EVALUATING AND IDENTIFYING THE PHARMOCOLOGICAL ACTIVIES OF *Cissus quadrangularis* USING MOLECULAR DOCKING AND ANIMAL MODEL

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ABSTRACT:

Cissus quadrangularis is a succulent plant belongs to the family of *Vitaceae*. It is a fleshy, cactus in nature. It is additionally acknowledged as *Vitis quadrangularis*. It is commonly observed in tropical and sub-tropical xeric wood. It is an historical remedy plant native to hotter components of Ceylon and India. This plant has been said to include flavonoids, triterpenoids, phytosterols, glycosides and wealthy supply of calcium. This learns about targets to deliver a systematic evaluate of *Cissus quadrangularis* in a variety of pharmacological mechanisms. Evidences from the preceding research recommended the efficacy of *Cissus quadrangularis* with anti-microbial, anti-diabetic, anti-inflammatory, anti-obesity, bone turnover, cell reinforcement, ache relief, gastro-intestinal treatments, cardiovascular and hepatoprotective activities. In conclusion, the plant seems helpful of pharmacological investigations for new drug formula for inflammation.

Keywords: *Cissus quadrangularis*, bone turnover, calcium, pharmacological, anti-inflammatory, flavonoids, *Vitis quadrangularis*

INTRODUCTION:

Cissus quadrangularis is a perennial plant of the grape family. It is usually recognized as Veldt Grape or Devil's Backbone (Ganesan & Choi 2016). The plant generally acknowledged as a "bone setter"- it is referred to as Asthisandhani in Sanskrit and Hodjod in Hindi due to the fact of its potential to join bones. *Cissus quadrangularis* is used as a medicinal plant (Abu Bakar et al., 2020). In siddha medication, it is regarded as tonic and analgesic. It is additionally used to heal damaged bones, injured ligaments and tendons (Liebsch et al., 2011). Therefore, it is identified as Asthisamharaka (that which prevents the destruction of bones). The clean stem and leaves of *Cissus quadrangularis* are used for the therapy of hemorrhoids, menstrual disorders, scurvy and flatulence. It requires a heat tropical climate. It is propagated the usage of the stem slicing techniques in the months of June to July (Kubo et al., 2006). The plant is successfully reproduced using its mature stem cuttings. Sickness free, healthful and mature plant of Cissus quadrangularis was once used as

a supply of stem cuttings for further development (Evans, W. C. 2009). It can immediately grownup in the organized beds with average supply of water and appropriate substratum to climb). A developed plant of *Cissus quadrangularis* is beneficial to switch the plant life is easily from one region to any other as per need. The above activities no longer solely support the plants for fast multiplication (Balouiri et al., 2016). However, additionally for their dissemination. The *Cissus quadrangularis*, invites the interest of the global researchers for its pharmacologic things to do such as anti- analgesic, anti-inflammatory, anti-oxidant, and free radical anti-scavenging activities, anti-osteoporosis endeavor and bone restoration activity. It is a tendril-climbing shrub barring stout, fleshy quadrangular stems. The important that are components discovered in the plant are ascorbic acid, carotene, ketosteroid, calcium, triterpenoids (Wagner & Ulrich-Merzenich 2009). It was mentioned to include three unsymmetric tetracyclic triterpenoids alongside with the β-sitosterol, β-amyrin, and β-amyrone. In addition, It carries the flavonoids, phytosterols, δ-amyrin, δ-amyron, resveratrol, piceatannol, pallidol, parthenocissine, quadragularins and water-soluble glycosides (Raj, et.al., 2011).

MATERIALS AND METHODS:

The fresh stem parts of the plant Cissus quadrangularis was collected from the Kilakarai, Ramanathapuram, Tamilnadu. Collected stems were rinsed with distilled water and allowed to dry in shadow for 20-30 days. The completely dried stem was powdered for further studies (John et al., 2011). The studies such as extraction, phytochemical analysis, thin-layer chromatography, gas chromatography – mass spectrometry was accomplished (Kagan et al., 2014).

The identified phytocompounds from the gas chromatography – mass spectrometry was undergone with the molecular docking with the aid of PyRx Python Virtual Screening Tool (version 0.8) against Human C-Reactive Protein, an inflammatory agent in human (Morris, G. M., Lim-Wilby, M. 2008). The method hemolysis was implemented using an animal model (Albino Rat) with different concentrations of the extract (Du Clos, T. W. 2000). The antibacterial activity was conducted using both Gram-positive and Gram-negative strains of bacteria, including Bacillus subtilis, Staphylococcus aureus, Klebsiella sp., and *Escherichia coli (*Mohanty et al., 2011*).* By observing the results of all the above studies, the anti-inflammatory gel-based product was developed with the help of activators, buffers and preservative (Grada et al., 2018).

