Anthelmintic and Quantitative Analysis of Phytocompounds from Methanolic Extract of an Endangered Medicinal Plant Decalepis hamiltonii

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ABSTRACT

Over the centuries, traditional cultures around the world have learned how to use phytomedicines to fight illness and maintain health. Medicinal plants contain a wide range of metabolites that can be used to treat chronic as well as infectious diseases. In spite of tremendous development in the field of synthetic drugs and antibiotics during the 20th century, plants still continue to be a major source of drugs in modern as well as traditional systems of medicine throughout the world. The present study is focused on the extraction of secondary metabolites from the medicinal plant **Decalepis hamiltonii** (Wight and Arn.) (family:Asclepiadaceae), is an endemic and endangered to the southern peninsula. The study was designed to screening the phytocompounds qualitatively, quantitatively and evaluates the anthemintic and anti microbial activity of methanolic root extract of the selected plant against the Indian Earthworm **Pheretima posthuma and pathogenic microorganisms respectively.** Different concentrations of methanolic extract were tested and results were expressed in terms of time for paralysis and time for death of worms for antihelmenthic and zone of inhibition for antimicrobial activity. The methanolic roots extract shows the better results compare to the control.

Introduction:

Nature is a nice source of salvation for human being by providing different remedies from its plants, animals and other sources to treat all ailments of mankind. Among all of the natural sources, medicinal plants are important contributors to the medicinal preparations. Several thousands of plants containing medicinal values have been identified by expert scientists for treating different ailments. So, medicinal plants always play an important and crucial role for the development of health in mankind. Plants are an exemplary source of traditional medicine and pharmaceutical drugs for human kind since time immemorial. About 80% of world population still dependent upon the herbal drugs for their health care. Roots account for 60% of the medicinal plants used in the traditional systems of medicine (Ayurveda,

Siddha, Unani) as the principle material for drug preparation [1]. *Decalepis hamiltonii* Wight & Arn (Asclepiadaceae) is a monogeneric climbing shrub native of Deccan peninsula and forest areas of Western Ghats of India, commonly known as Shwet Sariva. This medicinal plant play a key role in Ayurveda, the ancient Indian traditional system of medicine because of having the plenty of medicinal values to the rhizome. The rhizome is largely used for pickling along with curd or limejuice [2], used as demulcent, diaphoretic, diuretic and tonic. It is useful in the loss of appetite, fever, skin diseases, diarrhoea, nutritious disorders, as blood purifier [3],[4], antimicrobial [5], [6] and in treatment of epilepsy and central nervous system disorders. The roots have been used locally to stimulate the appetite and to relieve flatulence and act as a general tonic [7] all these activities of the rhizome is having the abundant bioactive compounds such as alkaloids, flavanoids, ellagic acid [8], flavor compound 2-Hydroxy 4-methoxybenzaldehyde as a major compound (97%)[9]. aldehyde, inostiols, saponins, amyrins and lupols[10]. In addition 4-hydroxyisopthalic acid, 14- aminotetradecanoicacid, 4-(1-hydroxy-1-methylethyl)-1-methyl- 1,2- cyclohexane diol, 2-(hydroxymethyl)-3-ethoxybenzaldehyde, 2,4,8-trihydroxybicycle (3.2.1) octan-3-one, bis-2,3,4,6-galloyl-α/β- D-glucopyranoside have been identified in swallow root [11],[12]. The roots have also been used as a substitute for Hemidesmusindicus in ayurvedic preparations of ancient Indian medicine [13]. It possess potent antioxidant properties [14], antiulcer, anti – inflammatory [15] and antipyretic [16], gastroprotective [17] activities. The present study was focus on the qualitative and quantitative analysis of phytocompounds and evaluation of the bioactivities such as antimicrobial and anthelmenthic of crude methanolic root extract of Decalepis hamiltonii.

Materials and Methods:

Plant material Collection

The roots were collected from Tirumala Hills, Tirupati, AP, India. The root (plant material) was identified and authenticated by Taxonamist Dr.Madhava Shetty, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Preparation of Root extract

Young, fresh and non diseased roots were washed under tap water, air dried under shade, powdered and stored in airtight bottles. 100g of **D.hamiltonii** root powder was subjected to successive extraction with different solvents of increasing polarity viz., ethyl acetate, chloroform, methanol, ethanol, amyl alcohol and distilled water and further analysis of phytochemicals were carried out in the second cycle of soxhlet apparatus.

