

In Vitro Evaluation of the Anthelmintic Activity on the Aqueous and Alcoholic Bark Extract of Swietenia Macrophylla.

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ABSTRACT: Swietenia macrophylla, a plant from the Meliaceae family, has long been utilized in traditional medicine for its antimicrobial, anti-inflammatory, antioxidant, and other beneficial properties. This study aimed to investigate the anthelmintic activity of its bark extract in alcoholic and aqueous solvents using Eisenia andrei. The bark was collected, dried, crushed, and subjected to Soxhlet extraction using water and alcohol as solvents. Different concentrations (10, 20, and 40 mg/ml) of both aqueous and alcoholic extracts were tested for their vermifugal effects, with albendazole used as a reference standard at 20 mg/ml. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in both extracts, which are potentially responsible for the observed anthelmintic activity. The paralysis and death times of Eisenia andrei were recorded for each extract and the reference standard. Statistical analysis showed significant differences between the extracts and the reference, with both alcoholic and aqueous extracts demonstrating dose-dependent vermifugal activity. The higher concentration (40 mg/ml) of both extracts exhibited stronger anthelmintic effects. The study identified tannins and triterpenoids in the bark of Swietenia macrophylla as key active components responsible for its potent anthelmintic properties. In conclusion, the 40 mg/ml concentration of both the alcoholic and aqueous extracts exhibited the highest anthelmintic activity. Among these extract the alcoholic extract at 40mg/ml show more potent anthelmintic activity

KEYWORDS: Swietenia macrophylla, anthelmintics, Eisenia andrei

I. INTRODUCTION

Helminthiasis, also known as worm infection or intestinal helminthiasis, is a parasitic disease caused by parasitic worm called helminths that affect the various body part of animal and

humans. Soil-transmitted helminthiasis are the most common transmitted disease in the majority of the human population worldwide. One well-known example of soil transmitted helminthiasis is ascariasis. the infection that spread via soil is mostly responsible for the helminthiasis. The worms are transmitted to the human beings through the eggs present in the human faeces that contaminated in the soil. The mostly infections are caused by round worm, whipworms and hookworms [1]. The various symptoms of helminthiasis infections are abdominal pain, diarrhoea, weakness. Chronic intestinal blood loss is caused by hook worms that can lead to chronic fatigue and anaemia. Matured eggs of Hookworms present in the soil that has an ability to penetrate the skin of humans. Barefoot walking in contaminated soil contain infectious parasite is the primary cause of hookworm infestation. Ingesting eggs or larvae causes whipworm [2]. According to the WHO there are almost nearly 880 million of people requires the treatment of helminthiasis and this disease is mostly spread in the African region due to poor sanitation [3]. Anthelmintics are the group of drugs that used to treat helminthiasis by killing or expel the worm from the intestine through faeces. The most commonly used anthelmintic drugs are albendazole, mebendazole, pyrantel pamoate, praziquantel, diethylcarbamazine citrate [4]

Plant profile of Swietenia macrophylla

Swietenia macrophylla (Meliaceae) is an endangered and medicinally important plant that used in the tropical and subtropical regions of the World [5]. Swietenia macrophylla has been widely used as a folk medicine by various groups of people to treat various infectious diseases. The review reveals that limonoids and its derivatives are the major constituents of Swietenia macrophylla. There are several review data of

Swietenia macrophylla that indicating a great variety of pharmacological activities of *Swietenia macrophylla*, which exhibits antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, anticancer, antitumor and antidiabetic activities. Various other activities like anti-nociceptive, hypolipidemic, antidiarrheal, anti-infective, antiviral, antimalarial, acaricidal, antifeedant and heavy metal phytoremediation activity have also been reported [6].



Whole tree of *Swietenia macrophylla*

Taxonomical classification of *Swietenia macrophylla* [7]

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	<i>Swietenia</i>
Common name	Mahogany

Common Names: The species *Swietenia macrophylla* is also known under various other common names, including bastard mahogany, broad-leaved mahogany, Brazilian mahogany, large-leaved mahogany, genuine mahogany, tropical American mahogany, and sky fruit, Honduran mahogany, Honduras mahogany, or big-leaf mahogany [8].

