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Insilico evaluation of pharmacological activities of *Kalarchi Chooranam*

Research Article

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Abstract

The Indian traditional medicine practices such as siddha and ayurveda records various medicinal plants for the betterment of human health. Recent reports shows that this system of medicine has potential plants and formulation to treat various diseases and ailments. In the present study, we extracted and prepared the *Kalarchi Chooranam* of various ratios and chosen 4:1 ratio from our previous work. The aqueous extract of 4:1 ratio *Kalarchi Chooranam* was subjected to GCMS study and the compounds Inositol, Xanthosine (CAS), alpha.-D-Glucopyranoside, methyl 2,3-bis-O- were identified as lead 3 compounds. The Xanthosine (CAS), alpha.-D-Glucopyranoside, methyl 2,3-bis-O- have passed the drug likeness and ADME properties and further docked against various receptors involved in disease pathogenesis such as cancer (Bcl2, Bcl-X), PCOS (Cyp17, Follistatin), Inflammation & Analgesic (Cox1, Cox2), and Diabetics & Cardiovascular diseases (Er Alpha, Er Beta). The overall study reveals that the compounds have more binding affinities against the poly cystic ovarian syndrome receptors as the Xanthosine showed significant binding energies of -8.9 and -8.2 Kcal/mol and the Alpha.-D-Glucopyranoside, methyl 2,3-bis-O showed -8.2 and -9.7 Kcal/mol against CYP17 and Follistatin. The *Kalarchi Chooranam* has to be studied more reveal all its pharmacological activities.

Key Words: *Kalarchi Chooranam, Siddha medicine,* PCOS, Molecular Docking.

Introduction

The Indian traditional medicine systems such as siddha, ayurveda and unani represents alternative and complementary medicine in the modern world (1, 2). These medicinal practices especially siddha consists of various medicinal valued plants with versatile medicinal properties to treat several diseases and ailments (3, 4, 5). These indigenous culture were followed in ancient days in several countries and some follow it even in the current era of medicine (6, 7, 8). The medicinal plants and other formulations reported in the Indian medicinal systems were reported to have various pharmacological activities including antimicrobial, antioxidant, antidiabetic, anti-inflammatory, antiulcer, anticancer, antiproliferative, antidepressant and antitumor potential (9, 10, 11, 12). These medicinal herbs and plants have versatile phytoconstituents such as alkaloids, flavonoid, polyphenol, etc., which posses these medicinal properties to support and enhance the human health (13, 14, 15). Many polyherbal formulations (chooranas) have been reported to have combination of medicinal plants which shows relatively high amount of phytoconstituents when compared when used as a single composition, thus showing high medicinal properties (16, 17, 18).

The bioinformatic tools is helping the speedy process of drug discovery in present days. The computer aided drug discovery also helps to identify the medicinal value and the pharmacological potential of traditional medicinal plants in more details with clear understanding of their molecular targets in various diseases including cancer (19, 14). The drug likeness properties by Lipinski's rule of five and the adsorption, distribution, metabolism, excretion and toxicity (ADMET) evaluation of lead compounds also help in the identification of suitable drug candidates (20). The molecular modelling studies evidenced various phytoconstituents were further studied for their biological activities and showed great potential to various disease pathogenesis such as diabetics, cancer and cardiovascular diseases (21, 22).

In our previous study, we have reported that in total of 8 ratios of aqueous extract, the 4:1 (*Caesalpinia bonduc: Piper nigrum*) ratio of *Kalarchi Chooranam* have potential antioxidant, antibacterial and antifungal activities. In the present study, we quantified the phytoconstituents in the 4:1 ratio of *Kalarchi Chooranam* through GCMS analysis and predicted that the Inositol, Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O as the top 3 lead compounds. These compounds were evaluated for their drug likeness and ADME properties and Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O have passed. Further these 2 compounds were docked against various molecular targets of cancer, PCOS, inflammation & analgesic, and analgesic, antipyretic

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activity. The Bcl-2 and Bcl-X acts as an antiapoptotic proteins in cancer, Cyp17 involves in the androgen synthesis pathway and Follistatin promotes PCOS, Cox1 and Cox2 involves in the inflammation and analgesic, Er Alpha and Er Beta receptors in the diabetics and CVD pathogenesis respectively (23, 24, 25). Overall, the 2 lead compounds showed good binding affinities against all the receptors and showed significant binding affinities against the PCOS receptors. Xanthosine showed significant binding energies of -8.9 and -8.2 Kcal/mol and the Alpha.-D-Glucopyranoside, methyl 2,3-bis-O showed -8.2 and -9.7 Kcal/mol against CYP17 and Follistatin. From this study, we conclude that these compounds from the aqueous extract of 4:1 ratio of *Kalarchi Chooranam* have potential binding affinities against PCOS and further molecular mechanisms of these compounds against PCOS has to be studied in future.

