Evaluation of Anthelmintic and Neuropharmacological Activities of the leaves of*Chassalia*

Abstract

Chassaliacurviflora belongs to the family Rubiaceae has been documented for its anticancer, anthelmintic&neuropharmacological effects. Literature review of the plant revealed that some research works are performed during this plant. That's why; this study was performed to gauge the anticancer, anthelmintic and neuropharmacological activities of the methanolic extract from the leaves of *Chassaliacurviflora*(MECC). Anthelmintic activity was investigated using earthworms (*Pheretimaposthuma*) and fresh leaf juice of the plant. Anticancer test was done using cell viability assay. During anticancer test in the concentration of 1000µg/mL the percent of inhibition was 29.16%. In anthelmintic activity, the time of paralysis for fresh leaf juice was begin from 8 min 19 s and end to 38 min 21 s while the time for tested drug Albendazole was started from 9 min 19 s and end to 46 min 52 s. Besides, the time of death start from 10 min 35 s and end to 63 min 19 s for leaf juice and for Albendazole the time of death start from 22 min 36 s and end to 75 min 3 s respectively at several doses which suggest considerable anthelmintic activity of the plant. Neuropharmacological activity was performed by hole cross and light/dark box tests at the doses of 200 mg/kg and 400 mg/kg in *Swiss albino* mice contrast to the test drug Diazepam (2mg/kg). The extract significantly decreases the locomotor activity as shown by the results of hole cross test which indicate significant antidepressant property. The results also matching the activity in light/dark box test. Thus, the obtained results in the research work provide a support for the utilization of this plant for medicinal purposes and encourage further investigations for more fruitful results.

Keywords:*Chassaliacurviflora*, Anticancer, Neuropharmacological, Antidepressant, Anthelmintic activity.

1. INTRODUCTION

From the start of civilization, Medicinal plants are playing a crucial role for the wellbeing of human. *Chassaliacurviflora* is one among the foremost important medicinal plants which are yet to be explored pharmacologically. *C. curviflora* belongs to the Rubiaceae, could also be a little shrub or tree with white or pinkish flower and glossy black fruits. It's native to India, South China and Phillippine. It's widely growing in western guts of India. The plant is ordinarily conversant in crank flower woody Vellakurinji and Chassalia, Yamari or Mundanchedi in Malayalam[1]. The plant can reach up to 1-2 m; branches weakly flattened to subterete, glabrous or rarely sparsely puberulent. Leaves opposite; petiole 1-4 cm, glabrous; blade drying membranous to thinly papery and sometimes yellowish green, oblong-elliptic, elliptic, oblanceolate, or narrowly lanceolate. *C. curviflora* contain important phytochemical constituents like carbohydrates, saponins, alkaloids, steroids, terpenoids, tannins, phenolic compounds, flavonoids, and amino

acids. Chemical constituents from this plant utilized in various traditional systems within the universe[2].*C. curviflora,* an important ethno-medicinal plant utilized by the Kurichia people in Wayanad's Western Ghats area, has yet to be pharmacologically investigated. It's used as a paste to the bodies of cattle and birds to treat skin ailments[3].The whole plant is put to use for medicinal purposes. The leaves boiled with water are used for ear and eye diseases, ulcer and pharyngitis. Whole plant is employed for skin diseases. Essence of root is given as a remedy in phlegm, rheumatism, and pneumonia. Root is an antidote against the sting or bite of serpents. It's used for treatment of malaria, coughs, wounds, and ulcers. Chakma tribes of Bangladesh make use of burst crushed leaves to the injuries for treating snake and bug bites. It's also proven for antimicrobial, antihypertensive, antioxidant and acaricidal activity[4].Flowers sub sessile, trim orphic: with anthers excreted and stigmas included, with anthers covered and stigmas exerted, or with anthers and stigmas both excreted. Calyx with hypanthia part ellipsoid to bovid, 1-1.5 mm, glabrous; limb 5-lobed, 0.5-1 mm; lobes 0.3-0.5 mm, acute. Corolla white with pink, red, or orange on lobes, outside glabrous to sparsely puberulent and longitudinally ridged to pinnated towards tube then midribs of lobes; tube shallowly to markedly cranked, straight or bent at base, 10-15 mm, pubescent inside; lobes (4 or)5, ovate-triangular, 2-2.5 mm, at vertex thickened. Infructescence axes becoming swollen and red. The fruit is purple color, oblate to globose or weakly didymous, 5-7 × 6-9 mm[5].The genus Chassalia is examined in Asia. There are 23 species recognized in total, including one novel combination and a validation of a previously submitted combination. Some 17 names in Chassalia that are pertinent to the Asia-Pacific area are classified as Psychotria, Eumachia, or Geophila instead of Chassalia. The region's Cephaelis names are also examined. In Psychotria, one new combination and two new names are proposed. All names are given synonyms and types, including 29 lectotypifications[6].

