Original Article

Anthelmintic Activity And Insilico Study Of Phytoconstituents In Mirabilis Jalapa Root Extract

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Introduction

Traditional system of medicine is devoid by hazardous impacts on health, and it will be safer which ensures the healthy lifestyle. Several effective medicines were used in their naturalstate as therapeutic agents in different parts of the world, based on empirical study of their application.

The revolution aspect in industry has made a second generation of plant drugs emerged based on scientific processing of the plant extracts to isolate ″their active constituents″. Plant materials remain an important component in combating serious diseases in the world, for the therapeutic approach to several pathologies. Interest in medicinal plants has been overwhelming in the recent times especially as an important source of medication/health care. By 2000, World Health Organization had assessed that 80% of inhabitants of the world were estimated who were only relies on traditional medicines for the needs of primary health care and it also presumed that the major portion of traditional healing involves by utilizing the extracts or activeconstituents of plants [1].

By 2003, Indian Council of Medical Research estimated that the global market for medicinal plants has been around US $62 billion, and the demand is growing rapidly even still[2].The goals of using plants as sources of therapeutic agents were to isolate bioactive compoundsfor direct use as drugs, to produce bioactive compounds of novel or known structures as lead compounds, to use agents as pharmacologic tools, to use the whole plant or part of it as a herbalremedy[3].

The use of herbal medicine for the treatment of diseases and infections is as old as mankind. Indian medicinal plants also provide a rich source for oxidants that are known to prevent delay different diseased states. The antioxidant protection is observed at different levels. Medicinal plants have been used since time immemorial for treatment of various elements of skin and dermatological disorders especially cuts, wounds, and burns. The world health organization estimated that 80% of people worldwide rely on herbal medicines for someaspect of their primary health care.

According to World Health Organization (WHO); traditional, complementary, alternative, or non-conventional medicines are used by 70-95% of global population particularly in developing countries for their healthcare[4]. Moreover, the use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies[5]. The growing use of botanicals (drug and other products derived from plants) by the public is forcing moves to assess the health claims of these agents and to develop standards of quality and manufacture.

The Indian system of medicine, mainly comprising of Ayurveda, Siddha and Unani, is one of the oldest holistic management systems with thoroughly documented remedies. Ayurveda, a part of cultural heritage of India, is widely respected for its uniqueness and global acceptance as it offers natural ways to treat diseases and promote healthcare[6].

India can emerge as a major country and play the lead role in production of standardized, therapeutically effective Ayurveda formulations. India needs to explore the medicinally important plants, and this can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. Liver disorders are considered among the major world health problems[7]. A single herbal drug extract was standardized on the basis of its active principles. As per literature review, only very few chemical or analytical methods are available for herbal drug standardization[8].

Standardization of herbal medicines is the process of pre- scribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Moreover, many dangerous and lethal side effects have recently been reported, including direct toxic effects, allergic reactions, effects from contaminants, and interactions with herbal drugs[9]. On this background, standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of a herbal drug[10].

Unfortunately, standardization and quality control have remained grey areas in the preparation of Ayurvedic medicines. Till date, most of the ayurvedic formulations are lacking in their defined quality control parameters and method of its evaluation[11].

Medicinal plants are playing very active role in traditional medicines for the treatment of various ailments[12]. However a key obstacle, which has hindered the promotion in use of alternative medicines in the developed countries, is no evidence of documentation and absence of stringent quality control measures. There is a need for the record of all the research work carried out on traditional medicines in the form of documentation. With this drawback, it becomes extremely important to make surety about the standardization of the plant and parts of plant to be used as a medicine. For the process of standardization, we can use different techniques and methodology to achieve our goal in the stepwise manner e.g. pharmacognostical and phytochemical studies. These steps and processes are helpful in identification and standardization of the plant material.

Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy[13].

Diseases caused by helminths are chronic. Helminthiasis is infested to human beings with worm's likely pinworm, round worm, or tapeworm[14]. The diseases caused by parasites results in morbidity and leads to the condition onchocorciasis 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 choosen. 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.

The current study is focussed to evaluate anthelmintic activity of three extracts viz., Chloroform, Ethylacetate and Ethanol extract of *Dechaschistia crotonifolia Wight & Arn.*

MATERIALS AND METHODS

MATERIALS:

SOFTWARES REQUIRED:

Autodock 4.0, Chimera & SWISS ADMETSELECTION OF LIGAND:

The 3D structure of protein β-tubulin (PDB ID: 1oj0) is collected from Protein Data Bank (www.rcsb.org/pdb) shown in Figure 2.

PHYTOCONSTITUENTS PRESENT IN *Dechaschistia crotonifolia*:

Dechaschistia crotonifolia Wight & 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. The Twig of the D. crotonifolia is shown in Figure 1.