II. MATERIAL AND METHODS

Extraction Procedure

The bark was taken and cleaned using distilled water and dried the bark using Hot Air oven under a moderate temperature between 40°C to 60°C. After drying crush the bark into fine

powder in a mixer grinder without inducing temperature and passed the crushed material through the mesh (mesh size 20& 44) and store the sieved particles in a sterile air tight container. The powdered bark powder was subjected to Soxhlet extraction using water and alcohol as solvent. 10g of the powdered bark was taken in a thimble and place inside the Soxhlet apparatus and 200ml of solvent taken the round bottom flask and heat the round bottom flask using heating Mantle at a temperature suite for the solvent. Reflux it for 27 hours the mixture obtained is evaporated to obtain a crude extract. The Obtained semi-solid extract was preserved in a sterile glass container at a refrigerator until further use of the extract. Solvent was recovered under evaporation to obtain crude extract [9-10].



Soxhlet extraction of bark

Phytochemical screening test of the Aqueous and alcoholic bark extract of *Swietenia macrophylla*

The water and alcoholic bark extract of *Swietenia macrophylla* was tested to identify the presence of various phytochemical by various chemical tests such as

Test for phenols

Iodine test

To the 1mL bark extract add few drops of dil. Iodine solution. A transient red colour is obtained

Test for Tannins

Gelatin test

Plant extract is dissolved in 5mL distilled water and add 1%gelatin solution and 10% NaCl. A white precipitate is obtained

Braymer's test

To the 1mL filtrate add 3mL distilled water and 3 drops 10% Ferric chloride solution a Blue-green colour is obtained

Test for Saponins

Foam test

To the 0.5gm plant extract add 2mL water and vigorously shaken it A Persistent foam for 10 min.

Test for Terpenoids

Add 2ml chloroform to 5mL plant extract and evaporated on water bath then add 3mL conc. H_2SO_4 while boiled on water bath. A grey coloured solution is obtained

Test for Triterpenoids

Salkowski's test

To the Filtrate add few drops of conc. H_2SO_4 Shaken well and allowed to stand for some time A Golden yellow layer is obtained at the bottom

Test for alkaloids

Dragendorff's test

Few mL filtrates are added with 1-2 mL Dragendorff's reagents A reddish-brown precipitate

Iodine Test

3mL extract solution add few drops of iodine solution into the bark extract A blue colour, which disappears on boiling and reappears on cooling

Detection of Flavonoids

Ferric chloride test

To the extract aqueous solution add few drops 10% ferric chloride solution A green precipitate is obtained

Lead acetate test

1mL plant extract add few drops of 10% lead acetate solution A yellow precipitate [11].

ANTHELMINTIC ASSAY

The anthelmintic assay was carried out as per the method reported by Ajaiyeoba et al with minor modification. The assay was performed on adult earthworm species *Eisenia Andrei* due to its anatomical and physiological resemblance with the intestinal roundworm parasite on human being. Extract with different concentration (10mg/ml, 20mg/ml, 40mg/ml). The time taken for complete

paralysis time and legal time was ascertained by death were recorded. The time taken by worm to become motionless is considered as the paralysis time and legal time was ascertained by death of motionless worm followed by changing the colour of the wormbody.

Administration of the extract

The suspension of different solvent extract (water & alcohol) of *Swietenia macrophylla* of different concentration (10mg/ml, 20mg/ml, 40mg/ml) were prepared and final volume is made up to 10ml. Three water extract and three alcoholic extract each contain three earthworm individuals.[12]

	10mg/ml	20mg/ml	40mg/ml
Aqueous extract			
Alcoholic extract			

Administration of Reference standard

Albendazole of 20mg/ml was prepared from 200mg powder in 10 ml of distilled water with 0.1 g of acacia as suspending agent

