

# Assessment of Anthelmintic Property and *Insilico* study of phytochemicals in roots of *Dechaschistia crotonifolia* Wight & Arn.

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## Research Article

**Keywords:** Dechaschistia crotonifolia Wight & Arn., Ebanaceae, Pheretima and Anthelmintic, Docking, Lipinski rule

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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 *Dechaschistia crotonifolia* Wight & Arn. belongs to the family Ebanaceae against *Pheretima posthuma*. Further *Insilico* study was carried out for phytochemicals present in *Dechaschistia*.

**Results:** The chloroform, ethylacetate and ethanol extract of *Dechaschistia crotonifolia* Wight & Arn. 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 (Pt) and Death Time (Dt) 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. The study resulted that ethanolic extract was significant when compared with the Albendazole 10 mg/ml. Docking studies revealed the all phytochemicals in *Dechaschistia* shown binding affinity, however comparatively scopoletin and stigmasterol had shown a good binding affinity 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 *Dechaschistia crotonifolia* Wight & Arn. 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 phytochemicals in *Dechaschistia* as the phytochemicals were shown excellent docking score when compared with standard Albendazole.

## Background

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 onchocerciasis and Schistosomiasis. The 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.

*Dechaschistia crotonifolia* Wight & Arn. is a shrub consists of dense whitish woolly on stems and branches [2]. 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 [3, 4]. As the Investigations on *Dechaschistia crotonifolia* Wight & 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 *Dechaschistia crotonifolia* Wight & Arn.

## Methods

### Plant material

The roots of *Dechaschistia crotonifolia* Wight & 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 *Dechaschistia crotonifolia* Wight & Arn. was extracted by successive solvent extraction using chloroform, ethylacetate and ethanol by Soxhlet extraction for 72 hours. The extract was made concentrated by rotary evaporator and placed in desiccator for further use.

### Evaluation of Anthelmintic Property

Anthelmintic property of chloroform, ethylacetate and ethanol root extracts of *Dechaschistia crotonifolia* Wight & Arn. was examined by using an Indian earthworm *Pheretima posthuma* [5,6]. Choosing of *Pheretima posthuma* is made as it resembles identical towards anatomy and physiology of round worm parasite which occurs in alimentary tract of *Homosapiens*.

Adult earth worms measures an average size 4-7cm in length and 0.3 – 0.7 cm of 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 & 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 & 80mg/ml of each Chloroform, Ethylacetate and Ethanol extract of *Dechaschistia crotonifolia* Wight & 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 [7,8]. Prior docking studies of Phytochemicals, it is very much needed to qualify drug-likeness test, i.e., they have to obey Lipinski rule[9]. The canonical smiles of phytochemicals 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](http://pubchem.ncbi.nlm.nih.gov)) predicted their drug likeness test using SwissADME ([SwissADME](http://SwissADME)) and their physico chemical parameters.

### *In-silico study*

For molecular docking study [10,11,12] Autodock vina is used for prediction of potent phytochemicals of *Dechaschistia* viz., Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol against active site of  $\beta$ -tubulin. The chemical structures of phytoconstituents Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol were obtained from Pubchem Project Database shown in Fig. 3. They were structurally plotted in Discovery Studio Biovia. The 3D structure of protein  $\beta$ -tubulin (PDB ID: 1oj0) is collected from Protein Data Bank ([www.rcsb.org/pdb](http://www.rcsb.org/pdb)) shown in Fig. 2. The x, y & z attributes along with radius is noted. Further the structure is prepared by removing water, adds up polar hydrogen bond and made torsion free.

## Statistical Analysis

The values were represented as mean  $\pm$  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

### Anthelmintic activity

Table 1, 2 & Fig 1 represents the mean time of Paralysis (Pt) and Death (Dt) by various concentration of chloroform, ethylacetate 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 phytochemicals shown the zero violation except stigmasterol as it shown 1 violation. The standard drug Albendazole also showed zero violation. The results were depicted in table 4.

## ***In-silico* Study**

Docking revealed that out of 6 phytochemicals Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol with protein  $\beta$ -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 phytochemicals had shown hydrogen bond interactions with amino acid and the results disclose the hydrogen bond interactions are associated with amino acids in each ligand & protein complex except with Syriacusin C. The outcomes are depicted in table 3 and the complexes are made visualized in Fig no 4.

