

## GC-MS characterization and computational assessment of phytochemicals in *Heliotropium indicum* ethanolic leaves extract

### 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 (+)-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.

## 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 (Gurib-Fakim and Schmelzer, 2008) 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.* (2016) 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 (Muthu *et al.*, 2016), malaria, cholera, wound-healing (Togola *et al.*, 2005; Wiart, 2006), 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 (Muthu *et al.*, 2016) and whooping cough (Pranabesh *et al.*, 2018). 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 (Boye *et al.*, 2012). *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 (Dolui *et al.*, 2012), and analgesic activity (Ashutosh *et al.*, 2017).

The pharmaceutical potentials of *H. indicum* may be related to its chemical constituents, consequently responsible for their extensive bioactivities indicated in folk medicine (Koffuor *et al.*, 2012). 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 (Grollman, 2007). 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.

## **MATERIALS AND METHODS**

### **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.

### **Reagents and chemicals**

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

### **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.

### **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.

### **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 ChemSketch/ACD/Labs software. The structures were saved in MOL format. File conversion from MOL format to PDB format was done using PyMOL v2.0.7.

### ***In silico* pharmacokinetics**

The ligands SMILES were used for *in silico* pharmacokinetics on SwissADME server (Daina *et al.*, 2017) at default settings, to predict the absorption, distribution, metabolism, and excretion (ADME) parameters.

### ***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 (Daina *et al.* 2019).

### **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.* (2022). Briefly, the ligands and protein targets were prepared for docking using AutoDock Tools (ADT) v1.5.6 (Morris *et al.* 2009) at default settings and the output file was saved in pdbqt format. Molecular docking simulations were done using AutoDock Vina v1.1.2 (Trott and Olson 2010), and the outputs of ligand-proteins interaction poses were visualized on PoseView webserver available at <https://proteins.plus/> (Stierand *et al.*, 2006).

### **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<sup>-1</sup>) and -5.1 (kcal.mol<sup>-1</sup>) respectively on androgen receptor. Figure 1 showed selected molecular docking interactions.

## 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 (Muthulakshmi *et al.*, 2012). 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 (Gkika *et al.*, 2015). A study has suggested that TRPM8 is possibly a vital new endoplasmic reticulum  $\text{Ca}^{(2+)}$  release channel, that hypothetically involved in a number of  $\text{Ca}^{(2+)}$ - and store-dependent processes in prostate cancer epithelial cells, including those that are imperative for prostate carcinogenesis, such as apoptosis and proliferation (Thebault *et al.*, 2005). 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 (Shtivelman *et al.*, 2014). 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 (Taylor 2010; Grasso *et al.*, 2012). 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 (Fujita and Nonomura, 2019). Blocking the production of androgens with or without inhibition the action of the AR by therapeutic drug, prevented the growth of PCa (Tan *et al.*, 2015). AR is a validated molecular target in PCa with enzalutamide as approved standard drug (Shtivelman *et al.*, 2014).

**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

SN	Identified Constituents	Pubchem CID	Predicted ADME Parameters									
			Physicochemical properties			Lipophilicity	Water solubility		Pharmacokinetics			Drug-likeness
			MW	MR	TPSA (Å <sup>2</sup> )	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	Poorly soluble	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	Moderately soluble	High	No	No	0.55
4	6,10,14-trimethyl 2-Pentadecanone	10408	268.48	88.84	17.07	5.66	-5.09	Moderately soluble	High	No	Yes	0.55
5	Thunbergol	5363523	290.48	95.92	20.23	4.75	-4.77	Moderately soluble	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	Poorly soluble	Low	No	No	0.55
9	1,4-Eicosadiene	5365774	278.52	97.31	0.00	7.34	-6.72	Poorly soluble	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	Poorly soluble	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	Moderately soluble	High	Yes	No	0.55
12	Nonadecane	12401	268.52	93.45	0.00	7.54	-6.69	Poorly soluble	Low	No	No	0.55
13	Phytol	5280435	296.53	98.94	20.23	6.22	-5.98	Moderately soluble	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	Poorly soluble	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
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

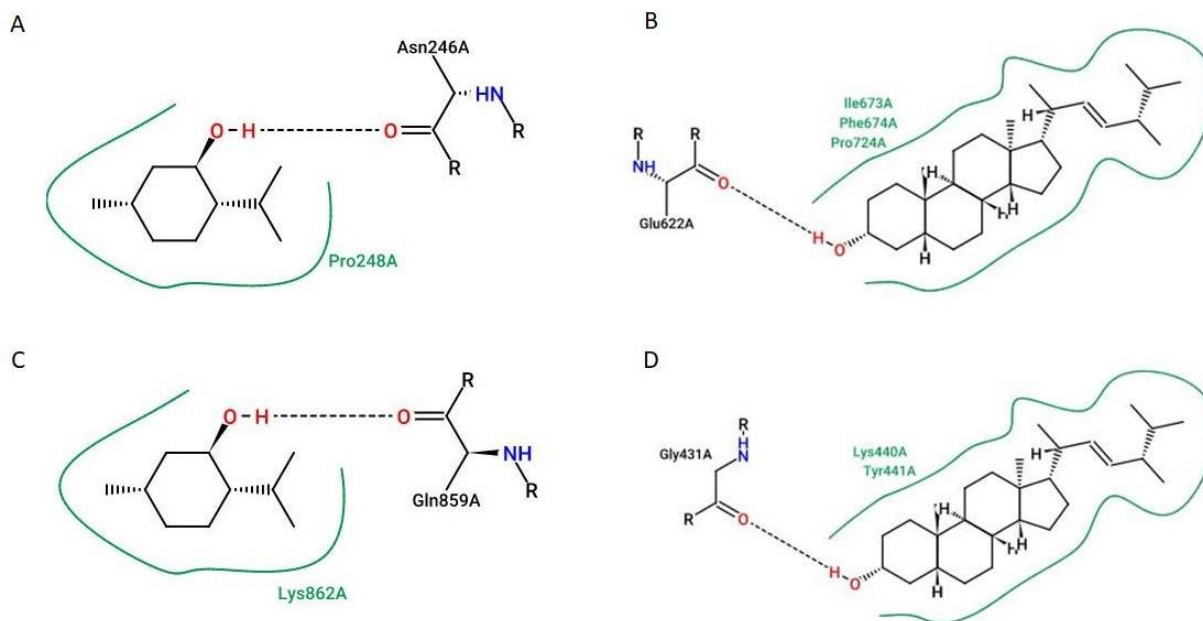
S.N	Target Proteins	Gene Code	UniProt ID	% Probability of Target		
				L1	L2	L3
1	Androgen receptor (AF-P10275-F1)	AR	P10275	65	20	
2	Aromatase (AF-P11511-F1)	CYP19A1	P11511	30		
3	Sodium-dependent noradrenaline transporter (AF-P23975-F1)	SLC6A2	P23975	25		
4	Cytochrome P450 2C19 (AF-P33261-F1)	CYP2C19	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 channel subfamily 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 $\Delta G$ (kcal.mol <sup>-1</sup> )		
			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	
7	Transient receptor potential cation channel subfamily 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



**Figure 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.

## 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.

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