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Evaluation of Anthelmintic Activity of Caesalpinia Pulcherrima Leaf Extract by In-Silico and In-Vitro Studies

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Abstract: The Greek term for "helminthes" means "worm." Human-infecting parasites fall into one of two categories: keepsakes or heirlooms. Heirlooms are parasites that have been passed down from African ancestors, while souvenirs are parasites that humans have picked up from animals through migration, evolution, and agricultural activities. These helminthic infections are the most prevalent human infectious agents in poor nations. The helminthic parasite affects around 2 billion people worldwide, or more than 25% of the total population. It is a big problem in developing nations, particularly for young people. Therefore, the goal of the current study was to extract the plant's active ingredients and test the crude extract's anti-helminthic properties. In this study, we worked on the ceasalpeniapulcherima plant with a focus on natural remedies for the treatment of helmintiasis. The chief scientist of Sri Konda Laxman Telangana State Horticulture University, Floriculture Research Station, Rajndranagar, authenticated the true nature of the plant. Water and ethanol were utilized in the extraction procedure. To find out what chemicals are in the alcoholic extract, a screening test is performed on it. The flavonoid test on the extract came out positive. Using Albendazole as the prescribed medication, the anthelmintic action was carried out on Indian earthworms. In Indian earth worms, we found that alcoholic extract exhibits good antihelmintic action. By Insilco studies we found the interaction of flavanoids with antihelmentic proteins.

Key Word: Helminthes, ceasalpeniapulcherima, flavonoids, extractioin, in silico study.

1. Introduction

Plant profile: CaesalpiniaPulcherrima

Classification:

Kingdom:PlantaeOrder: FabalesFamily:Fabaceae
Subfamily:CaesalpinioideaeGenus:Caesalpinia
Species:CaeaslpiniaPulcherrima

It is a shrub growing to 3 m tall. In climates with few to no frosts, this plant will grow larger and is semievergreen. In Hawaii this plant is evergreen and grows over 5 m tall. Grown in climates with light to moderate freezing, plant will die back to the ground depending on cold, but will rebound in mid- to late spring. This species is more sensitive to cold than others. The leaves are bipinnate, 20–40 cm long, bearing three to 10 pairs of pinnae, each with six to 10 pairs of leaflets 15–25 mm long and 10–15 mm broad. The flowers are borne in racemes up to 20 cm long, each flower with five yellow, orange, or red petals. The fruit is a pod 6–12 cm long.[18]

The Pride of Barbados is an evergreen shrub or small tree that is a member of the Fabaceae or legume family. It is originally from Mexico and the Caribbean and has beautiful showy orange-red flowers, fern-like leaves, and prickles on its stems and branches. It is the national flower of Barbados [19]

Chemical constituents:

Various Phytoconstituents have been isolated from the various parts of Caesalpinia Pulcherrima Linn. Caesalpinia pulcherrima is rich source of polyphenols, Flavonoids [20]