Materials and Methods

Extraction

The finely powdered samples with various ratios such as 1:1, 2:1, 3:1, 4:1, 1:2, 2:2, 3:2, 4:2 of powdered *Caesalpinia bonduc* and *Piper nigrum* respectively were prepared. The Soxhlet's extraction procedure by distilled water as solvent was followed and the extracts were filtered as mentioned in our previous work (26). We reported that the 4:1 (*Caesalpinia bonduc:Piper nigrum*) ratio of *Kalarchi Chooranam* shower significant antioxidant and antimicrobial activities due to the high presence of phytoconstituents.

Quantification of phytochemicals through GC-MS

The phytoconstituents present in the 4:1 ratio of *Kalarchi Chooranam* was quantified through the GCMS-QP2010 system. The RTX stationary column with a diameter of 0.3µm and length of 30m was used for the separation and Helium was used as mobile phase with a flow rate of 1 mL/ min. Then oven temperature was set at 105°C and the inject section temperature was set at 275°C respectively. The ionizing temperature in the MS was set at 205°C and the compound interface temperature was set at 285°C respectively. The voltage in the detector was set at 0.15kV and the mass range was set as 30-290 m/z in the MS. The detected compounds were then matched in the NIST library (27, 19).

Molecular modelling studies

The three-dimensional structures of receptors such as Bcl-2 (4IEH), Bcl-X (3ZK6), Cyp17 (3RUK), Follistatin (2BOU), Cox1 (6Y3C), Cox2 (5KIR), Er Alpha (1GWR), Er Beta (5TOA) were retrieved from PDB and the compounds such as Inositol (892), Xanthosine (CAS) (33510), alpha.-D-Glucopyranoside, methyl 2,3-bis-O- (64959) were retrieved from PubChem respectively. The physiochemical properties, drug likeness properties and the ADME properties of the compounds were evaluated using Molinspiration. For the molecular docking, the grid box was set with respected to the receptors and the energy minimized compounds were docked against those receptors with default parameters using Autodock software (28, 29). The binding affinities of the protein-ligand complexes were evaluated and their binding energies were calculated.

Result and Discussion

GCMS Analysis

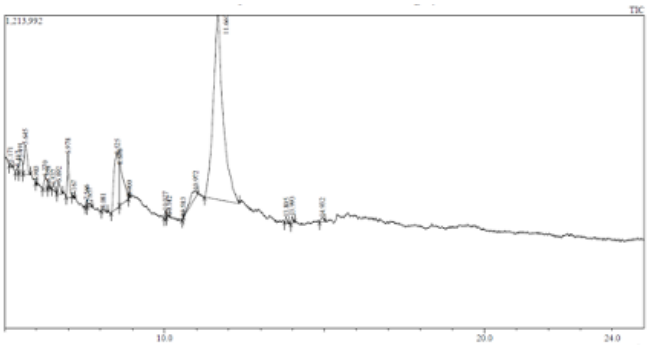


Figure 1: Chromatogram of aqueous extract of 4:1 ratio of the *Kalarchi Chooranam*

The aqueous extract of 4:1 ratio of the *Kalarchi Chooranam* was subjected to GCMS analysis to determine the phytoconstituents composition present in it. Nearly 25 individual compounds were identified with specific peak area % and unique retention time (RT) as shown in Fig 1 and Table 1. Inositol, Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O were identified as the top 3 predominant compounds (Bolded compounds in the peak details table). The area % and the RT of these compounds were identified as 67.37, 9.83, 6.08 and 11.660, 8.525, 8.608 respectively. These top 3 compounds were further taken for the *Insilico* molecular modelling studies.

