo D, Dembélé S, Barsett H, Paulsen BS. Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. J Ethnobiol Ethnomedicine. 2005;1(1):7.
5. Wiart C. Medicinal plants of the Asia-Pacific: Drugs for the future? World Scientific Publishing Co. Pte. Ltd., Singapore; 2006.
6. Pranabesh G, Prakriti D, Chandrima D, Supratim M, Sirshendu C. Morphological characteristics and phyto-pharmacological detailing of hatishur (*Heliotropium indicum* Linn.): A concise review. Journal of Pharmacognosy and Phytochemistry. 2018;7(5):1900-1907.
7. Boye A, Koffuor GA, Ameyaw EO, Abaitey AK. Analgesic activity and safety assessment of *Heliotropium indicum* Linn. (boraginaceae) in rodents. International Journal of Pharmacology. 2012;8(2):91-100.
8. Dolui AK, Atul K, Manabendra D. Reproductive activities of *Heliotropium indicum* isolate against *Helopeltis theivora* and toxicity evaluation in mice. *Journal Environmental Biology*. 2012;33:603-607.
9. Ashutosh S, Rosario del Carmen F, Alexandre C, Maria L. Antibacterial activities of medicinal plants used in Mexican traditional medicine. Journal of Enthnopharmacology. 2017;208:264-329.
10. Koffuor GA, Boye A, Amoateng P, Ameyaw EO, Abaitey AK. Investigating the site of action of an aqueous extract of *Heliotropium indicum* Linn (boraginaceae) on smooth muscles. Research Journal of Pharmacology. 2012;6(1):12-19.
11. Grollman AP. Role of environmental toxins in endemic (balkan) nephropathy. Journal of American Society on Nephrology. 2007;18:2817-2823.
12. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Report. 2017;7(1):42717.
Available:https://doi.org/10.1038/srep42717
13. Daina A, Michielin O, Zoete V. Swiss Target Prediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Research. 2019;47(W1):W357-W364.
Available:https://doi.org/10.1093/nar/gkz382
14. Fatoki T, Chukwuejim S, Ibraheem O, Oke C, Ejimadu B, Olaoye I, Oyegbenro O, Salami T, Basorun R, Oluwadare O, Salawudeen Y. Harmine and 7,8-dihydroxyflavone synergistically suitable for amyotrophic lateral sclerosis management: An *insilico* study. Research Results in Pharmacology. 2022;8(3):49-61.
DOI 10.3897/rrpharmacology.8.83332
15. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and Auto Dock Tools 4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry. 2009;30(16):2785–2791.
Available:https://doi.org/10.1002/jcc.21256
16. Trott O, Olson AJ. Auto Dock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 2010;31(2):455–461.
Available:https://doi.org/10.1002/jcc.21334
17. Muthulakshmi A, Margret JR, Mohan VR. GC-MS Analysis of Bioactive components of *Feonia elephantum* correa (rutaceae). J. App. Pharm. Sci. 2012; 2(2):69-74.
18. Gkika D, Lemonnier L, Shapovalov G, Gordienko D, Poux C, Bernardini M, Bokhobza A, Bidaux G, Degerny C, Verreman K, Guarmit B, Benahmed M, de Launoit Y, Bindels RJ, Fiorio Pla A, Prevarskaya N. TRP channel-associated factors are a novel protein family that regulates TRPM8 trafficking and activity. J Cell Biol. 2015;208(1):89-107.
DOI:10.1083/jcb.201402076
19. Thebault S, Lemonnier L, Bidaux G, Flourakis M, Bavencoffe A, Gordienko D, Roudbaraki M, Delcourt P, Panchin Y, Shuba Y, Skryma R, Prevarskaya N. Novel role of cold/menthol-sensitive transient receptor potential melastatine family

member 8 (TRPM8) in the activation of store-operated channels in LNCaP human prostate cancer epithelial cells. J Biol Chem. 2005;25;280(47):39423-35.
DOI: 10.1074/jbc.M503544200

20. Shtivelman E, Beer TM, Evans CP. Molecular pathways and targets in prostate cancer. Oncotarget. 2014;5(17):7217-7259.
21. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M. Integrative genomic profiling of human prostate cancer. Cancer cell. 2010;18(1):11-22.
22. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M. The mutational landscape of lethal castration-resistant prostate cancer. Nature.2012;487(7406):239-243.
23. Fujita K, Nonomura N. Role of Androgen Receptor in Prostate Cancer: A Review. WorldJ Mens Health. 2019;37(3):288-295.
DOI: 10.5534/wjmh.180040
24. Tan MH, Li J, Xu HE, Melcher K, Yong EL. Androgen receptor: Structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin. 2015;36(1):3-23.
DOI: 10.1038/aps.2014.18

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/99581