Earlier preliminary phytochemical assessment was made[15]. 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.*

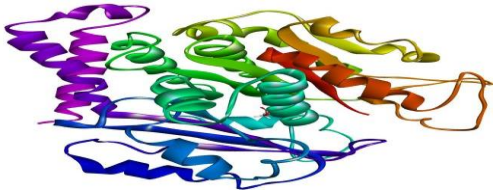


Figure 2: β-tubulin **(**Protein ID: 1OJ0)

The phytocompounds present in *Dechaschistia crotonifolia* consists of Parvifloral A, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol.

METHODS:

Docking studiesADME Analysis

Pharmacokinetic Evaluation of phytoconstituents is necessary as it effects binding of compounds in specific active target site.[16,17] Prior docking studies of Phytochemicals, it is verymuch needed to qualify drug-likeness test, i.e., they have to obey Lipinski rule.[18] The canonicalsmiles 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 *Dechaschistia* viz., Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol against active site of β-tubulin.[19,20] The chemical structures of phytoconstituents Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol were obtained from Pubchem Project Database shown in Figure 3. 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 3. The x, y & z attributes along with radius is noted. Further the structure is prepared by removingwater, adds up polar hydrogen bond and made torsion free.

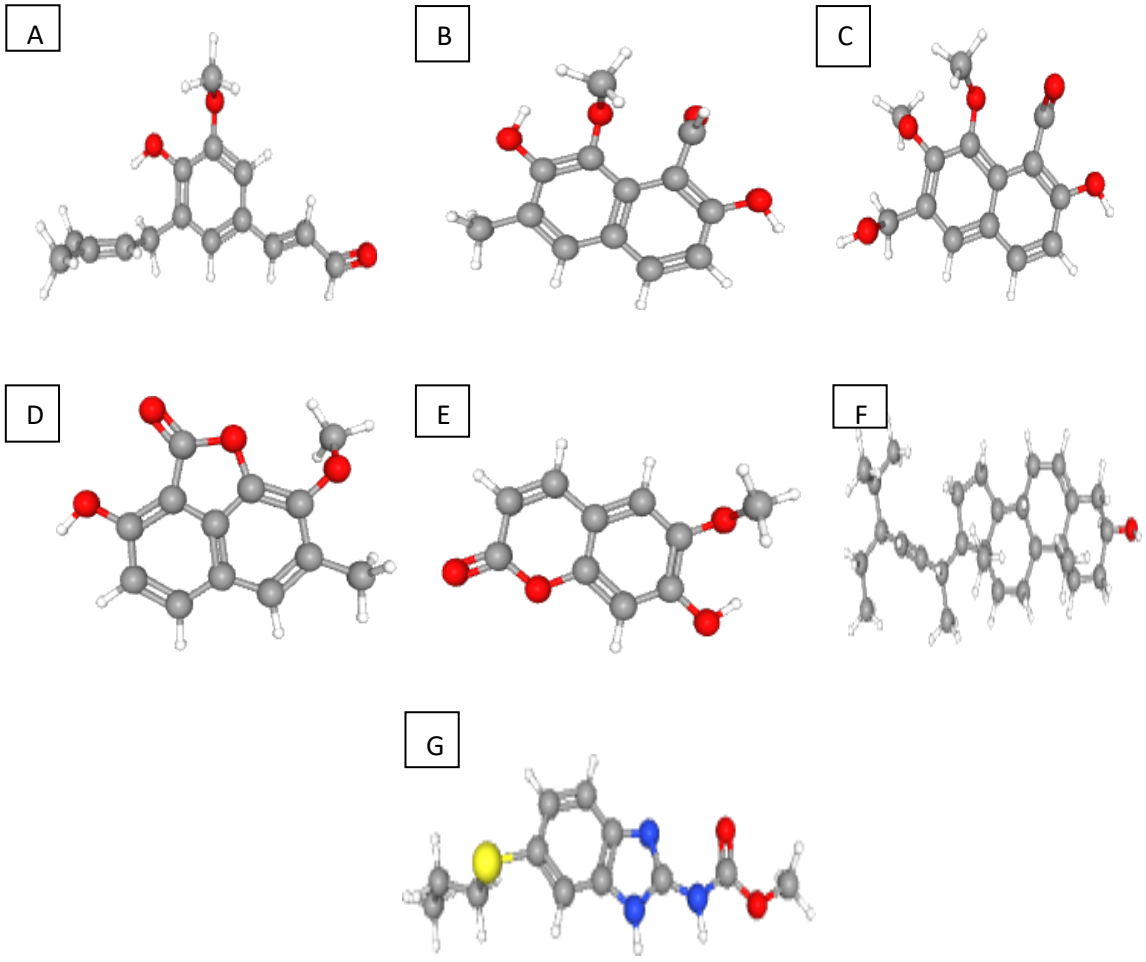


Figure 3: Chemical Structures of phytocompounds present in *Dechaschistia* A)Parvifloral B)Syriacusin A C) Syriacusin B D) Syriacusin C E) Scopoletin D) Stigmasterol & G) Albendazole (Standard drug)

RESULTS AND DISCUSSION

ADME analysis of Phytoconstituents in *D. crotonifolia:*

All the phytocompounds shown the zero violation except stigmasterol as it shown 1 violation.The standard drug

Albendazole also showed zero violation were depicted in Table 1.