Screening of Phytoconstituents

Preparation of reagents:

Bromocresol green solution*:* solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. ***Phosphate buffer solution (pH 4.7):*** buffer solution was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na2HPO4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Folin- Ciocalteu's (FC) reagent: 10ml of of Folin- Ciocalteu's solution was dissolved in 90ml of double distilled water.

Iron (III) chloride solution*:* 500mg of ferric chloride was weighed and was dissolved in 100ml of distill water.

Potassium hexacyanoferrate (III) solution*:* 500mg of potassium hexacyanoferrate was weighed and was dissolved in 100ml of distill water.

Preliminary phytochemical screening:

1. Test for steroids:

Salkowski Test*:* Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour.

Liebermann Burchard's Test*:* To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.

2. Tests for triterpenoids:

Salkowski Test: Few drops of concentrated sulphuric acid is added to the extract, shaken and on standing, lower part turns golden yellow colour.

Lieberman Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a red ring indicates triterpenes.

Ischugajiu Test: Excess of acetylchloride and pinch of zinc chloride are added to the extract solution, Kept aside for reaction to subside and warmed on water bath, cosin red colour is produced.

Brickorn and Brinar Test: To the extract, few drops of chlorosulfonic acid in glacial acetic acid(7:3) are added, red colour is produced.

3. Test for Saponins:

Foam Test: Small amount of extract is shaken with little quantity of water, the foam produced persists for 10 minutes. It confirms the presence of saponins.

Haemolysis Test*:* To 2ml of 1.8% Sodium chloride solution in two test tubes, 2ml distilled water is added to one and 2ml of 1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract indicates the presence of saponins

4. Test for Steroidal Saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

5. Tests for Triterpenoidal Saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

6. Tests for Alkaloids:

Mayer's Test: The acid layer when mixed with Mayer's reagent (Potassium mercuric iodide solution) gives creamy white precipitate.

Dragendroff's Test*:* The acid layer with few drops of Dragendroff's reagent(Potassium bismuth iodide) gives reddish brown precipitate.

Wagner's Test: The acid layer when mixed with few drops of Wagner's reagent(solution of iodide in potassium iodide) gives brown to red precipitate.

Hager's Test*:* The acid layer when mixed with few drops of Hager's reagent(Saturated solution of pricric acid)gives yellow coloured precipitate.

7. Test for Carbohydrates:

Fehlings's Test: The extract when heated with Fehling's A and B solutions gives an orange red precipitate showing the presence of reducing sugar.
Molischs's Test: The extract is treated with Molisch's reagent and conc .sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.
Benedict's Test: The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.
Barfoed's Test: Barfoed's reagent is added and boiled on water bath for few minutes, reddish precipitate is observed for the presence of carbohydrate.

8. Test for Flavonoids:

Shinoda Test: The extract solution with few fragments of magnesium ribbon and concentrated hydrochloric acid produced magenta colour after few minutes.

Ferric chloride test: Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and given blackfish green color.
Lead Acetate Test: Alcoholic solution of extract reacts with 10% lead acetate solution and given yellow precipitate.

9. Test for Glycosides:

Anthraquinone test: Drug is powdered and extracted with either ammonia or caustic soda. The aqueous layer shows pink color
Keller-killiani test*:* This is for cardiac glycosides. The extract and 0.4 glacial acetic acid are mixed with ferrous chloride and 0.5 mi of concentrated sulphuric acid. The acetic acid layer shows blue color.

10. Test For Phenolic Compounds:-

Ferric chloride test*:-*Treat the extract with ferric chloride solution then blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Gelatin test:- To the test solution add 1% gelatin solution containing 10% Nacl, and then ppt is formed.