Drug likeness and ADME properties

The drug likeness and ADME properties of the top three compounds from the GCMS analysis was performed using molinspiration webserver. The physiochemical and drug likeness properties such as Molecular formula, Molecular weight (g/mol), H-bond donor, H-bond acceptor, Lipophilicity (LogP) were predicted and given in the Table 2, and the ADME properties such as Lipophilicity (LogP), Hydrophilicity (LogS), Topological polar surface area (TPSA), Gastrointestinal absorption, Blood-Brain Barrier permeability, Bioavailability, along with its Synthetic accessibility were also predicted and given in the Table 3 respectively. The compounds Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O. have passed all the drug likeness properties but Inositol failed to pass the drug likeness properties evaluated by Lipinski's rule by high LogP value. So, the compounds Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O were only further taken for the molecular docking study.

Table 1: Peak details of GCMS analysis

Peak No.	RT	Area%	Height%	A/H	Name
1	5.171	0.16	0.66	3.01	Cyclohexanamine, N-3-butenyl-N-methyl-
2	5.417	0.45	1.35	4.18	Linalool oxide (cis-anhydrofuran)
3	5.491	1.78	3.25	6.93	4,5-Dimethyl-4-hexen-3-one
4	5.645	3.14	6.29	6.30	2,3-Dihydroxypropyl acetate
5	5.983	0.05	0.52	1.14	1-Propyne (CAS) Propyne
6	6.270	1.20	2.62	5.77	Acetic acid, pentyl ester (CAS) n-Amyl acetate
7	6.358	0.32	1.57	2.58	N-(P-ANISIDINOMETHYL)-4-METHYLPH
8	6.537	0.36	1.09	4.12	1,2-Butanediol, 1-(2-furyl)-2,3-dimethyl-
9	6.692	0.97	2.43	5.04	BICYCLO[4.2.0]OCTA-1,3,5-TRIENE-7,8-D
10	6.978	3.11	9.24	4.25	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxy
11	7.167	0.07	0.39	2.39	2(5H)-Furanone, 3,5-dimethyl-
12	7.566	0.28	1.33	2.63	trans-3-Hexen-1-ol, trimethylsilyl ether
13	7.657	0.37	0.91	5.08	[10B]-TRIETHYLBORANE
14	8.081	0.35	0.60	7.22	1-(3-FLUOROBENZYL)-2(1H)-IMINO-3-M
15	8.525	9.83	11.47	10.81	**Xanthosine** (CAS)
16	8.608	6.08	8.83	8.68	**Alpha.-D-Glucopyranoside, methyl 2,3-bis-O**
17	8.900	0.08	0.42	2.30	N-(P-ANISIDINOMETHYL)-4-METHYLPH
18	10.027	0.32	1.52	2.63	Phthalic acid, di-(1-hexen-5-yl) ester
19	10.142	0.21	0.47	5.59	BUT-3-YNOIC ACID
20	10.583	0.06	0.22	3.62	(1H-Imidazol-4-yl)oxoacetic acid
21	10.972	2.37	1.49	20.12	3,5-Dihydroxy-6-(hydroxymethyl)oxan-2-one
22	11.660	67.37	39.62	21.45	**INOSITOL**
23	13.805	0.30	1.18	3.25	Oxiranecarboxamide, 2-ethyl-3-propyl- (CAS)
24	13.993	0.33	1.52	2.72	Hexadecanoic acid (CAS) Palmitic acid
25	14.932	0.45	1.01	5.60	6,8-DIOXABICYCLO(3.2.1)OCTAN-2L-OL-

Table 2: Physiochemical and Drug likeness properties

Properties	INOSITOL	Xanthosine	Alpha.-D-Glucopyranoside, methyl 2,3-bis-O
MF	C6H12O6	C10H12N4O6	C5H10O4
MW (g/mol)	180.16	284.23	134.13
HBD	6	5	2
HBA	6	7	4
LogP	2.67	-1.92	-0.46

Table 3: ADME properties

Properties	INOSITOL	Xanthosine	Alpha.-D-Glucopyranoside, methyl 2,3-bis-O
LogP	-2.67	-1.92	-0.46
LogS	1.38	-0.32	0.22
TPSA (Å²)	121.38	153.46	66.76
GIA	Low	Low	High
BBBP	No	No	No
BA	0.55	0.55	0.55
SA	3.53	3.84	2.09