EXPERIMENTAL DESIGN

The earthworms were collected and initially cleaned by using normal saline (NS 0.9%w/v), Eight Petri dish were cleaned by using the distilled water and sterilized by using the hot water and one petri dish was used as control which contain 0.1g of acacia in water

The other six petri plates contain alcoholic and water extract in each three petri dishes at different concentration of 10mg/ml, 20mg/ml, 40mg/ml

The first three Petri dish contain water extract was named as W1, W2, W3 and the other three Petri dish was named as A1, A2, A3

The earthworm was taken and wash the worm using normal saline water and place three earth worms in each petri plates containing the extract solution

And the rest one petri dish was place with the standard of albendazole with three earthworms

From this study we conclude that the plant extract shows anthelmintic activity. Higher concentration shows better anthelmintic activity in

both alcoholic and water extract. The plant extract shows better anthelmintic activity compared to

reference standard of albendazole [13-14].

EXPERIMENTAL DATA

Concentration	Paralysis Time of Earthworm (In mins)	Death Time of Earthworm (In mins)	Total Time Taken of Earthworm (In mins)
ALCOHOLIC EXTRACT			
10mg/ml	77 81 87	79 86 92	156 167 179
20mg/ml	41 52 56	42 57 58	83 109 114
40mg/ml	31 33 34	33 35 36	64 68 70
WATER EXTRACT			
10mg/ml	181 182 185	183 184 188	364 366 373
20mg/ml	65 66 67	73 74 75	138 140 142
40mg/ml	54 62 63	63 69 72	117 131 135
STANDARD			
20mg/ml	10 11 10	13 15 13	23 26 23
CONTROL (0.1g acacia with 10ml water)	No response	No response	No response

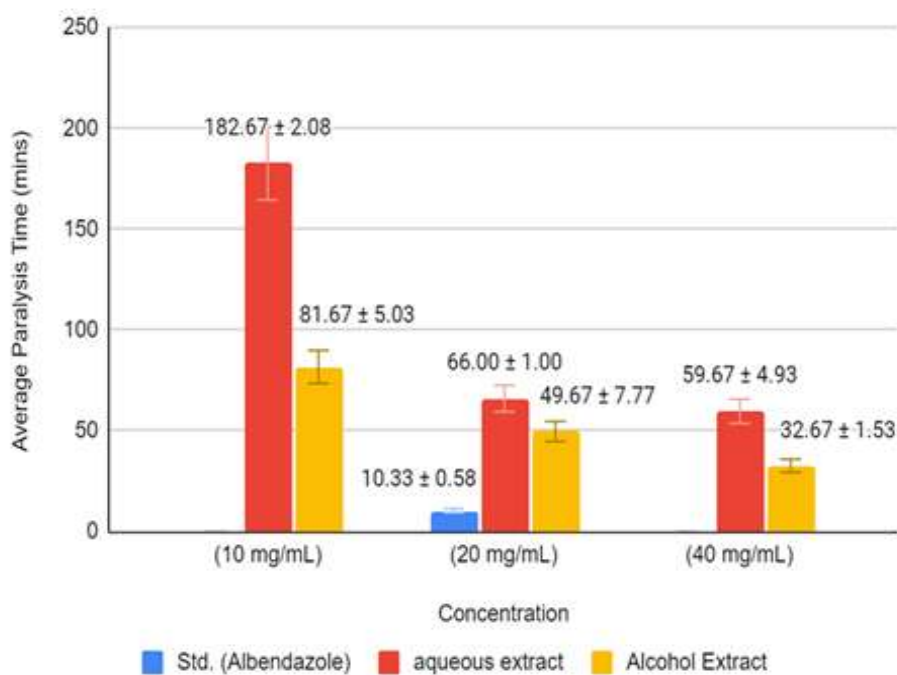
STATSITICAL ANALYSIS

Statistical data

Comparison of time for paralysis using one way ANOVA

	10mg/ml		20mg/ml		40mg/ml	
DRUG EXTRACT	MEAN	SD	MEAN	SD	MEAN	SD
Aqueous extract of SM	182.67	2.08	66.00	1.00	59.67	4.93
Alcoholic extract of SM	81.67	5.03	49.67	7.77	32.67	1.53
Standard drug (albendazole)	0	0	10.33	0.58	0	0