RESULTS AND DISSCUSSION:

Phytochemical Analysis:

Preliminary phytochemical analysis of the aqueous, ethanol and methanol extracts of *Cissus quadrangularis* indicated the presence of certain metabolites in a different manner.

Table 1: Phytochemical Analysis of Aqueous, Methanol and Ethanol Extracts

S.NO	TEST		REAGENT	AQUEOUS	METHANOL	ETHANOL
1	Alkaloids	Mayer's Test	Mayer's Reagent	+	+	-
		Wagner's Test	Wagner's Reagent	+	+	-
2	Coumarins	Sodium Hydroxide Test	NaOH	+	+	+
3	Terpenoids	Salkowski's Test	$CHCl_3$ H_2SO_4	+	+	+
4	Phenols	Ferric Chloride Test	$FeCl_3$	+	+	-
5	Quinones	Alkali Test	10% NaOH	+	-	-
6	Anthraquinones	Hydrochloric Acid Test	HCl	+	-	-
7	Tannins	Gelatin Test	NaCl	+	+	+
8	Phlobatannins	Ammonia Test	NH_4OH	-	+	+
9	Carbohydrates	Molish's Test	Alphanapthol	+	+	-
		Benedict's Test	Benedict's Reagent	+	+	-
		Fehling's Test	Fehling's Reagent	+	+	-
10	Glycosides	Legal's Test	$CHCL_3$ NH_4OH	-	-	+
11	Cardiac Glycosides	Keller-killani Test	CH_3COOH $FeCl_3$ H_2SO_4	-	-	+
12	Proteins	Million's Test	Million's Reagent	+	+	-
		Biuret's Test	Biuret's Reagent	+	+	-
13	Amino Acids	Ninhydrin Test	Ninhydrin Reagent	+	+	+
14	Steroids	Ferric Chloride – Acetic acid test	$CHCL_3$ $FeCl_3$ CH_3COOH H_2SO_4	-	+	+
15	Phytosteroids	Chloroform – Sulphuric Acid Test	$CHCL_3$ H_2SO_4	-	+	-

16	Acids	Sodium Bicarbonate Test	$NaHCO_3$	+	+	-
17	Saponins	Foam Test	H_2O	+	+	-
18	Carboxylic Acids	Phenolphthalein Test	$C_{20}H_{14}O_4$ NaOH	-	+	+
19	Fixed Oils	Oil Detection Test	Whattman Filter Paper	-	-	-
20	Fats	Saponification Test	KOH $C_{20}H_{14}O_4$	-	-	-
21	Gums And Mucilages	Distilled water and Alcohol Test	C_2H_5OH	+	+	+
22	Flavanoids	Aqueous Test	NaOH	+	+	+
		Sulphuric Acid Test	H_2SO_4	+	+	+

TABLE 2: Phenol and Flavonoid compounds quantified in the extract

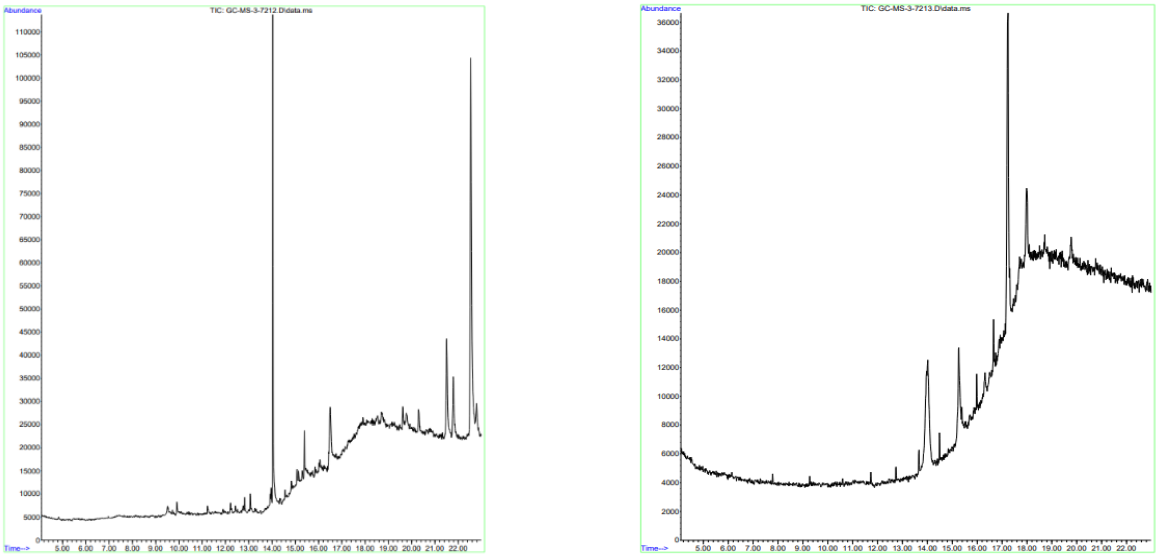
S. No	Phytochemicals	Amount (µg/mg)
1.	Phenols	744.2 ±1.05
2.	Flavonoids	152.5 ±0.22