Molecular docking

The molecular docking analysis was performed for the compounds Xanthosine (CAS) (33510), alpha.-D-Glucopyranoside, methyl 2,3-bis-O- (64959) against Bcl2 (4IEH), Bcl-X (3ZK6), Cyp17 (3RUK), Follistatin (2BOU), Cox1 (6Y3C), Cox2 (5KIR), Er Alpha (1GWR), Er Beta (5TOA) by Autodock software. The binding energies (Kcal/mol) of the above compounds against the various proteins were predicted along with the Interacting Hydrophobic residues around 4 Å of the active pocket and the data were given in the table 4. The surface model and the cartoon models of Xanthosine with all the proteins were shown in Fig 2 and Fig 3 respectively. The surface model and the cartoon models of alpha.-D-Glucopyranoside, methyl 2,3-bis-O- with all the proteins were shown in Fig 4 and Fig 5 respectively. Comparatively, both the lead compounds showed good binding affinities against the PCOS receptors CYP17 and Follistatin with significant binding affinities.

Table 4: Binding affinities of protein-ligand complexes

Compound Name	Protein name	Mean binding energy (Kcal/mol)	Interacting Hydrophobic residues around 4 Å
Xanthosine	Bcl2	-6.1	ALA59, ASP62, PHE63, ARG66
	Bcl-X	-6.3	ALA89, GLU92, ALA93, GLU96
	CYP 17	-8.9	ARG45, ASP207, ASN208, LEU209, SER210, LYS211, ASP216, TRP220
	Follistatin	-8.2	PHE97, ARG100, TYR101, GLY138, VAL141, TYR195, ASN197
	Cox1	-6.8	ALA199, ALA202, GLN203, THR206, HIS207
	Cox2	-5.7	HIS39, CYS41, GLN42, ASN43
	Er Alpha	-6.7	MET343, LEU346, LEU349, ALA350, GLU353
	Er Beta	-6.8	GLU332, SER333, TRP335, MET336, GLU337
Alpha.-D-Glucopyranoside, methyl 2,3-bis-O-	Bcl2	-7.3	ALA59, ASP62, PHE63, ARG66, TYR67
	Bcl-X	-6.4	PHE97, ARG102, PHE105, SER106
	CYP 17	-8.2	ALA105, ALA113, ASN202, ILE205, ILE206, VAL236, ARG239, GLY301, ALA302
	Follistatin	-9.7	ARG22, CYS23, PRO25, GLY26, PHE27, SER28, SER29, SER31, GLU32, ILE44
	Cox1	-7.3	LEU96, ASN102, ARG105, VAL107, ALA108
	Cox2	-8.0	CYS36, HIS39, CYS41, GLY45, VAL46, CYS47
	Er Alpha	-7.8	GLU323, PRO324, PRO325, ILE326, GLU353
	Er Beta	-7.9	GLU276, PRO277, PRO278, HIS279

Fig 2: Surface model interaction of Xanthosine against
A) Bcl2, B) Bcl-X C) CYP17 D) Follistatin, E) Cox1, F) Cox2, G) Er Alpha, H) Er Beta

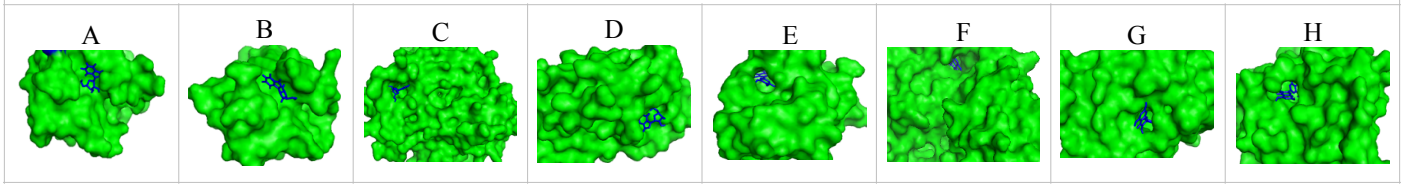


Fig 3: Cartoon model interaction of Xanthosine against
A) Bcl2, B) Bcl-X C) CYP17 D) Follistatin, E) Cox1, F) Cox2, G) Er Alpha, H) Er Beta

