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Original Article

Assessment of Anthelmintic Activity and *in silico* Study of Phytoconstituents in *Decaschistia crotonifolia Wight & Arn.* Root Extract

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ABSTRACT

Background: Worm infections in developing countries were reported high. Phytoconstituents have been a vital role for the treatment of many ailments. The current study was aimed assess for anthelmintic activity of different root extracts of *Decaschistia crotonifolia* belongs to the family Ebanaceae against *Pheretima posthuma*. Further *Insilico* study was carried out for phytocompounds present in *Dechaschistia*. **Methods:** The chloroform, ethylacetate and ethanol extract of *Decaschistia crotonifolia* were considered for the study of anthelmintic property on earthworms at concentrations 20 mg/ml, 40 mg/ml and 60 mg/ml. During this study, the parameters paralysis time and Death Time of adult Indian earthworms was observed. As a standard and control Albendazole 10 mg/ml and 2% Tween 80 in distilled water were taken respectively. **Results:** The study resulted that ethanolic extract was significant when compared with the Albendazole 10 mg/ml. Docking studies revealed all phytocompounds in *Dechaschistia* shown binding affinity, however comparatively scopoletin and stigmasterol had shown a good binding affinitiy about -7.7 Kcal/mol and -7.6 Kcal/mol compared to standard drug Albendazole which was shown about -8.7 Kcal/mol. **Conclusion:** The study revealed that the ethanol extract of *Decaschistia crotonifolia* at a concentration of 60mg/ml exhibited a stronger anthelmintic property compared to Albendazole 10mg/ml. A dose dependent anthelmintic activity is exerted by all the extracts in an ascending manner Chloroform<Ethyl acetate<Ethanol. These observations were made evidenced by docking studies of phytocompounds in *Dechaschistia* as the phytocompounds were shown excellent docking score when compared with standard Albendazole.

Key words: *Decaschistia crotonifolia* Wight and Arn., Ebanaceae, *Pheretima* and Anthelmintic, Docking, Lipinski rule.

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INTRODUCTION

Diseases caused by helminths are chronic. Helminthiasis is infested to human beings with worm's likely pinworm, round worm, or tapeworm.[1] The diseases caused by parasites results in morbidity and leads to the condition onchocorciasis and Schistosomiasis. A more number of worm infections has been reported in developing countries due to lack of proper hygienic conditions. By considering the affordability and various side effects of synthetic compounds, a preferability towards herbal medicines were chosen. An adult Indian earthworm *Pheretima posthuma* is selected for assessment of anthelmintic property as it shows similarity in anatomy and physiology of round worm parasites resides in intestine of human beings.

Decaschistia crotonifolia Wight and Arn is a shrub consists of dense whitish wooly on stems and branches. The leaves are in ovate lance shaped measures 3-6 cm long, 2-4 cm width. The base of leaf is heart shaped or rounded, pointed apex with coarsely toothed margins. Leaves are velvety, bears 1.5cm long stalks. It represents with yellow flowers with dark maroon centered in single leaf axils. The Sepal cup is bell in shape, 1-1.5cm long cup encloses capsules and seeds. The seeds are kidney shaped. It is most common in the deciduous forests of peninsular India. Flowering takes place in the month of March to June.

Earlier preliminary phytochemical assessment was made.[2,3] As the Investigations on *Decaschistia crotonifolia* Wight and Arn. were very limited based on literature survey and existence of insecticidal activity in the family Ebanaceae. The current study is focussed to evaluate anthelmintic activity of three extracts viz., Chloroform, Ethylacetate and Ethanol extract of *Decaschistia crotonifolia Wight and Arn.*

METHODS

Plant Material

The roots of *Decaschistia crotonifolia* Wight and Arn belonging to the family to Ebaenaceae were collected from surroundings of Tirumala, Andhra Pradesh, India in the month of June and it was authenticated by Dr. K. Madhava Chetty, Head of Department, Department of Botany, SV University, Tirupati. Voucher Specimen (PHCOG/VVIPS/056) were preserved. The roots of *Decaschistia crotonifolia* were shade dried, powdered and stored in well closed container.