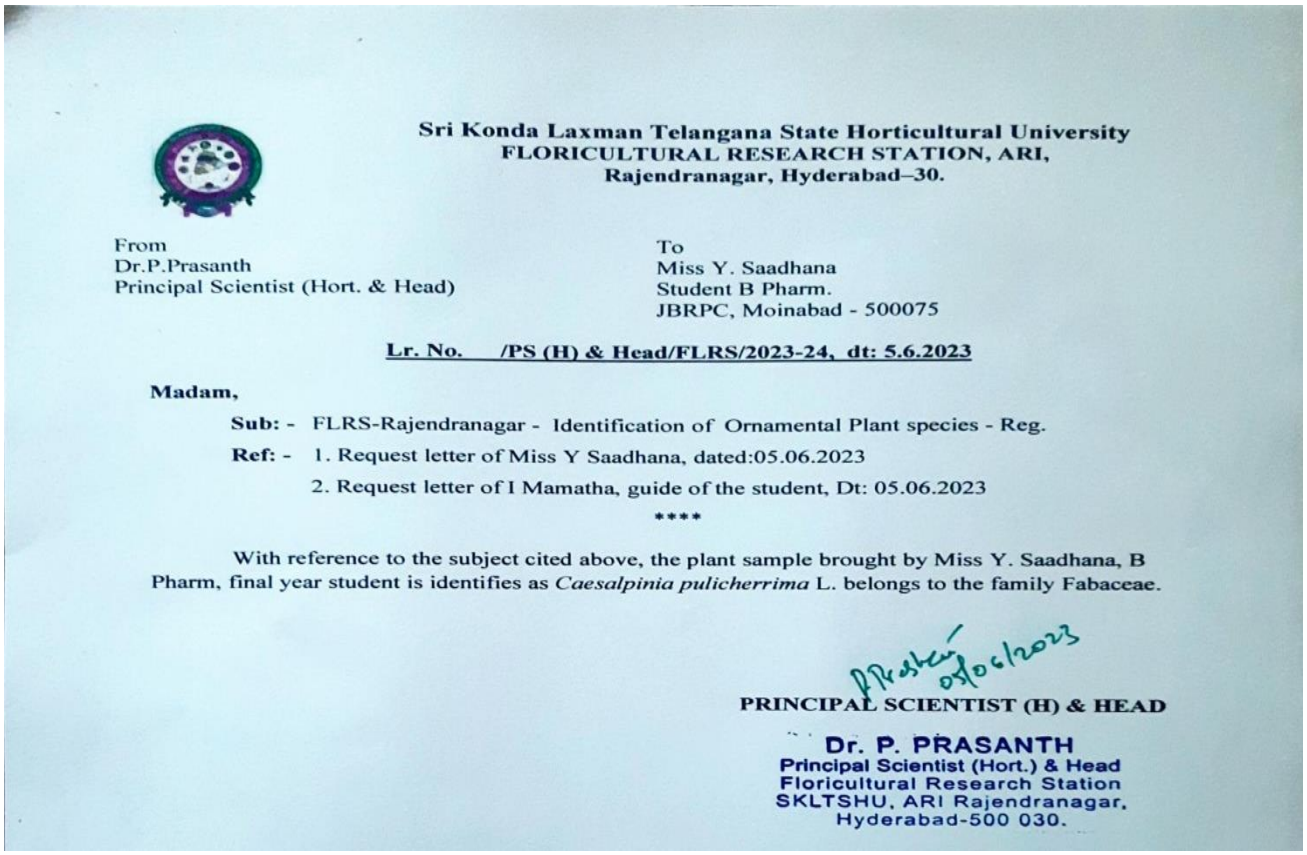
Pharmacological actions: Antioxidant Activity, Anticancer Activity, Immunosuppressive Activity, Antidiabetic Activity, Anti-Inflammatory Activity, Anti-Microbial Activity, Vaso relaxing Effect, Abortifacient , Cathartic [20]

2. Meterials and Methods

Materials: Plantmaterial,Grinder, beakers, soxletapparatus, Whattman Filter paper ,funnel, rotary evaporator, chinadish, desiccators, Petridishes, IndianEarthworms.

Plant material:

The leaves of Caesalpinia Pulcherrima were collected from local areas of Ranga Reddy,Hyderabad,India.The plant material was collected during morning hours. They were authenticated, by Dr.P.Prasanth, Principal scientist (HortiandHead) Sri Konda Laxman Telagana state Horticultural University, Floricultural Research Station, ARI, Rajendra Nagar,Hyderabad-500030,India.



Sri Konda Laxman Telangana State Horticultural University
FLORICULTURAL RESEARCH STATION, ARI,
Rajendranagar, Hyderabad–30.

From
Dr.P.Prasanth
Principal Scientist (Hort. & Head)

To
Miss Y. Saadhana
Student B Pharm.
JBRPC, Moinabad - 500075

Lr. No. /PS (H) & Head/FLRS/2023-24, dt: 5.6.2023

Madam,

Sub: - FLRS-Rajendranagar - Identification of Ornamental Plant species - Reg.

Ref: - 1. Request letter of Miss Y Saadhana, dated:05.06.2023
2. Request letter of I Mamatha, guide of the student, Dt: 05.06.2023

****

With reference to the subject cited above, the plant sample brought by Miss Y. Saadhana, B Pharm, final year student is identifies as *Caesalpinia pulicherrima* L. belongs to the family Fabaceae.

05/06/2023

PRINCIPAL SCIENTIST (H) & HEAD

Dr. P. PRASANTH
Principal Scientist (Hort.) & Head
Floricultural Research Station
SKLTSHU, ARI Rajendranagar,
Hyderabad-500 030.

ChemicalsUsed:99.9%Ethanolwas collected from lab needs, Hyderabad, Telangana.