Table 1: ADME Analysis of Phytocompounds

S. No.	Phyto compounds	Molecular Weight[a] (g/mol)	H-donor[b]	H-acceptor[c]	Log Value d	PMolar Refractivity[e]	Drug likeness
1.	Parvifloral	246.30	1	3	3.06	73.78	0
2.	cusinA	232.23	2	4	2.24	64.84	0
3.	cusinB	262.26	2	5	1.84	70.47	0
4.	cusinC	230.22	1	4	2.53	62.39	0
5.	Scopoletin	192.17	1	4	1.52	51.00	0
6.	ıasterol	412.17	1	1	6.97	132.75	1
7.	ıdazole	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 & 130

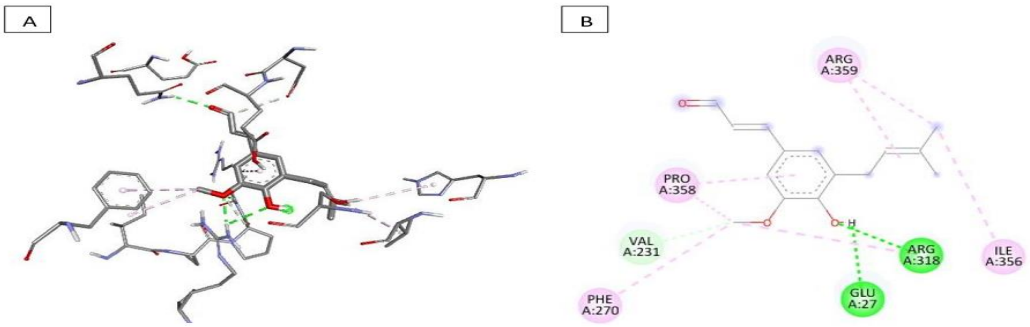
In silico Study

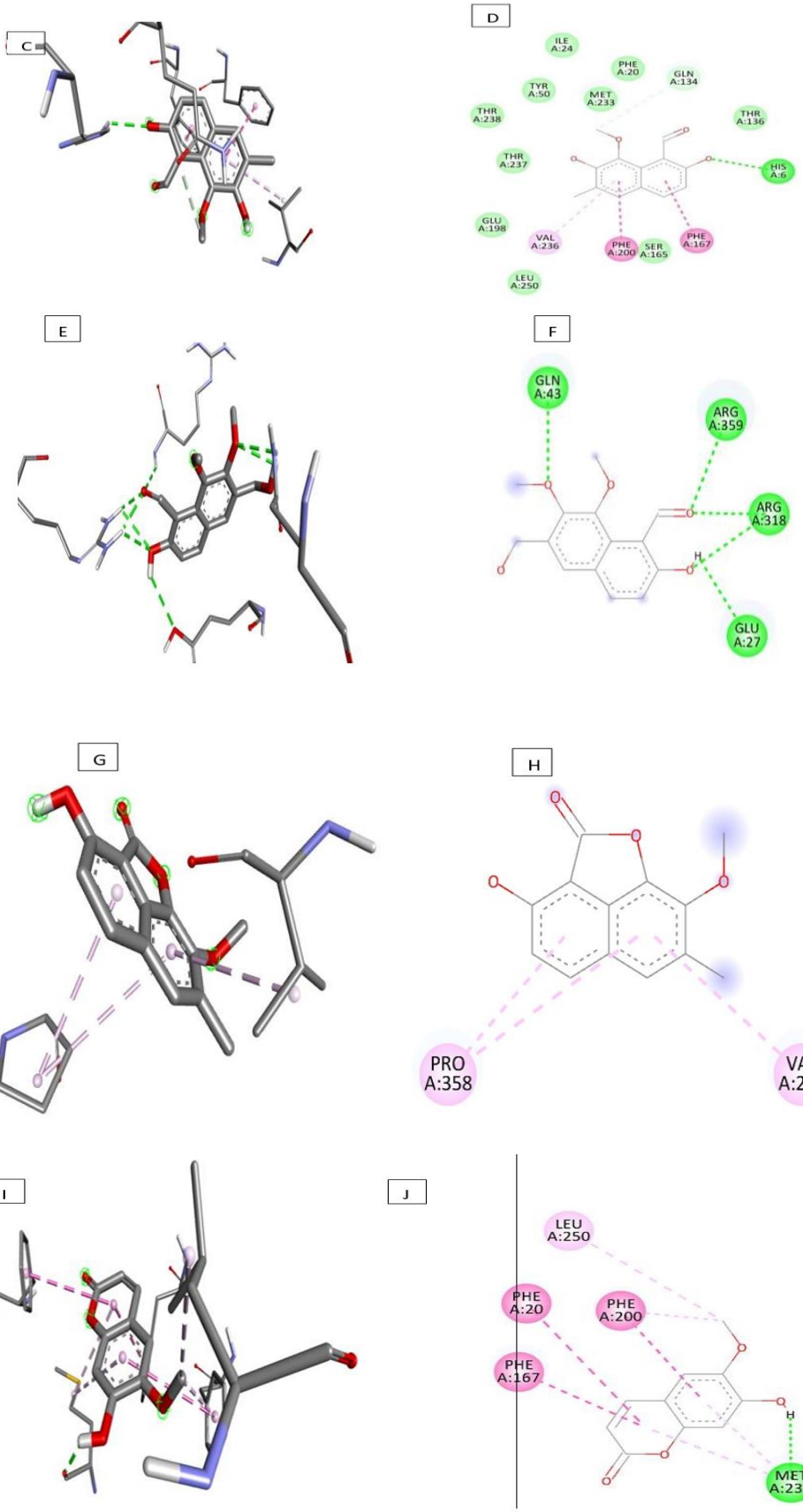
Docking revealed that out of 6 phytocompounds Parvifloral, Syriacusin A, Syriacusin B, Syriacusin C, Scopoletin and Stigmasterol with protein β-tubulin had shown docking scores of -6.3 kcal/mole, -6.9 kcal/mole, -6.0 kcal/mole, -6.7 kcal/mole, -7.7 kcal/mole, -8.7 kcal/mole and standard drug Albendazole 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 & protein complex except with