Preparation of Extracts

About 300gm of dried root powdered drug of *Decaschistia crotonifolia* Wight and Arn. was extracted by successive solvent extraction using chloroform, ethyl acetate and ethanol by Soxhlet extraction for 72 hr. The extract was made concentrated by rotary evaporator and placed in desiccator for further use.

Evaluation of Anthelmintic Property

Anthelmintic property of chloroform, ethyl acetate and ethanol root extracts of *Decaschistia crotonifolia* Wight and Arn. was examined by using an Indian earthworm *Pheretima posthuma*.[4-5] Choosing of *Pheretima posthuma* is made as it resembles identical towards anatomy and physiology of roundworm parasite which occurs in alimentary tract of *Homosapiens*.

Adult earth worms measure an average size 4-7cm in length and 0.3–0.7 cm in width was collected from medicinal garden of

V. V. Institute of Pharmaceutical Sciences and proper washings are carried out to remove extraneous matter. The extract at concentration of 30mg/ml, 60mg/ml and 80mg/ml was used to examine the time of paralysis (Pt) and Death (Dt). The selected earthworms are categorized into 11 groups of 6 each viz., control group treated with 2% Tween 80 in distilled water, 9 Test groups treated with concentrations of 30mg/ml, 60mg/ml and 80mg/ml of each Chloroform, Ethylacetate and Ethanol extract of *Decaschistia crotonifolia* Wight and Arn. and standard group treated with 10mg/ml concentration of Albendazole. Earthworms are treated with volume of 10ml of each concentration of standard, control, and test solutions respectively. The time taken for Paralysis (Pt) and Death (Dt) was noted.

Docking Studies

ADME Analysis

Pharmacokinetic Evaluation of phytoconstituents is necessary as it effects binding of compounds in specific active target site.[6-7] Prior docking studies of Phytochemicals, it is very much needed to qualify drug-likeness test, i.e., they have to obey Lipinski rule.[8] The canonical smiles of phytocompounds Parvifloral A (PubChem CID: 90470346), Syriacusin A (PubChem CID: 9991528), Syriacusin B (PubChem CID: 10015552), Syriacusin C (PubChem CID: 10105245), Scopoletin (PubChem CID: 5280460), Stigmasterol (PubChem CID: 5280794) and Standard drug Albendazole (PubChem CID: 2082)was obtained from Pubchem (pubchem.ncbi.nlm.nih.gov) predicted their drug likeness test using SwissADME (SwissADME) and their physicochemical parameters.

In silico Study

For molecular docking study, Autodock Vina 1.5.7 is used for prediction of potent phytocompounds of *Decaschistia* viz., Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol against active site of β-tubulin.[9-10] The chemical structures of phytoconstituents Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol were obtained from Pubchem Project Database shown in Figure 1. They were structurally plotted in Discovery Studio Biovia 2021. The 3D structure of protein β-tubulin (PDB ID: 1oj0) is collected from Protein Data Bank (www.rcsb.org/pdb) shown in Figure 2. The x, y and z attributes along with radius is noted. Further the structure is prepared by removing water, adds up polar hydrogen bond and made torsion free.