Methods: plant extraction is done by maceration and Soxhletion process. Anthelmintic activity was determined by molecular docking study (Auto dock & discovery studios) to identify compounds having maximum activity against Tubulin Colchicine protein & in vitro studies were carried out on Indian earthworm

Preparation of extract:

The sufficient amount of plant materials was collected and brought to the laboratory and washed thoroughly [3times] with clean tap wate. After that, it was air dried completely under s hade area. The resulting powder was sieved, weighed and store dincleans topper bottles and kept in dry place until the extraction process was started. In plant prepartion, extraction is first crucia step. The powdered plant material was prepared and kept in dried place were subjected to 99.9% Ethanol as solventusing cold macerationand soxhlet extraction technique.

Ethanol was the most preffered solvent based on the access to the alcohols and general solvent and most perferred solvent for plant extraction possibly owing to its polar nature that ensure there lease of several bioactive compounds from the plants. It has been proven that high polarity solvents should be used to extract different bioactive compounds with high accuracy. Fruitful results of active compound in plant mainly depend up on the solvent used for herbal formulation.

The powdered specimen was then subjected to extraction using 99.9% Ethanol by cold maceration and soxlet extraction technique. A total of 300g of the powdered material was separately soaked and then followed by soxlet extraction where the plant material was properly wrapped within the filer paper [WhatmanNo.1]with ethanolin R.Bflaskat70℃for72hrs.Then,the extract was again filter educing filter paper and was extracted using Rotary Evaporator, to evaporate Ethanol at 60℃wit 60 RPM and kept in hot air oven to obtain pure crude extract .The extract were taken weighed to know the yield of plant and recored .

SoxhletExtraction ofC.pulcherrimaLeavesbyethanol

Rotary vaccum evaporator

Phytochemical study:

Phytochemical analysis:

Preliminary phytochemical screening of aqueous and ethanolic extracts of CaesalpiniaPulcherrima leaves was performed for the detection of the Constituents that were responsible for the activity.

1 Testforalkaloids:

To the extracts dilute hydrochloric acid was added and filtered. The filtrate was treated with various alkaloid reagents. [61]

a) Mayer's test:

When the filtrate was treated with Mayer's reagent, Potassium Mercuric iodine solution, appearance of cream colored precipitate indicated the presence of alkaloid. [61]

b) Dragendorff's test:

When the filtrate was treated with Dragendroff's reagent, potassium bismuth iodine solution, appearance of orange brown precipitate indicated the presence of alkaloids. [61]

c) Hager's test:

The filtrate when treated with Hager's reagent, picric acid, appearance of yellow color precipitate indicated the presence of alkaloids. [61]

2 TestforCarbohydrates:

Small quantities of the extracts were dissolved in 4ml of distilled water filtrated. The filtrate was subjected to following tests.

a) Fehling's test:

The extracts were treated with Fehling solution A and B. The appearance of reddish brown color precipitate indicated the presence of reducing sugars. [61]

b) Benedict's test:

The extracts were treated with Benedict's reagent; the appearance of reddish orange color precipitate indicates the presence of reducing sugars. [61]

3 **TestforSteroids:**

a) Liebermann Burchard test:

The extracts were treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green color indicated the presence of steroids. [61]

b) Salkowski's test:

The extracts were treated with 3ml of acetic anhydride, concentrated sulphuric acid drops. Appearance of yellow color indicated the presence of steroids. [61]

4 TestforTannins:

a) The extracts were treated with 10% lead acetate solution. Appearance of white precipitate indicated the presence of tannins. [61]

b) The extracts were treated with aqueous bromine solution. Appearance of white color precipitate indicated the presence of tannins. [61]

7.3.5 Test for Phenolic compounds:

a) The extracts were treated with neutral ferric chloride solution. Appearance of violet color indicated the presence of phenolic compounds. [61]

b) The extracts were treated with 10% sodium chloride solution. Appearance of cream color indicated the presence of phenolic compounds. [61]

5. TestforFlavonoids:

a) Zinc hydrochloride test:

To the test solution, add a mixture of zinc dust and concentrated hydrochloric acid. It gives red color after few minutes. [61]

b) Shinoda's test:

The extracts were dissolved in alcohol to which few magnesium turnings were added followed by concentrated HCl drop wise and heated. Appearance of magenta color shows the presence of flavonoids. [61]