## **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 activity [13-17]. The considerations of anthelmintic activity due to flavonoids and steroids were stated earlier. The flavonoids biochanin A and genistein was shown effective anthelmintic activity against *Aspicularis tetraptera*. Anthelmintic tests according to the procedure of Hounzangbe Adote et al were conducted for the phytochemicals against *Haemonchus contortus*. The best activity was obtained with flavonoids [18].

Aqueous extract of whole plant of *Amaranthus spinosus* had exerted anthelmintic activity against *Pheritima posthuma* in dose dependent manner due to presence of steroids and flavonoids [19]. The study aimed to evaluate anthelmintic activity of chloroform ethylacetate and ethanolic root extract of *Dechaschistia crotonifolia*. The pharmacognositical investigations were carried out. The qualitative chemical screening of *Dechaschistia* was studied and revealed the presence of steroids, flavonoids and tannins more in ethanolic extract. In earlier studies Trinorcadalenes, parviflorals A, Syriacusin A, B & C, Scopoletin and Stigmasterol were isolated and their structures along with resonance were elucidated by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy [4].

The chloroform ( $44.00 \pm 0.89$ ,  $99.00 \pm 1.26$ ), ethylacetate ( $32.83 \pm 87.16 \pm 0.75$ ) and ethanol extract ( $21.00 \pm 0.89$ ,  $25.33 \pm 0.81$ ) of *Dechaschistia crotonifolia* Wight & 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 phytotherapies for various diseases. A various phytochemicals for anthelmintic activity was investigated [20, 21, 22]. Docking studies signify the fact that out of 6 phytochemicals stigmasterol and scopoletin shown -8.7 kcal/mole and syriacusin B with least among 6 phytochemicals was -6 kcal/mole. The docking score of scopoletin and stigmasterol had shown at -7.6 kcal/mole and -8.7 kcal/mole representing good binding affinity between phytochemical and  $\beta$ -tubulin than between the protein ( $\beta$ -tubulin) and standard drug albendazole, shown the docking score at -7.6

kcal/mole. ADME analysis of phytochemicals and standard revealed that zero violation of drug likeness and obeyed the Lipinski rule.

## Discussion:

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## Conclusions

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

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  $\beta$ -tubulin complexes parviflorals A, Syriacusin A, Syriacusin B & 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 *Dechaschistia* need to investigate for the discovery of new generation of drugs as they will be remedies against organisms causing helminths.

## **Declarations**

### **Ethics approval and consent to participate**

This study did not take include samples from humans. So no content was required.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

All data generated or analyzed during the study are included in this article.

### **Competing interests**

Authors declare that they have no competing interest.

### **Funding**

Not Applicable.

### **Authors' Contributions**

RP is the designer of the research project and supervised the work. LRA, KPA, KSS, JK and RSA participated in study implementation. All the authors participated in the data collection, reading and validation of the manuscript.

### **Acknowledgements**

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### **Plant Authentication**

The plants were used in this study were authenticated at Sri Venkateswara University under the number PHCOG/VVIPS/056 for *Dechaschistia crotonifolia*.

# Abbreviations

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

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## Tables

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

S. 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
1.	Albendazole	25.53±0.51	25.53±0.51	25.56±0.53
1.	Chloroform Extract (CEDC)	94.83±1.16	64.33±1.21	44.00±0.89**
1.	Ethylacetate Extract (EAEDC)	81.66±1.63	47.00±1.26**	32.83±0.75**
1.	Ethanol Extract (EEDC)	51.16±1.16**	34.83±0.75**	21.00±0.89**

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

**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)	49.50±1.04**	33.33±1.21**	20.33±0.81**

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

**Table 3** Docking Simulation of  $\beta$ -tubulin and Phytocompounds

S. No.	Phytocompounds	Binding Energy (kcal/mole)	Hydrogen bonds
1.	Parvifloral	-6.3	ARG (A:318), GLU (A: 27) & VAL (A:231)
1.	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)
1.	Syriacusin B	-6.0	GLN (A:43), ARG (A:359), ARG (A: 318) and GLU (A:27)
1.	Syriacusin C	-6.7	-
1.	Scopoletin	-7.7	MET (A:233)
1.	Stigmasterol	-8.7	GLN (A:43)
1.	Albendazole	-7.6	SER (A:615) & VAL (A:236)