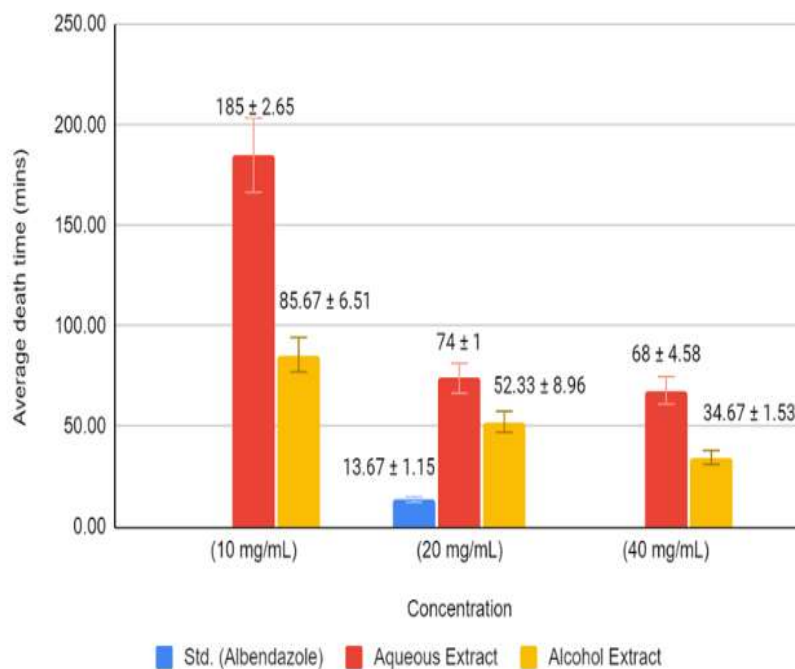
The graphical representation of the paralysis time



Comparison of time for death using one way ANOVA

	10mg/ml		20mg/ml		40mg/ml	
DRUG /EXTRACT	MEAN	SD	MEAN	SD	MEAN	SD
Aqueous extract of SM	185	2.65	74.00	1.00	68	4.58
Alcoholic extract of SM	85.67	6.51	52.33	8.96	34.67	1.53
Standard drug (albendazole)	0	0	13.67	1.15	0	0

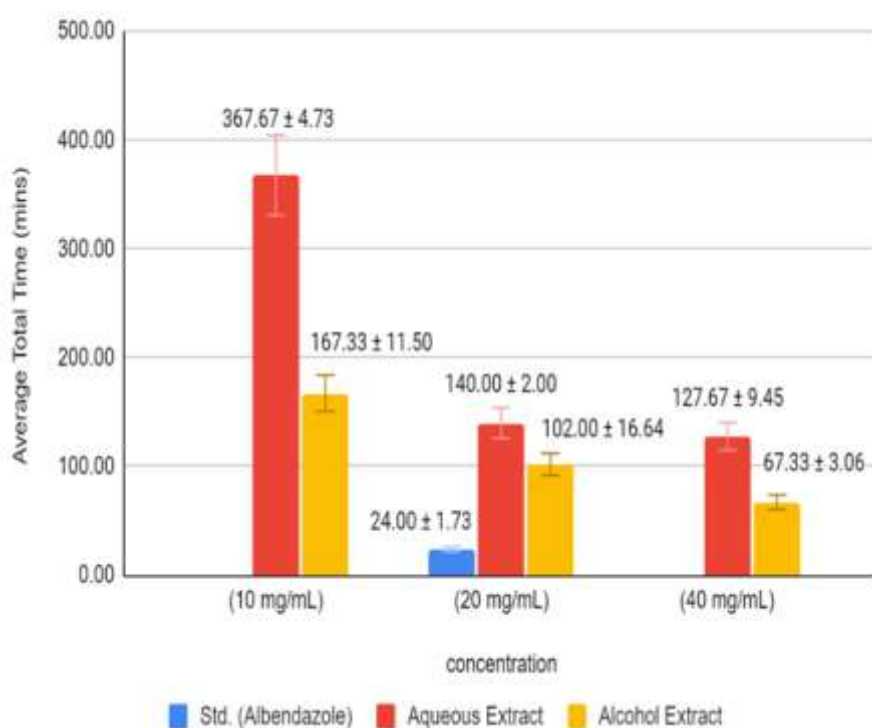
The graphical representation of the death time