6 .TestforGlycosides:

When a pinch of the extracts were treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides. [61]

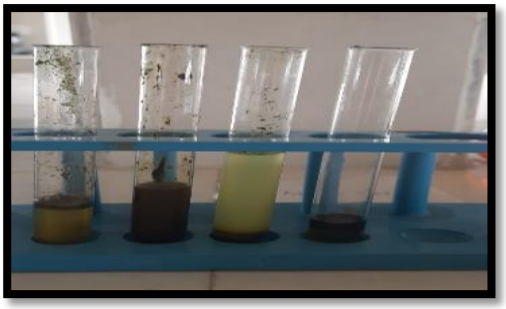
7. TestforSaponins:

Foam test:

About 1ml of the extracts were diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicated the presence of saponins. [61]

8.TestforFixedoils:

Mix 1ml of 1% copper sulphate solution and 5 drops of the extract. Then add 5 drops of 10% sodium hydroxide solution. A clear blue colour solution was obtained which indicates the presence of fixed oils.[61]



Identification test of flavonoids

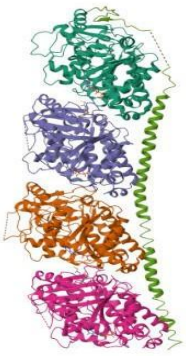
Insilicomoleculardockingstudy

For molecular docking study, Autodock is used to predict the potent active compound of Caesalpiniapulcherima against the active site of TUBULIN COLCHICINE enzymes.

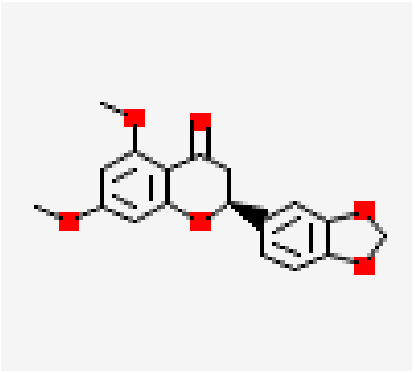
ProteinandLigandPreparation:

In case of the protein preparation, the 3D structure of TUBULIN-COLCHICINE receptor was obtained from the Protein Data Bank (PDB: 1SAO). Afterward, the structure was prepared and refined using the protein preparation wizard [Discovery studios] [Biovia] where charges and bond orders were assigned, hydrogens were added to the heavy atoms, selenomethionines were converted to methionine, and all waters portion were removed. On the othe hand, certain thiol and hydroxyl groups we rereoriented, and amide groups of a sparagines, glutamine, and imidazolering of his tidines, protonation states of his tidines, glutamic acidandaspartic acids were optimized at neutralpH. 5,7-dimethoxy-3'4'-

methylenedioxy flavanone, was the ligand selected by collected literature review. Molecular Formulais $C_{18}H_{16}O_6$. Molecular weight:328.3g/mol. Computed by pubchem release 2021.05.07. It is natural product found in Caesalpinia Pulcherriama[63].Ligand optimizationor refined using chemdraw3D.



Protein: TUBULIN-COLCHICIN



Ligand

3. Result & Discussion

Preliminary Phytochemical Analysis:

The results of preliminary phyto chemical screening is present in Table3. Qualitative Phytochemical studies wrew performed one thano licandaqueou sextract of CaesalpiniaPulcherrima leaves using suitable chemicals and reagents to confirm the presence of phenolics, steroids, flavonoids, lipids, and tannins.

Phyto chemical constituents	Aqueou sextract	Ethano licextract
Carbohydrates	-	+
Phenols	+	-
Glycosides	+	-
Alkaloids	+	-
Flavonoids	+	-
Tannins	+	-
Steroids	-	+

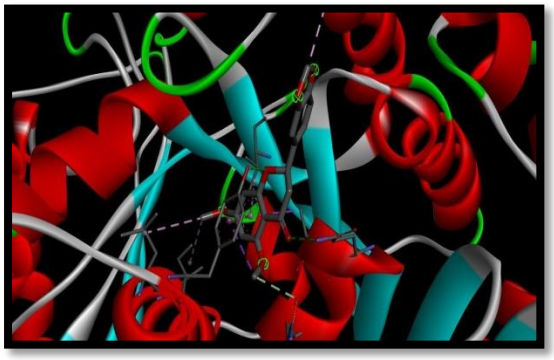
*"+"indicatesthepresence"-" indicates the absence**Table3***