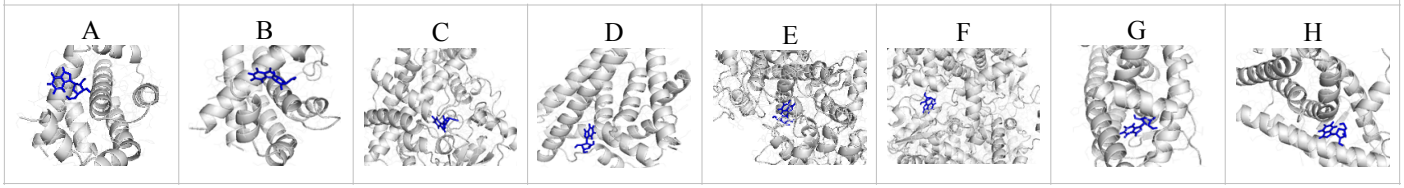


Fig 4: Surface model interaction of Alpha.-D-Glucopyranoside, methyl 2,3-bis-O against
A) Bcl2, B) Bcl-X C) CYP17 D) Follistatin, E) Cox1, F) Cox2, G) Er Alpha, H) Er Beta

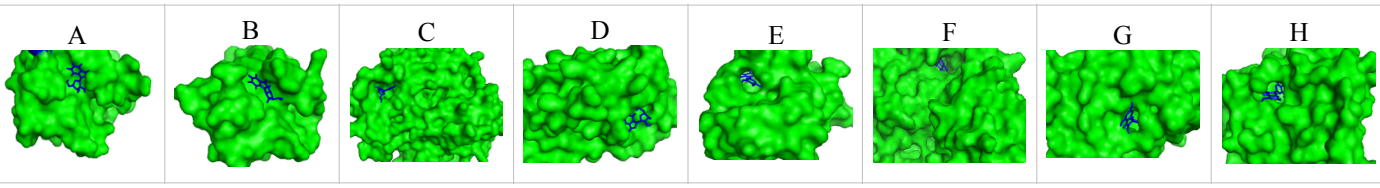
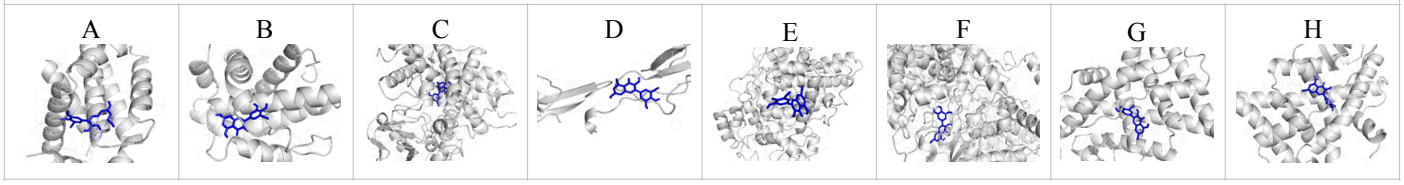


Fig 5: Cartoon model interaction of Alpha.-D-Glucopyranoside, methyl 2,3-bis-O against
A) Bcl2, B) Bcl-X C) CYP17 D) Follistatin, E) Cox1, F) Cox2, G) Er Alpha, H) Er Beta



Conclusion

The aqueous extract of 4:1 ratio of *Kalarchi Chooranam* was prepared and subject to the GCMS analysis revealed that Inositol, Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O are the top 3 lead compounds. The Xanthosine, and Alpha.-D-Glucopyranoside, methyl 2,3-bis-O alone passed the drug likeness and ADME properties and further molecular docking of these 2 compounds against various receptors showed the good binding affinities and comparatively they showed strong and significant activities against the PCOS receptors. Xanthosine showed significant binding energies of -8.9 and -8.2 Kcal/mol and the Alpha.-D-Glucopyranoside, methyl 2,3-bis-O showed -8.2 and -9.7 Kcal/mol against CYP17 and Follistatin. From this study, we conclude that these compounds from the aqueous extract of 4:1 ratio of *Kalarchi Chooranam* have potential binding affinities against PCOS and further molecular mechanisms of these compounds against PCOS has to be studied in future.

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