The total phenol content was 744.2 ±1.05 µg/mg of GAE and the total flavonoid content was 152.5 ± 0.22 µg/mg in the extract.

Gas chromatography mass spectrometry:

The total phytocompounds identified in the GC-MS analysis was about Aqueous = 13 phytocompounds and Methanol = 16 phytocompounds.

Figure 1: Chromatogram of the result observed from GC-MS Analysis of Aqueous Extract and Methanol Extract of *Cissus quadrangularis*



Molecular docking studies:

The molecular docking was done between the phytocompounds observed in the Gas Chromatography – Mass Spectrometry with the Human C-Reactive Protein.

TABLE 3: Results of the molecular docking (Methanol Extract)

Compound	Binding Affinity (Kcal/Mol)	Mode	Rmsd Lower Bounding	Rmsd Upper Bounding
5-Methyl-2-Phenylindolizine	-6.8	0	0.0	0.0
	-6.8	1	0.801	2.402
	-6.4	2	1.183	1.647
	-6.3	3	1.204	2.944
	-5.6	4	19.015	19.975
	-5.6	5	18.391	19.579
	-4.4	6	19.344	20.154
	-3.9	7	20.172	21.263
Acetic acid	-3.2	0	0.0	0.0
	-3.1	1	20.398	20.946
	-3.1	2	15.483	15.534
	-3.0	3	20.408	20.881
	-2.9	4	23.811	24.267
	-2.7	5	16.383	16.597
	-2.7	6	22.404	22.911
	-2.6	7	17.26	17.631
Dimethylaminoethyl	-4.9	0	0.0	0.0
	-2.8	1	1.123	2.331
	-2.0	2	8.535	11.037
Benzoxazepine	-6.7	0	0.0	0.0
	-5.8	1	2.188	3.17
	-5.2	2	2.533	5.116
	-5.0	3	2.18	40705
	-4.0	4	3.046	5.77
	-3.8	5	17.797	18.501
Cyclotrisiloxane	-4.7	0	0.0	0.0
	-4.1	1	26.415	27.355
	-3.8	2	12.973	16.116
	-3.6	3	1.034	21.91
	-3.6	4	9.935	13.071
	-3.2	5	9.76	13.557
	-3.0	6	1.526	5.660
	-2.7	7	12.912	16.139
	-2.4	8	22.655	27.677
Benzo [H] quinoline	-5.2	0	0.0	0.0
	-5.1	1	2.334	5.29
	-5.1	2	18.856	19.669
	-5.1	3	1.422	2.436

	-4.6	4	3.136	5.677
	-4.3	5	3.432	5.655
	-4.3	6	1.042	2.011
	-4.3	7	10.993	12.233
	-4.2	8	20.371	21.146
	-6.0	0	0.0	0.0
1,2-Benzisothiazol	-3.0	1	1.885	2.425
	-3.9	0	0.0	0.0
	-3.9	1	0.27	0.277
	-3.9	2	0.259	2.528
	-3.4	3	2.466	3.395
Benzene	-3.4	4	2.391	3.927
	-3.3	5	20.106	20.769
	-3.3	6	20.117	20.724
	-3.3	7	20.048	20.701
	-3.3	8	20.194	20.885
Cycloheptatrien	-.3.8	0	0.0	0.0
	-3.8	1	0.264	1.447
	-3.8	2	0.248	2.3
	-3.8	3	19.652	20.794
	-3.7	4	19.567	20.467
	-3.7	5	0.243	2.293
Heptamethyltrisiloxane	0	0	0	0
	-4.7	0	0.0	0.0
	-4.1.	1	26.415	27.355
	-3.8	2	12.973	16.116
	-3.6	3	1.034	4.91
Cyclotrisiloxane	-3.6	4	9.935	13.071
	-3.2	5	9.76	13.557
	-3.0	6	1.526	5.666
	-2.7	7	12.912	16.139
	2.4	8	26.655	27.677