Test for chlorogenic acid*:-*Treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

Table 1: Qualitative analysis of methanolic root extract of Decalepishamiltonii

S. No	Screening Tests	Ethanolic extract
1	**Steroids**	
a)	salkowski	+ve
b)	Libermann	**+ve**
2	**Triterpenoids**	
a)	Salkowski	+v e
b)	Liberman tests	**+Ve**
3	**Saponins**	
a)	Foam	_ve
4	Steroidal saponin	
5	**Triterpenoid saponin**	+ve
6	**Alkaloids**	
a)	Picric acid	_ve
7	**Carbohydrates**	
a)	Benedicts	**+ve**
b)	Molisch	_ve
8	**Flavanoids**	
a)	Ferric chloride	**+ve**
9	**Phenols**	
a)	$Fecl_3$	**+ve**
b)	chlorogenic	**-ve**

Estimation of phytochemicals:

Quantitative Estimation of Alkaloids:

To 1ml of extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Table 2: Total alkaloid:

S.NO	Concentration in µg/ml	absorbance
1	40	0.391
2	50	0.481
3	60	0.559
4	70	0.631
5	80	0.721
6	90	0.799

Estimation of Alkaloids:

S. NO	Name of the part	Solvent used	Absorbance Found	Amount found mg/gram of the extract
1	*D.hamiltonii*	methanol	0.116	**5.26**

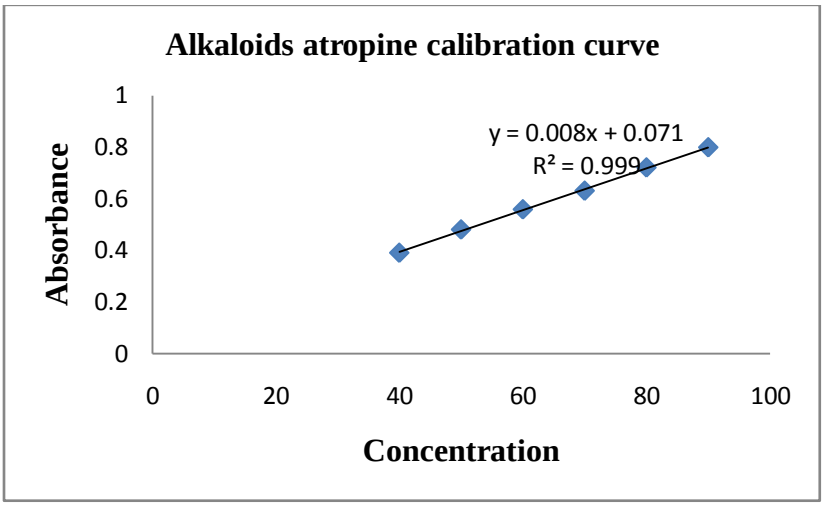

Fig: 1 Alkaloids atropine calibration curve

Quantitative Estimation of Steroids:

1ml of extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Table 3: Total Steroids

S.NO	Concentration in µg/ml	absorbance
1	0.2	0.258
2	0.4	0.605
3	0.6	0.889
4	0.8	1.215
5	1	1.507
6	1.2	1.878

Estimation of Steroids:

S. NO	Name of the part	Solvent used	Absorbance Found	Amount found mg/gram of the extract
1	*D.hamiltonii*	methanol	0.159	**13.39**

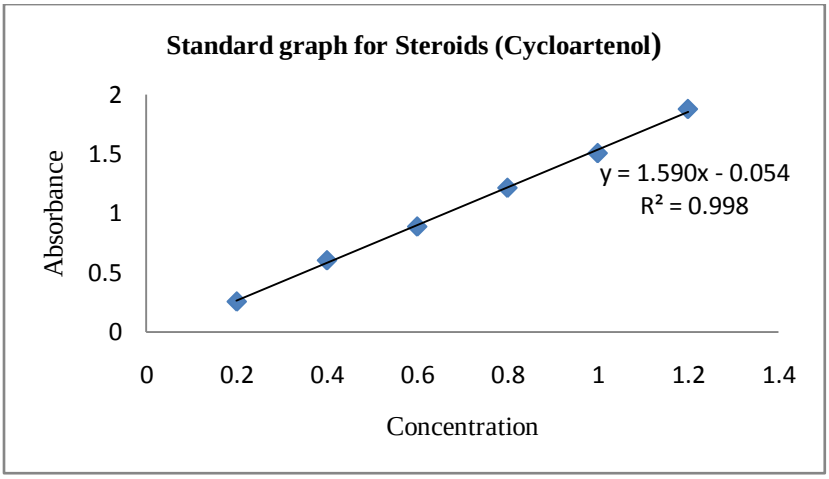

Fig 2: standard graph for steroids

Determination of total flavonoid content

Total flavonoid content was determined using aluminium chloride (AlCl3) according to a known method, 15 using quercetin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% NaNO2 (0.03ml). After 5 min at 25°C, AlCl3 (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm.