**Table 4** ADME Analysis of Phytocompounds

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

<sup>a</sup>Molecular weight accepted range <500

<sup>b</sup>Hydrogen bond donor acceptable range  $\leq 5$

<sup>c</sup>Hydrogen bond acceptor acceptable range  $\geq 10$

<sup>d</sup>High Lipophilicity (expressed as LogP, acceptable range < 5

<sup>e</sup>Molar Refractivity should be between 40 & 130

## Figures

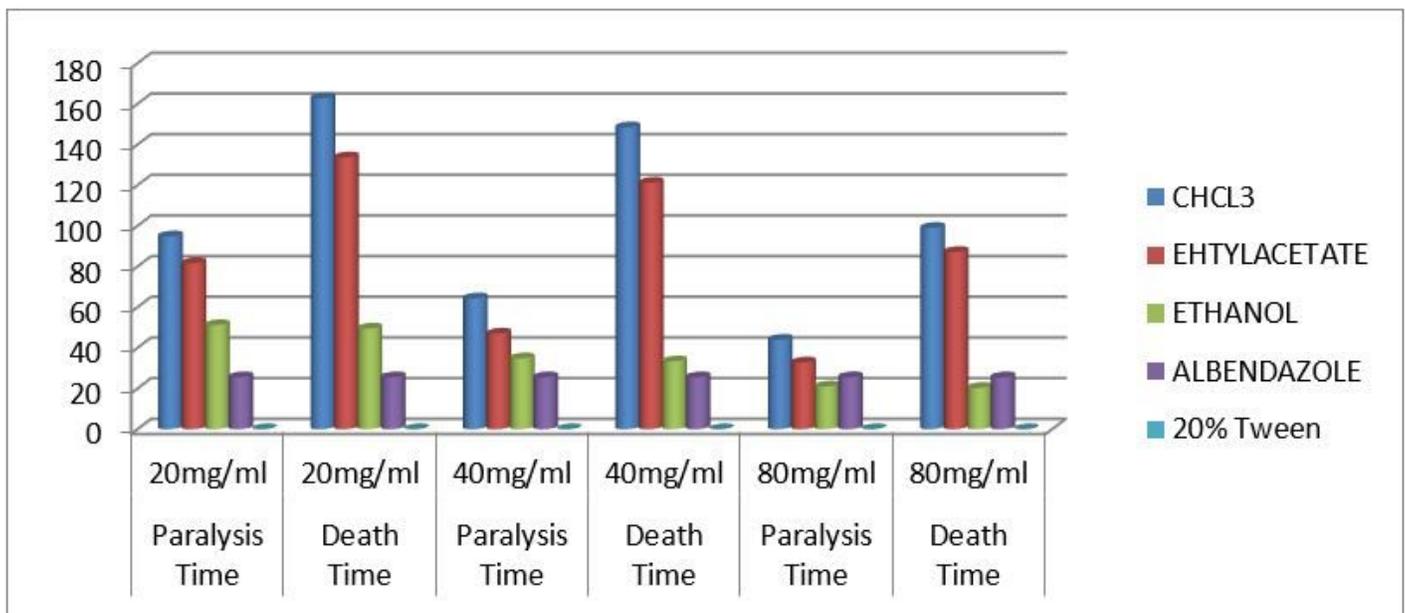
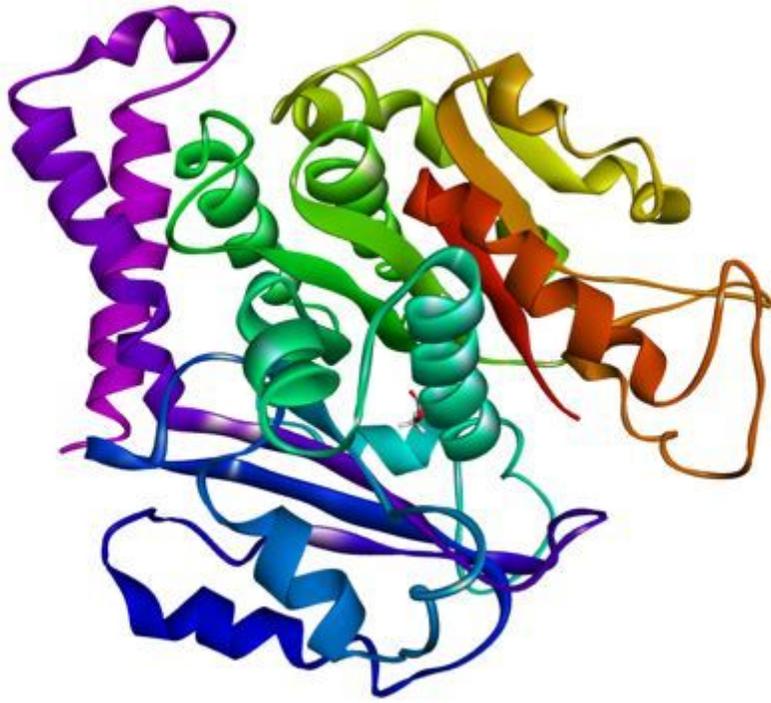