TABLE 4: Results of the molecular docking (Aqueous Extract)

Compound	Binding Affinity (Kcal/Mol)	Mode	RMSD Lower Bounding	RMSD Upper Bounding
Phytol	-4.3	0	0.0	0.0
	-4.1	1	3.612	10.606
	-3.1	2	9.552	14.333
	-2.4	3	5.424	10.287
	-2.0	4	3.797	6.336
	-1.7	5	3.88	6.134

Isophytol	-4.1	0	0.0	0.0
	-3.8	1	1.454	2.116
	-3.1	2	4.764	12.006
	-2.9	3	1.475	2.239
	-1.7	4	3.132	5.64
	-1.4	5	3.941	11.203
1-Hexadecen-3-ol	5.0	0	0.0	0.0
	5.4	1	1.366	2.044
	55	2	2.943	9.801
	6.1	3	1.275	2.153
	6.7	4	3.503	10.146
	7.7	5	3.383	10.095
	7.8	6	1.568	2.569
Sulfurous Acid	-2.6	0	0.0	0.0
	-1.6	1	5.085	6.488
	-0.6	2	5.798	6.919
2,4-Dimethylcyclopentanol	-4.4	0	0.0	0.0
	-4.3	1	2.412	3.214
	-4.0	2	20.166	20.693
	-4.0	3	2.312	3.464
	-3.9	4	2.02	3.719
	-3.8	5	19.983	20.882
Butylcyclopentane	-4.6	0	0.0	0.0
	-4.3	1	19.003	19.817
	-4.2	2	18.661	19.336
	-4.0	3	19.698	20.288
	-3.8	4	19.342	19.907
	-3.7	5	3.014	5.96
1-Nonadecanol	-3.9	0	0.0	0.0
	-3.8	1	1.06	2.295
	-3.4	2	1.56	3.429
	-33	3	1.652	2.894
	-3.1	4	1.534	3.177
Isocholesteryl Methylether	0.5	0	0.0	0.0
	0.6	1	2.18	3.14
	1.2	2	8.116	6.784
Carbonic Acid	-5.6	0	0.0	0.0
	-5.4	1	1.352	2.202
	-5.3	2	20.765	21.535
	-5.3	3	20.393	21.196
	-5.1	4	19.304	20.16
	-5.1	5	19.741	20.643

	-5.1	6	19.277	20.174
2-Benzothiazolythio	-7.6	0	0.0	0.0
	-7.4	1	1.326	2.985
	-6.6	2	6.94	5.095
	-6.5	3	1.931	4.723
	-5.9	4	1.51	4.939
	-5.5	5	2.162	4.78
1,4-Oxazepine	-6.8	0	0.0	0.0
	-5.9	1	2.21	3.195
1-Methyl-2-Phenyl-1H Indole	-7.2	0	0.0	0.0
	-6.5	1	1.69	5.889
	-6.1	2	17.994	19.074
	-5.4	3	18.78	19.454
	-5.4	4	17.635	18.542

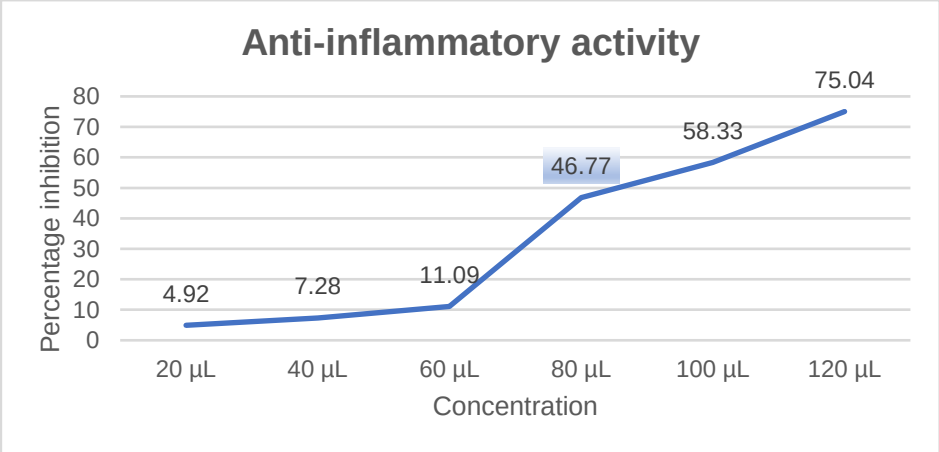
Animal Studies:

The outcome showed that extract has significant anti-inflammatory properties at different concentrations. The IC50 was 85.52 g/ml concentration, and the maximum hemolysis inhibition was 46.770.66 at 80 g/ml concentration. Asprin was used as the reference standard (IC50 = 137.14 g/ml concentration).