Table 4: Total flavanoid:

S.NO	Concentration in µg/ml	absorbance
1	0.5	0.214
2	1	0.387
3	1.5	0.558
4	2	0.704
5	2.5	0.864
6	3	0.997

Estimation of Flavanoids:

S. NO	Name of the part	Solvent used	Absorbance Found	Amount found mg/gram of the extract
1	*D.hamiltonii root*	methanol	0.353	**9.001**

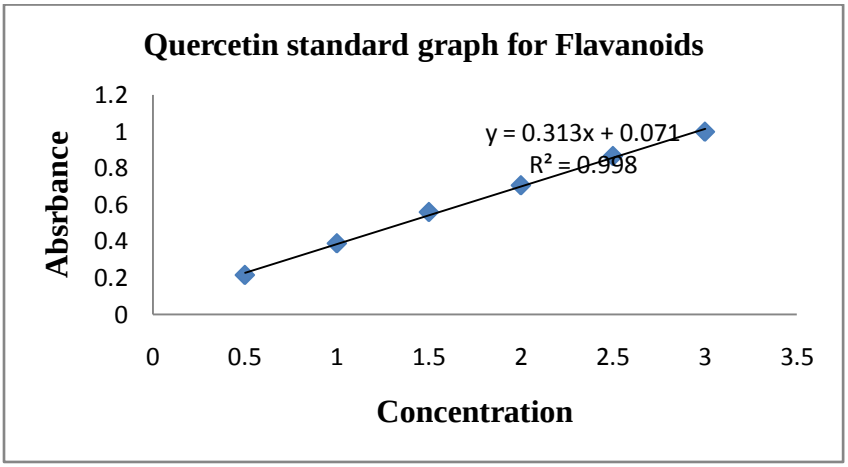

Fig: 3 Quercetin standard graph for flavanoids

Quantitative Estimation of Phenoilc Compounds:

The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, 1ml of extract was mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

Table 5: Total Phenolic compounds

S.NO	Concentration in µg/ml	absorbance
1	2	0.178
2	4	0.325
3	6	0.475
4	8	0.636
5	10	0.769
6	12	0.889

Estimation of Phenolic Compounds:

S. NO	Name of the part	Solvent used	Absorbance Found	Amount found mg/gram of the extract
1	*D.hamiltonii root*	methanol	0.225	**25.69**

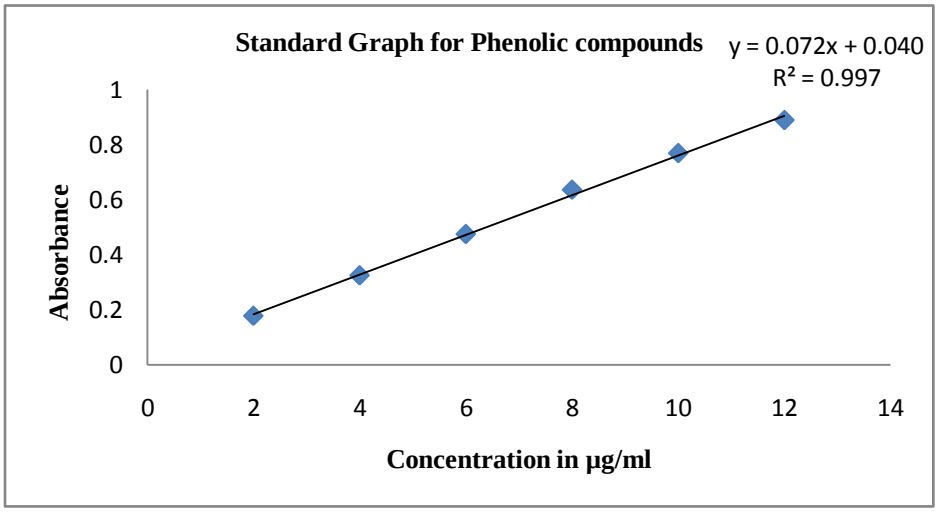

Fig:4 Standard Graph for phenolic compounds

Screening of anti-microbial activity for the selected plant D.hamiltonii

Agar well diffusion method was used to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 hours old broth culture of respective bacteria. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.5 ml of 75mg/ml concentrations of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37°C for18-24 hours for bacterial pathogens. Respective solvent control for root extracts was also maintained and the diameter of the zone of inhibition was recorded in mm and compared with standard values. It was

maintained in triplicates and the experiment was repeated thrice, and the average values were recorded for antimicrobial activity (M. Chandrasekhara Reddy and K. Sri Rama Murthy, 2013).