InsilicoStudies:

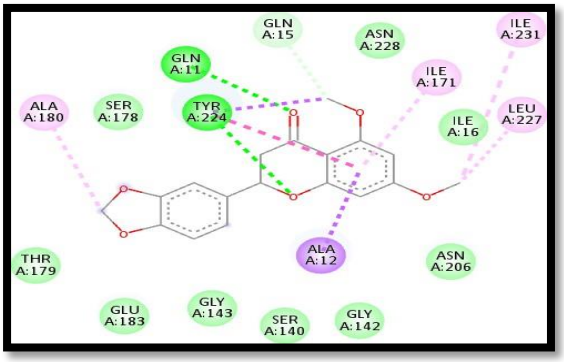
in this study, compound isolated from Caesalpinia pulcherima leaves were isolated for molecular docking study and the results shown in the table. Molecular docking showed that has the best docking score against TUBULIN COLCHICINE which is -7.37kcal/mol. Interactions between ligand and protein have been presented below

Binding Energy	-7.37
Ligandefficiency	-0.31
Inhibconstant	3.94
Inhibconstant units	uM
Intermolenergy	-8.27
Vdw_hb_deolv_energy	-8.4
Electrostatic_energy	0.13
Totalinternal	0.48
Torsionslenergy	0.89
Unboundenergy	0.48
Filename	dock.dlg
clRMS	0.0
refRMS	158.26
rseed1	None
rseed2	None

Table-2: Molecular Docking results of ligand and protein .The binding score was found tobe-7.37kcal/mol



3D MolecularDockingofProteinand Ligand



Dockingresultsof5,7-dimethoxy-3'4'-methylenedioxyflavanonewithTUBULIN-COLCHICINEenzyme[PDB:1SAO]foranthelminticactivity.Thecolorsindicatetheresidue.Interactions with the protein are marked with lines between ligand and proteinresidues.

In-vivostudies:

The shade dried leaves of Caesal piniapulcherrima were pulverized into coarse particles and extracted it habsoulte ethanol and distilled water using soxhlet extractor for 72 hrs and extract with absolute extarct by cold maceration for 72hrs. Both the aqueous and ethanol extracts were concerated in rotary evaporator at temperature less than 45°C and preserved in desiccator forfurtheruse. The yield fore than olic extract and aqueousextract were 49.6% and 46.08%,respectively. The preliminary phytochemical analyis were carried out to find phtoconstituents presntin crude extract. Indian earthworm heretimaposthuma were collected , the average size of earth wormbeing 6-8cm.They were washed with tap water tore move adhering dirt and soil particles.

Breifly,10ml formulations containing three different concentarions, each of crudealoholicextractof leaf [5,10,15ml] were prepared and six earthworms were placedin it. Both the test solution and standard drug solution were freshly prepared and time for paralys is was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The time of death of worms was recorded after ascertaining that the worms neither moved when shaken vigorouslynor when dipped in warm water at 50°C. A maximum time period of 120mins was ascertained forthe paralyzing as well as death time of pheretimaposthuma worms. Albendazol was used as reference standard with distilled water as the vehiclecontrol.