TABLE 5: The inhibition results of the extract

CONCENTRATION(µg/ml)	OD at 517nm	% OF INHIBITION
Control	0.17	-
20	0.179	4.92±0.58
40	0.184	7.28±1.03
60	0.195	11.09±2.41
80	0.322	46.77±0.66
100	0.411	58.33±0.46
120	0.698	75.04±0.53

Figure 2: Anti-inflammatory activity of the extract



Anti-Bacterial Activity:

The extract was investigated for in vitro antibacterial activity against microorganism including Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus) and Gram-negative bacteria (*Klebsiella Sp. and Escherichia coli*). The maximum zone of inhibition of combination extract against Bacillus subtilis was 12mm in 500µg/ml and Escherichia coli was 13mm in 500µg/ml.

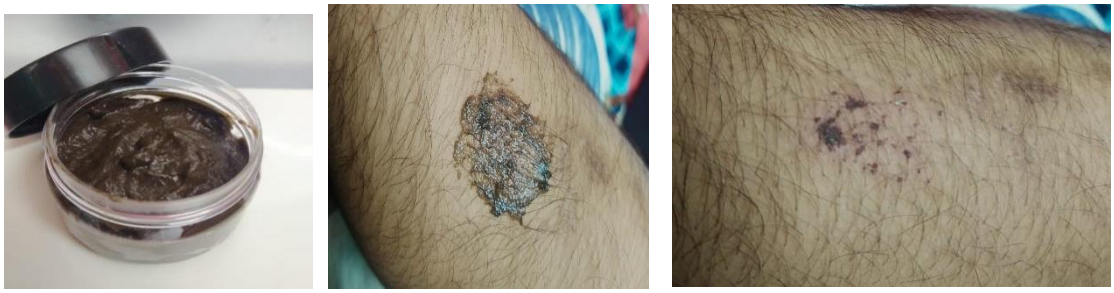
TABLE 6: Zone of inhibition in Anti-bacterial activity

S.NO	ORGANISM	ZONE OF INHIBITION			
		750 µg	250µg	375µg	500µg
1.	*Klebsiella Sp.*	16mm	10mm	12mm	13mm
2.	*Bacillus subtilis*	14mm	10mm	11mm	12mm
3.	*Escherichia coli*	15mm	10mm	11mm	13mm
4.	*Staphylococcus aureus*	21mm	11mm	13mm	15mm

Product Development:

The extract was treated with activators, buffers and preservatives results in the production of gel -based external use medication.

FIG 3: Gel-based external use product using plant extract



Patch test on skin:

A patch test is a skin test used to find the cause of a possible allergic reaction on the skin. The product applied in the inflamed skin for about one hour results in no allergic reactions.

CONCLUSION:

The active components that give medicinal plants their pharmacological potential are their phytochemicals. The extracts of *Cissus quadrangularis* in water, ethanol, and methanol showed varied patterns of the presence of certain metabolites according to preliminary phytochemical examination. Significant chemical components such flavonoids, alkaloids, coumarins, quinones, glycosides, and phytosteroids, among others, have been identified according to reports. The antioxidant activity appeared to be caused by the phenol and flavonoid components that were measured in the composition of the herbal extract. The extract's total phenol content was 744.2 1.05 g/mg and its total flavonoid content was 152.5 0.22 g/mg. The results of the molecular docking against the CRP shows the binding affinity which results in the potential to eradicate the inflammation. The results of the animal model demonstrated that extract, at various doses, has considerable anti-inflammatory activities. The maximal

hemolysis inhibition was 46.770.66 at an 80 g/ml concentration, while the IC50 was 85.52 g/ml. The reference standard was asprin (IC50 = 137.14 g/ml concentration).

The plant *Cissus quadrangularis* show the potential against inflammation. Thus, the plant can be modified and used as a natural source of medications which can heal the inflammation.

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