Comparison of total time (paralysis + death) using one way ANOVA

	10mg/ml		20mg/ml		40mg/ml	
DRUG /EXTRACT	MEAN	SD	MEAN	SD	MEAN	SD
Aqueous extract	367.67	4.73	140.00	2.00	127.67	9.45
Alcoholic extract	167.33	11.50	102	16.64	67.33	3.06
Standard drug (albendazole)	0	0	24.00	1.73	0	0

The graphical representation of the total time



III. RESULT AND DISSCUSSION

Phytochemical screening analysis report:

PHYTOCHEMICALS	AQUEOUS BARK EXTRACT	ALCOHOL BARK EXTRACT
Phenol	+ve	+ve
Tannins	-ve	+ve
Saponins	+ve	-ve
Terpenoids	+ve	-ve
Alkaloids	+ve	+ve
Flavonoids	- ve	- ve

ANOVA was used to analyse the significant difference between standard drug vs alcoholic extract of SM vs aqueous extract of SM. When comparing the paralysis time between the three group of 10mg/ml of aqueous and alcohol extract of SM vs 20mg/ml of standard ,the time taken by the standard is significantly low ($P < 0.01^*$) ,while comparing the 20mg/ml of all the three groups the time taken by the standard is significantly low ($P < 0.01^*$)and comparing the 40mg/ml of aqueous and alcohol extract of SM

with 20mg/ml of standard the time taken by the standard is significantly low ($P < 0.01^*$)

When analysing the significant difference of death time between the three group of 10mg/ml of aqueous and alcohol extract of SM vs 20mg/ml of standard ,the time taken by the standard is significantly low ($P < 0.01^*$) ,while comparing the 20mg/ml of all the three groups the time taken by the standard is significantly low ($P < 0.01^*$)and comparing the 40mg/ml of aqueous and alcohol extract with 20mg/ml of standard the time taken by the standard is significantly low ($P < 0.01^*$)

Also analysing the significant difference of total time between the three group of 10mg/ml of aqueous and alcohol extract of SM vs 20mg/ml of standard, the time taken by the standard is significantly low ($P < 0.01^*$), while comparing the 20mg/ml of all the three groups the time taken by the standard is significantly low ($P < 0.01^*$) and comparing the 40mg/ml of aqueous and alcohol extract of SM with 20mg/ml of standard the time taken by the standard is significantly low ($P < 0.01^*$).

From the statistical analysis by comparing the all the three groups at different concentration, it is evident that the higher concentration (40mg/ml) of alcoholic extract and aqueous extract shows significant difference and among this alcoholic extract of SM shows more anthelmintic activity.

IV. SUMMARY AND CONCLUSION

The main purpose of the study was the bark extract of *Swietenia macrophylla* shows anthelmintic activity and compare the time of death and paralysis with that of the reference standard (albendazole). The aqueous and alcoholic bark extract of *Swietenia macrophylla* at various concentration such as 10mg/ml, 20mg/ml, 40mg/ml, was prepared and extract was poured into Petri dishes containing *Eisenia andrei* and time shows paralysis or death of *Eisenia andrei*. The higher concentration shows less time taken for the paralysis or death of *Eisenia andrei*. By this study we came to conclusion that 40mg/ml concentration of alcoholic and aqueous extract of *Swietenia macrophylla* possess anthelmintic activity compare with the standard drug of albendazole. This study is helpful for the future research on *Swietenia macrophylla*.

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