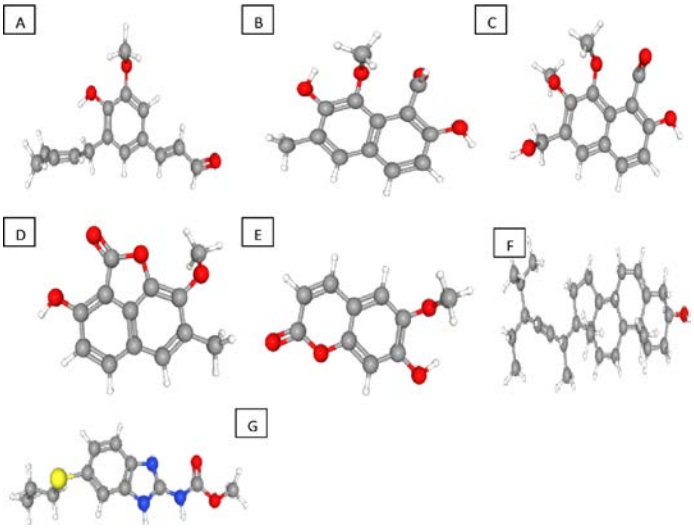

Figure 1: Chemical Structures of phytocompounds present in *Decaschistia* A) Parvifloral B) Syriacusin A C) Syriacusin B D) Syriacusin C E) Scopoletin D) Stigmasterol and G) Albendazole (Standard drug).

Figure 2: β-tubulin (Protein ID: 1OJ0).

Table 1: Time taken to paralyze by *P. posthuma* treated with CEDC, EAEDC and EEDC.

Sl. No.	Drugs Treatment	Time taken for Paralysis (Pt)		
		Dose taken at different Concentrations		
		20mg/ml	40mg/ml	80mg/ml
1	20% Tween	0	0	0
2	Albendazole	25.53±0.51	25.53±0.51	25.56±0.53
3	Chloroform Extract (CEDC)	94.83±1.16	64.33±1.21	44.00±0.89**
4	Ethylacetate Extract (EAEDC)	81.66±1.63	47.00±1.26**	32.83±0.75**
5	Ethanol Extract (EEDC)	51.16±1.16**	33.83±0.75**	21.00±0.89**

*All the values were expressed in Mean±Standard Deviation. Statistical significance $p<0.05$.

Statistical Analysis

The values were represented as mean ± S.D; via one-way ANOVA. The analysis was carried out by using Graph pad Prism (Version 3, U.S.A.) software program. $P < 0.05$ was taken into statistically significant.

RESULTS

Extraction

The roots of *Decaschistia crotonifolia* was collected in the month of June and it was made authenticated by the botanist. After the extraction and concentrated the percentage yield of chloroform, ethyl acetate and ethanol extract were found to be around 10.2%, 11.3% and 17.5%. It is observed that the highest yield was found to be in solvent ethanol around 17.5%.

Anthelmintic activity

Table 1, 2 and Figure 3 represents the mean time of Paralysis (Pt) and Death (Dt) by various concentration of chloroform, ethyl acetate and ethanol extract against earthworms. After scrutinizing the results obtained from experimental methods it was found that the higher concentrations of ethanol shown a faster paralytic and shorter death time of all earthworms.

ADME analysis

All the phytocompounds showed zero violation except stigmasterol as it showed 1 violation. The standard drug Albendazole also showed zero violation. The results were depicted in Table 3.

In silico Study

Docking revealed that out of 6 phytocompounds Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol with protein β-tubulin had shown docking scores of -6.3 kcal/mole, -6.9 kcal/mole, -6.0 kcal/mole, -6.7 kcal/mole, -7.7 kcal/mole, -8.7 kcal/mole and standard drug Albendazole shown at -7.6 kcal/mole. The phytocompounds had shown hydrogen bond interactions with aminoacid and the results discloses the hydrogen bond interactions are associated with aminoacids in each ligand and protein complex except with Syriacusin C. The outcomes are depicted in Table 4 and the complexes are made visualized in Figure 4.

Table 2: Time taken to kill *P. posthuma* treated with CEDC, EAEDC and EEDC.

Drugs Treatment	Time taken for Death (Dt)		
	Doses taken at Concentrations		
	20mg/ml	40mg/ml	80mg/ml
20% Tween	0	0	0
Albendazole	25.53±0.51	25.53±0.51	25.52±0.52
Chloroform Extract (CEDC)	162.83±1.72	148.5±1.04	99.00±1.26
Ethylacetate Extract (EAEDC)	133.66±1.03	121.16±0.75	87.16±0.75**
Ethanol Extract (EEDC)	52.50±1.04**	34.33±1.21**	25.33±0.81**

*All the values were expressed in Mean±Standard Deviation. Statistical significance $P<0.05$.