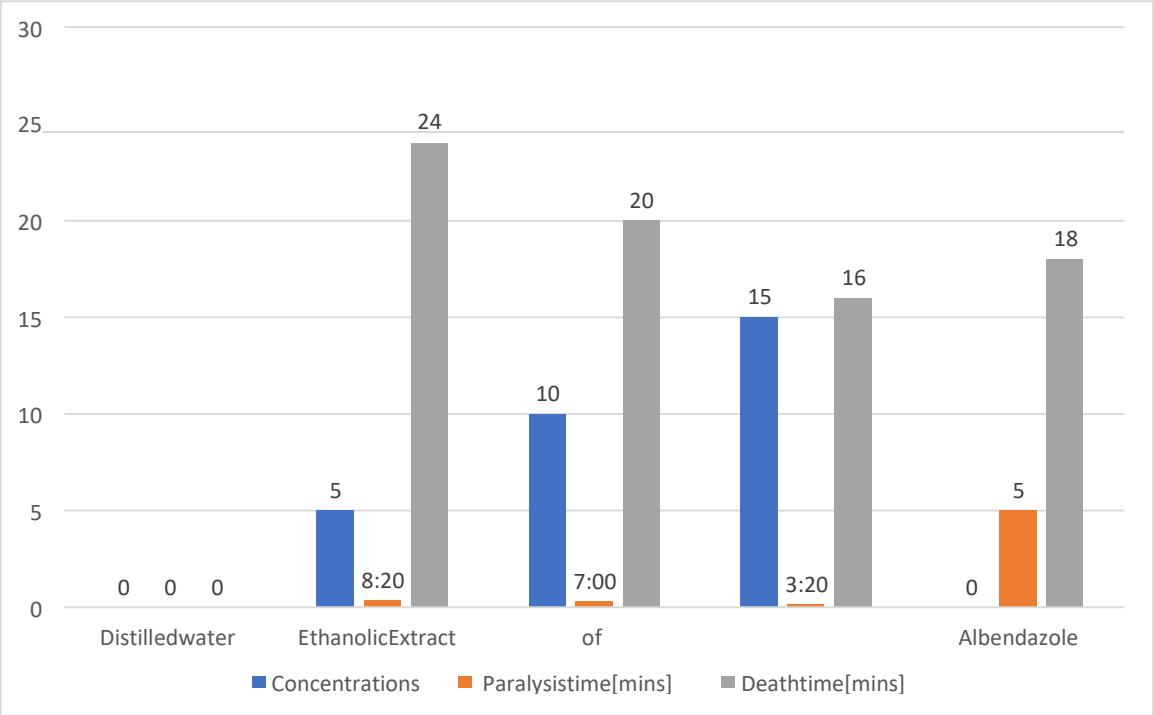
From the observations made, adose dependent paralytic effect much earlier and the time of death was observed inTable5.Although ethanolic extract appeared to be more effective for worms. Evaluation of anthelmintic was compared with standard Albendozole. The Ethanolic extract of leaf of Caesapinia Pulcherrima, caused paralysis at 08.20 mins and time of death at 24mins for pheretimaposthuma and standard drud Albendazoleshowed25.00and40mins,respectively.

The anthelmintic Activity of Ethanolic Extract of CeasalpiniaPulcherrima leaf on Indian Earth wormphere timaposthuma was indicatedintable :5 as follows

Groups	Concentrations [mg/ml]	Pheretima Posthuma	
		Paralyzing time [mins]	Death time [mins]
Distilled Water	NA	NA	NA
Ethanoloic Extarct of C.Pulcherrima leaf	5	8:20mins	24mins
	10	7:00mins	20mins
	15	3:20 mins	16mins
Albendazol	10mg	5mins	18mins

Table:3 Anthelminticactivity of ethanolic extract of Ceasalpinia pulcherrima leaf

The anthelmintic Activity of Ethanolic Extract of Ceasalpinia Pulcherrima leaf on Indian Earth wormpheretima posthumawas indicated in graph-1 as follows



Graph:1Anthelmintic activity of ethanolic extract of Caesalpinia Pulcherrima leaf on Indian Earth worm PheretimaPosthuma

Anthelminticaction of drug on different concentrations

Discussion:

The Problem of anthelmintic activity resistance, toxicity and the increasing on cernover the presence of drug residues in animal product has led to are newal of interest in the use of plant based drugs.Plantmaterial sevaluatedin the current study has been identified from various sources to serve as an the lmintic agent by traditional healers of. The invivo test using free living stages of parasiticnematodes offer a means of evaluating the anthelmintic activity of new plant compounds [63]. In vitrotechniques are preferred to in vivo methods due to their low cost, simplicity, and rapid turnover [64].Plant-derived natural products have gained attention as a potential source of new therapeutic agents. The medicinal properties of plant shave been investigated due to their potent pharmacological activities, low toxicity, and economic viability.Moreover, most of the clinical lyactive drugs are from natural products which indicate the importance of drugs having natural sources in drug discovery process. So, it is essential to study the medicinal plants so that the discovery of active natural product singredient can be identified for healing diseases and then the identified active ingredients could besyn the sized in the laboratory[64]. Helminths infection is considered to be a significant problem in human and animals that leads to achronic and devastating disease which ultimately leads to death and also causes drug resistance to other diseases. To prevent infection of helminths, there is a need for studies focusing on natural products such as medicinal plants which give new bioactive compounds having no or fewer side effects, easily available to the peoples of developing countries and more importantly, they have the best compatibility with human physiology than conventional drugs

In the current study, assign if icant association was noted between graded concentartions of the extract, the exposure test time interval and mortality of the earth worm.