Syriacusin C. The outcomes are depicted in Table 2 and the complexes are made visualized inFigure 4.

Table 2: Docking Simulation of β-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)
2.	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)
3.	Syriacusin B	-6.0	GLN (A:43), ARG (A:359), ARG (A:318) and GLU (A:27)
4.	Syriacusin C	-6.7	-
5.	Scopoletin	-7.7	MET (A:233)
6.	Stigmasterol	-8.7	GLN (A:43)
7.	Albendazole	-7.6	SER (A:615) & VAL (A:236)

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D
ILE A:24
PHE A:20
GLN A:134
TYR A:50
MET A:233
THR A:238
THR A:136
THR A:237
HIS A:6
GLU A:198
VAL A:236
PHE A:200
SER A:165
PHE A:167
LEU A:250
E
F
GLN A:43
ARG A:359
ARG A:318
GLU A:27
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PRO A:358
VAL A:231
I
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LEU A:250
PHE A:20
PHE A:200
PHE A:167
MET A:233

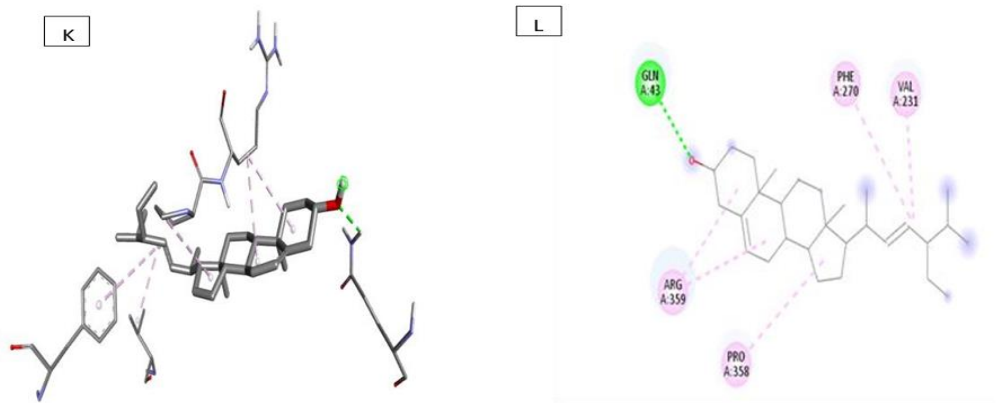

Figure 4: Visualization of 3D & 2D images of molecular docking between β-tubulin (Protein) and 6 phytocompounds present in *Dechaschistia* A,B)Parvifloral C,D) Syriacusin A E,F) Syriacusin B G,H) Syriacusin I,J) Scopoletin K,L) Stigmasterol.

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. [20-25] 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.[26]

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.[27]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}H and ^{13}C NMR spectroscopy.

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[28-30] 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 *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 β-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.

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