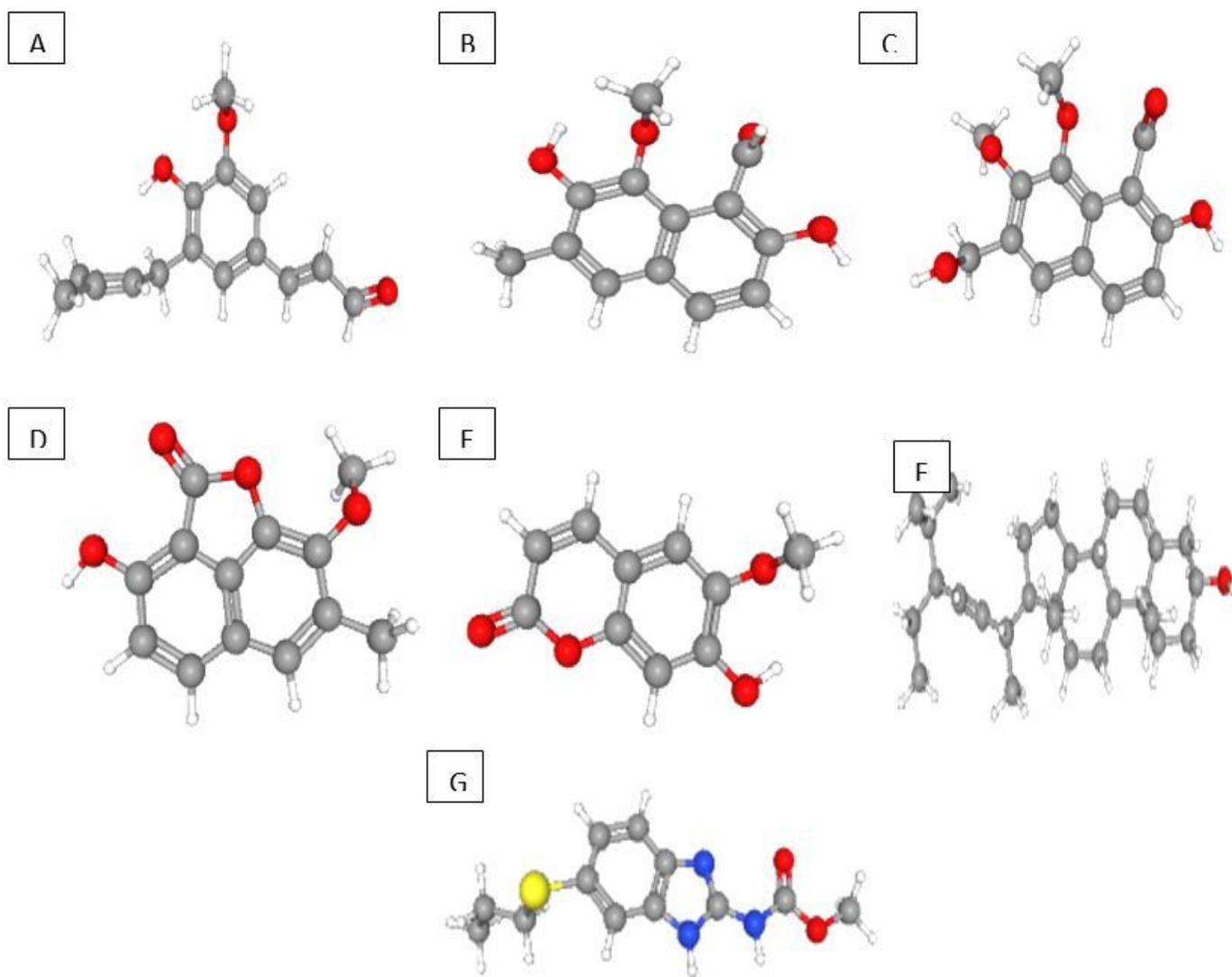
Figure 1

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



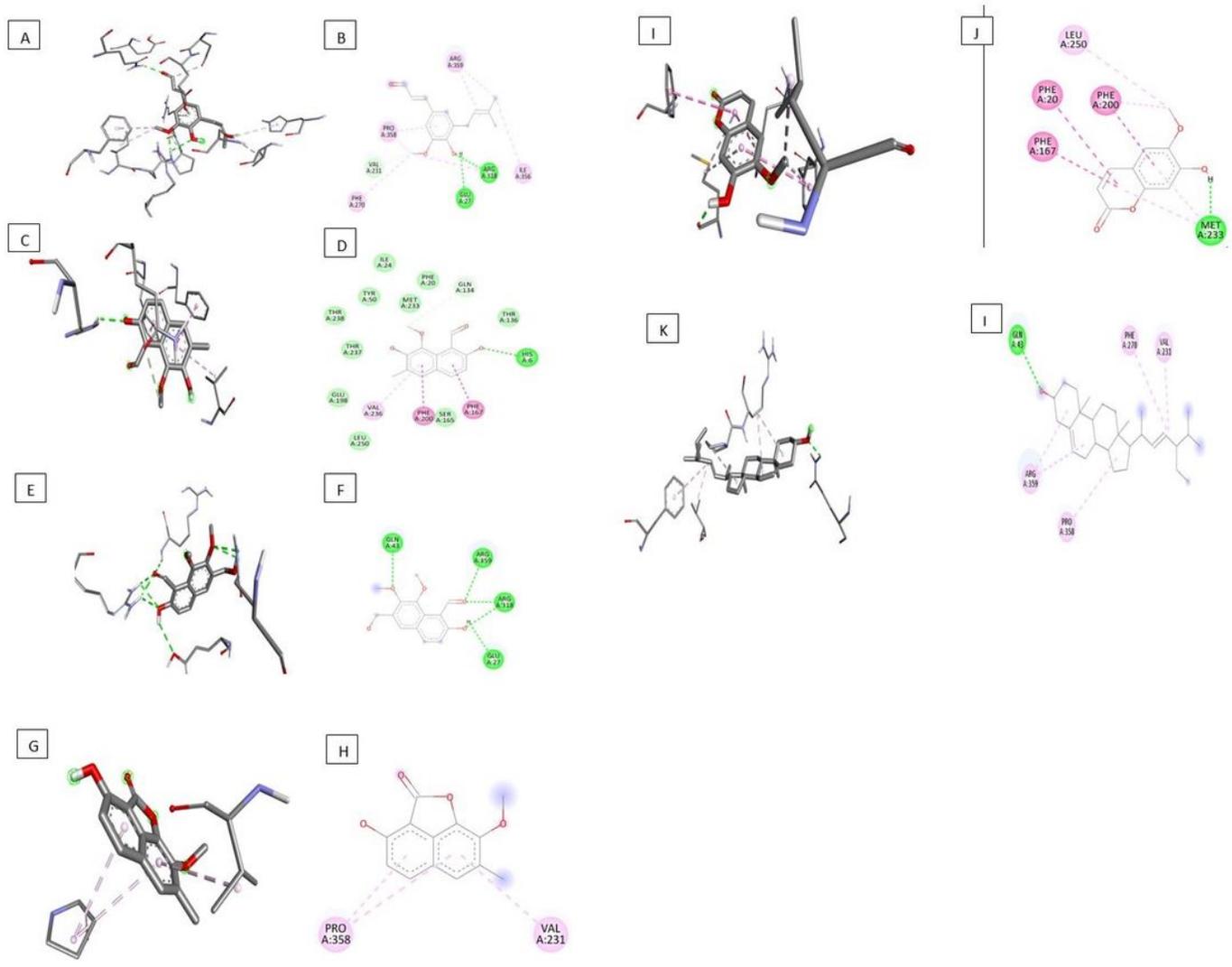
**Figure 2**

$\beta$ -tubulin (Protein ID: 10J0)



**Figure 3**

Chemical Structures of phytochemicals present in *Dechaschistia* A) Parvifloral B) Syriacusin A C) Syriacusin B D) Syriacusin C E) Scopoletin D) Stigmasterol & G) Albendazole (Standard drug)



**Figure 4**

Visualization of 3D & 2D images of molecular docking between  $\beta$ -tubulin (Protein) and 6 phytocompounds present in *Dechaschistia* A,B) Parvifloral C,D ) Syriacusin A E,F) Syriacusin B G,H) Syriacusin I,J) Scopoletin K,L) Stigmasterol