Considerable experimental data from earth worm studies have shown thaethanolic extract of CeasalpiniaPulcherrima leaf exposure produces pronounced an the lemintic activity. This experimental model is an suitable one to evaluate the potential of new anthelinitic agents withan anthelmintic effect. Hence the focus of the present work was to evaluate the effect and possible mechanis munder lined.

In the present study, showed 100% efficacy of the plant extract of leaves of Caeasalpinia Pulcherrima against the earth worms at the concentration of 15mg/ml which was the highest efficacy value and was comparable with the standard an the lmintic, Albendazole. Administration of ethanolic extract of Ceasalpinia Pulcherrima had significantly shown its an the liminticaction indosedependent manner.The whole plant of CesalpiniaPulcherrima is documented to possess medicinal properties antitumour(Cheetal.,1986,Pateletal.,1997)and antimicrobialproperties(Ragasaetal,2002). Some differences on the percentage yield of these extract materials among the plants might be due to the difference on the nature of plant species, chemical composition differences of the extracts, different environmental conditions which create differences in phytochemical constitution, and harvest time.Further more, the solvents and test protocols used during extraction promote difference in concentrations and classes of secondary bio actives present inextract.

Our current study concludes that EECPL has been found to possess significant anthelmintic potentialin a dose-dependent manner. This activity may be due to the presence of bioactive phytoconstituentssuch as alkaloids, tannins, flavonoids and saponins and also a considerable amount of condensedtannins. Some of these phytoconstituents such as alkaloids, tannins, phenols etc. may be responsible for the significant an the lminticactivity. Here, alkaloid scan produce paralysis by acting on the central nervous system(CNS)where as tannins and polyphenols electively bind to free proteinspresent in the GI tract (gastrointestinal tract) and eventually cause mortality. On the other hand, the anthelminticefficacy of saponins is due to its membrane permeabilising property. The anthelmintic activity of the EECPL may be due to a single compound or combined effect of these phyto chemicals.

Administration of 5mlconcentrationsofethanolicextarctof Caesalpiniapulcherrimaleafhadparalysistime of 8:20 mins and death time was 24mins.Administration of 10ml concentrations of ethanolicextarct of Caesalpiniapulcherrima leafhadparalysistimeof7:00minsanddeathtimewas20mins.Administrationof5mlconcentrationsofethanolicextarctof Caesalpiniapulcherrimaleafhadparalysis timeof 3:20minsanddeath timewas16mins.

Alltheconcentrationsarecomparedwithstanadard Albendazoledrug10mg,wheretheparalysistimewas 5 minsand deathtimewas18mins.

As far as traditional approach is considered “one drug, one target” theory of drug design is used, incontradictory network pharmacology which aims to explore the correlation of drugs and diseases,based on the multi-targeted therapy. Docking study was carried out to find the affinity as well as orientation of the selected active component by docking the magainst the selected receptors.

We have also evaluated the molecular docking of some compounds to demonstrate the collaboration between compounds and protein at the molecular level, which enables us to portray the conduct of molecule of those compounds in the coupling site of targeted proteins and toillustrate the biochemical process of the anthelminthic activity. From the result (as shown in Table4), it is concluded that[-7.37kcal/mol]showed the significant docking scores.From the result of docking study, it is clear that these compounds especially CaesalpiniaPulcherrima leaves extract can be a good candidate for new an the lminticagent.

4. Conclusion

FromthepresentInvestigation,The preliminary phytochemical estimation done shown the presence of alkaloids, phenols, Glycosides,Flavonoids, tannins. EECPL exhibited a dose-dependent and statistically significant anthelmintic activityon Indian earthworm .The best concentration of MEPSS for anthelmintic activity compare with reference standard albendazole(10mg/mL).On the other hand, our molecular docking study shows that has the best fitness score of − 7.37 kcal/mol with TUBULIN-COLCHICINE enzyme with the chemical constituents in the leaf.

Results of the present study confirmed potential an the lmintic activity of CaesalpiniaPulcherrima leaf extract and all compounds were found to be effective in computer aided drug design models.

From above discussion we can assume that this plant can play a prominent role for anthelminticactivity. We can suggest CaesalpiniaPulcherrimafor further research to amend the activity of an the lminticfor better effect

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