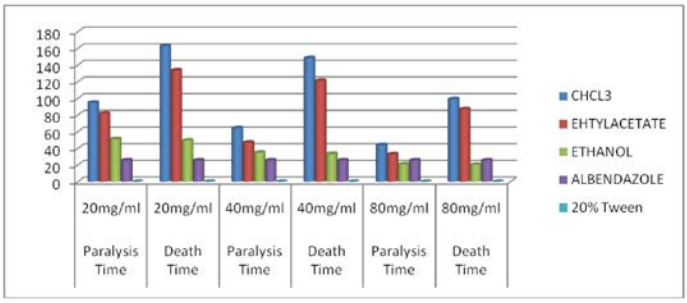

Figure 3: Representations of Paralysis time (Pt) and Death time (Dt) by *P. posthuma* treated with CEDC, EAEDC and EEDC.

DISCUSSION

Helmenthiasis is considered as disease in south Asia including India. Hence and investigation in larger no on alternative sources are made for their anthelmintic acitivity.[11-15] The considerations of anthelmintic activity due to flavonoids and steroids were stated earlier. The flavonoids biochanin A and genistein was shown effective anthlemintic activity against *Aspiculuris tetraptera*. Anthelmintic tests according to the procedure of Hounzangbe Adote *et al*. were conducted for the phytocompounds against *Haemonchus contortus*. The best activity was obtained with flavonoids.[16]

Aqueous extract of whole plant of *Amaranthus spinosus* had exerted anthlemintic activity against *Pheritima posthuma* in dose dependent manner due to presence of steroids and flavonoids.[17] The study aimed to evaluate anthelmintic activity of chloroform ethylacetate and ethanolic root extract of *Decaschistia crotonifolia*. The pharmacognositical investigations were carried out. The qualitative chemical screening of *Decaschistia* was studied and revealed the presence of steroids, flavonoids and tannins more in ethanolic extract. In earlier studies Trinorcadalenes, parviflorals A, Syriacusin A, B and C, Scopoletin and Stigmasterol were isolated and their structures along with resonance were elucidated by ^{1}H and ^{13}C NMR spectroscopy.[2]

The test solution and standard drug solutions were freshly prepared. The time for paralysis was noted as no movement is observed except when the worms were vigorously shaken. The 'time for death' of worms was recorded after confirming that the worms neither moved when shaken vigorously or merged in warm water at 50°. A maximum time period of 120 min was taken for the paralyzing as well as death time of *Pheretima posthuma* worms. Albendazole (10 mg/ml) was used as reference standard with distilled water as the vehicle control. The mean and SEM were analyzed statistically by ANOVA. From the observations, a dose dependent paralytic effect and the time of death was observed. Although, all the extracts showed anthelmintic activity in a dose-dependent manner but the ethanolic extract appeared to be more effective against worms. Evaluation of anthelmintic activity was compared with reference standard Albendazole. Comparative with all the extracts, the ethanolic extract of the roots of *Decaschistia crotonifolia*, caused paralysis at 51.16 min., 34.83 min. and 21.00 min. and time of death at 60.50 min., 43.33

Table 3: ADME Analysis of Phytocompounds.

Sl. No.	Phyto compounds	Molecular Weight[a] (g/mol)	H-donor[b]	H-acceptor[c]	Log p- Value[d]	Molar Refractivity[e]	Drug likeness
1	Parvifloral	246.30	1	3	3.06	73.78	0
2	Syriacusin A	232.23	2	4	2.24	64.84	0
3	Syriacusin B	262.26	2	5	1.84	70.47	0
4	Syriacusin C	230.22	1	4	2.53	62.39	0
5	Scopoletin	192.17	1	4	1.52	51.00	0
6	Stigmasterol	412.17	1	1	6.97	132.75	1
7	Albendazole	265.33	3	2	2.39	73.22	0

[a]Molecular weight accepted range <500
[b]Hydrogen bond donor acceptable range ≤ 5
[c]Hydrogen bond acceptor acceptable range ≥ 10
[d]High Lipophilicity (expressed as LogP, acceptable range < 5
[e]Molar Refractivity should be between 40 and 130.