Table 6 : Antimicrobial activity of various solvent extracts of *Decalepishamiltonii* (in mm)

Microorganisms	EthylAcetate	Methanol	Ethanol	Amyl alcohol	Chloroform	Water
Escherichia.coli	8	15	14	16	10	-
Salmonella.typhi	8	16	10	11	8	-
Streptococcus.aureus	10	20	15	18	10	-
Streptococcus.mutans	12	14	12	18	12	-
Salmonella.enterica	10	8	8	12	10	-
Bacillus.megaterium	15	12	16	16	8	-
Bacillus.subtilis	8	15	12	18	8	-
Lactobacillus.casei	12	8	10	12	12	-
Xanthomonas.campestris	10	8	8	10	10	-

Anthelimintic activity

The root extract of Decalepis was evaluated for anthelimintic activity in Pheretima posthuma (earth worm) of nearly equal size (6±1 cm). Pheretima posthuma is used due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human begins. Because of easy availability of earthworms, they have been used widely for the initial evaluation of the anthelmintic compounds. The anthelmintic activity was compared with Albendazole as the standard reference and with normal saline as the control. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into five groups of six earth worms in each and placed in eight Petri dishes containing the extract solutions are the reference drugs as mentioned below-

Group -1: Received Albendazole 50 mg/ml in 50ml solution as the standard

Group-2: Received Methanolic extract at a dose of 50mg/ml in 50ml solution

Group-3: Received Methanolic extract at a dose of 100mg/ml in 50ml solution

Group -4: Received Methanolic extract at a dose of 200mg/ml in 50ml solution

Group-5: Received Normal saline 100 mg/ml in 50ml solution as the control.

All Petri dishes were kept under room temperature. The living or viable worms were kept under close observation. Observations were made for time taken to complete paralysis (PT) and death (DT) for individual worms. Each worm was frequently applied with external stimuli which stimulates and induce

movement in earthworms, if alive. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading of the body color. The motionless worms were then transferred at 40º C to confirm that they were dead.

Table: 7 Anthelmintic activity of methanolic root extract of *Decalepishamiltonii*

Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Albendazole suspension (Standard)	50	34.8	54.4
Test (Methanolic root extract)	50	58.4	72.3
	100	37.5	58.5
	200	26.1	46.2
Normal Saline	100	—	—

RESULTS

The anthelmintic activity of methanolic root extract of *Decalepis* was carried out on earth warm. Different concentrations of the methanolic extracts were used for the studies. The time taken for paralysis and death of earthworms were recorded. The perusal of the data reveals that the methanolic extract at the concentration of 100mg, 200 mg/ml showed paralysis and death time in 37.5, 58.5 & 26.1, 46.2 minutes respectively. The effect increased with concentration. The extract caused paralysis followed by death of the worms at all tested dose levels. It was observed that the methanolic extract of *Decalepis* is more potent drug. The extract showed paralysis followed by death of the worms at all tested dose levels. The potency of the extract was found inversely proportional to the time taken for paralysis of death of worms, the antimicrobial activity of plant extracts was assayed *in vitro* by using agar well diffusion method. The results of antimicrobial activity of ethyl acetate, methanol, ethanol and amyl alcohol and chloroform root extracts of *D.hamiltonii* against various pathogens were studied. It was found that methanol and amyl alcohol root extract exhibited significant activity compared to the other extracts. Amyl alcohol showed the maximum activity against all the bacterial species

Conclusion:

The qualitative analysis of methanolic extract of *D.hamiltonii root* shows the presence of steroids, alakaloids, flavanoids and phenolic compounds. The above finding justify the anthlimintic and anti microbial properties of this plant further study regarding the isolation and characterization of the active principle responsible for anthelimintic and antimicrobial activity is currently under progress.

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