Table 4: Docking Simulation of β-tubulin and Phytocompounds.

Sl. No.	Phytocompounds	Binding Energy (kcal/mole)	Hydrogen bonds
	Parvifloral	-6.3	ARG (A:318), GLU (A: 27) and VAL (A:231)
	Syriacusin A	-6.9	ILE (A:24), PHE (A:20), GLN (A: 134), MET (A:233), TYR (A:50), THR A:238), THR (A: 237), THR (A:136), HIS (A:6), SER (A:165), LEU (A:250) and GLU (A:198)
	Syriacusin B	-6.0	GLN (A:43), ARG (A:359), ARG (A: 318) and GLU (A:27)
	Syriacusin C	-6.7	-
	Scopoletin	-7.7	MET (A:233)
	Stigmasterol	-8.7	GLN (A:43)
	Albendazole	-7.6	SER (A:615) and VAL (A:236)

min., 25.33 min., at concentrations of 20mg/ml, 40mg/ml and 80mg/ml for *Pheretima posthuma*. The reference drug Albendazole showed the time of paralysis and time of death as 25.53 and 45.53 min, respectively. Considering the ethanolic extract of roots showed comparable activity, it marks an important to identify the key phytoconstituents.

The chloroform (44.00±0.89, 99.00±1.26), ethylacetate (32.83±87.16±0.75) and ethanol extract (21.00±0.89, 25.33±0.81) of *Decaschistia crotonifolia* Wight and Arn. shown the anthelmintic activity at the concentration of 80mg/ml. Amongst Ethanolic extract had taken shorter duration of time to kill or paralyze and comparatively with standard drug Albendazole it is mere the same. All the extracts at 20mg/ml were taken too long to paralyze or to kill the adult earthworms.

It is possible to learn the mechanism of action of phytoconstituents in virtual screening methods. These methods make to design phytoremedies for various diseases. A various phytocompounds for antihelmintic activity was investigated.[18-20] Docking studies signify the fact that out of 6 phytochemicals stigmasterol and scopoletin shown a good docking score at -8.7 kcal/mole and -7.77 kcal/mole, which shown hydrogen bond interactions with GLN(A:43) and MET (A:233). syriacusin B with least among 6 phytochemicals was shown docking score at -6 kcal/mole, formed hydrogen bond interactions with GLN (A:43), ARG (A:359), ARG (A: 318) and GLU (A:27). The docking score of scopoletin and stigmasterol had shown good binding affinity between phytocompound and β-tubulin than between the protein (β-tubulin) and standard drug albendazole docking score at -7.6 kcal/mole, shown hydrogen bonding with SER(A:615) and VAL (A:236). The results disclose that, hydrophobic interactions are regulated by many amino acid deposits in each ligand-protein communication. ADME analysis of phytocompounds and standard revealed that zero violation of drug likeness and obeyed the Lipinski rule.

CONCLUSION

The current study aimed in evaluating anthelmintic activity of *Decaschistia crotonifolia*. The test revealed a significant anthelmintic activity of ethanolic root extract and the remaining extracts were also

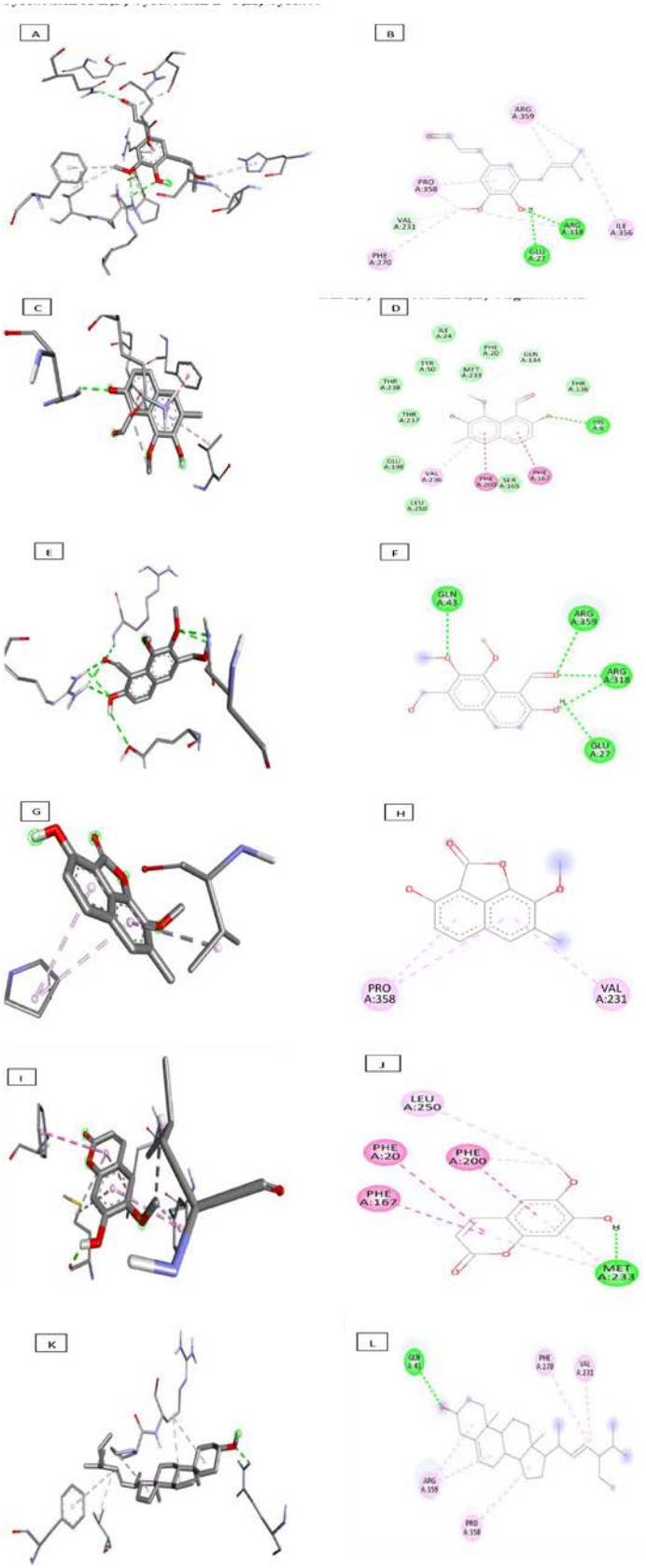

Figure 4: Visualization of 3D and 2D images of molecular docking between β-tubulin (Protein) and 6 phytocompounds present in *Decaschistia* A,B)Parvifloral C,D) Syriacusin A E,F) Syriacusin B G,H) Syriacusin I,J) Scopoletin K,L) Stigmasterol.

shown but it is considered as dose dependent manner. This activity is supported by docking studies. Docking studies shown that binding poses and distance measurement of β-tubulin complexes parviflorals A, Syriacusin A, Syriacusin B and Syriacusin C, Scopoletin and Stigmasterol reveals that the lead phytocompounds were in near proximity associated with most active site of aminoacids. This confirms the phytocompounds present in *Decaschistia* need to investigate for the discovery of new generation of drugs as they will be remedies against organisms causing helminths.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Pt: Paralysis time; **Dt:** Death time; **CEDC:** Chloroform extract of *Decaschistia crotonifolia*; **EAEDC:** Ethyl acetate extract of *Decaschistia crotonifolia*; **EEDC:** Ethanol extract of *Decaschistia crotonifolia*.

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