Hence there's a hopeful future for this medicinal plant as they're extensively distributed round the world, and most of the medical activities aren't yet investigated and their hidden potential of medical activities might be inevitable within the treatment of present and future studies[2].

2. MATERILAS AND METHODS

2.1 Plants materials

The plant leaves were collected from Moulvibazar, Syhlet, Bangladesh. Later the plant was identified by respective person of Bangladesh National Herbarium Institute, Mirpur, Dhaka, where a voucher specimen (DACB: 48247) has been deposited within the herbarium for future reference.

2.2 Methods of extraction

*Chassaliacurviflora*leaves were first separated from undesirable materials. They were dried for one and half week during a shaded place. The stem and other adulterants were removed first, then the leaves were washed with water to urge the fresh sample.

Then the collected samples were dried under shade for temperature for ten days.After drying, the leaves' part was grinded by Blender Machine (NOWAKE, JAPAN). Coarse powder was obtained after grinding. Powdered samples were placed in clean stagnant glass container. During Grinding of sample, the grinder was thoroughly cleaned to avoid contamination with the other substances that was grounded previously. The dries grinded powder was weighted by rough balance[7].

2.3 Extraction of plant materials

The dried leaves place separately by commercial grinder (Hammer mill) into a fine powder. The shade dried and powdered material (350 g) was separately extracted to exhaustion with Methanol (1050 ml) for 10 days. The extractive was filtered by clean cotton bed and eventually with Whatmann no-1 filters paper. The quantity of the filtrate was concentrated with a rotary evaporator at coldness (40-50° C) and reduced pressure[8].

2.4 Anticancer test

2.4.1 Sample preparation and treatment

The concentrate of *Chassaliacurviflora*, weighing exactly 100 mg, was dissolved in 1 ml of dimethyl sulfoxide (DMSO) and stored at -20 °C until ready for use, just like a pharmacologist would do. Cells were cultured until they reached approximately 70% confluence in a 24-well plate. They were then exposed to various concentrations of extract, including 0, 100, 300, and 500 µg/ml. The DMSO concentration in the treatment was carefully controlled to be below 0.25%. Every experiment was conducted multiple times to collect the necessary data[9].

2.4.2 Cell viability assay

Cells were cultured in their respective media in 96-well plates until they reached approximately 70% confluence. Subsequently, the cells were exposed to various concentrations of extract, in addition to a control containing the vehicle/DMSO, for approximately 24 hours. Afterward, the media was removed to cleanse the cells using phosphate buffer saline (PBS). A solution of MTT at a concentration of 0.5 mg/ml was added to each well of the plate. The plate was then incubated at 37 °C for 4 hours in the dark. After incubation, the MTT solution was replaced with 200 µl of DMSO. The plate was agitated at 150 rpm for 5 minutes and the optical density was measured at 490 nm using a plate reader (ELx 800; Biotek, Winooski, VT, USA). The experiment was conducted multiple times to ensure accurate data for graph plotting[9].

2.4.3 Morphology study

Cells were plated in 24-well plates and subjected to treatment with either DMSO or extract at the IC_{50} concentration for 24 hours. Following the treatment, the image was captured using phase contrast microscopy.

2.5Experimental Animals

2.5.1 For anthelminthic experiment

Adult earthworms (*Pheretimaposthuma*) were culled from wet soil of a private area of Bangladesh. Earthworms were washed with normal saline to get rid of all faceal matter. The earthworms of 3-8 cm long and 0.1-0.2 cm in breadth were used for all experimental protocol[10].

2.5.2 For nuropharmacological experiment

Young *Swiss-albino* mice having average weight 20-25 g was used for the experiment. The mice were collected from ICDDRB at Mohakhali in Dhaka, Bangladesh. They were kept in standard condition consistent with standard environment; the conditions are at 24±1°c temperature, 55-65% relative humidity and 12 hours light and 12 hours dark cycle. The above condition is maintained for one week after collection of mice and feed suitable mice food and pure water that are formulated by Jahangirnagar University to urge over food and water restriction incurred during transit and to urge them adapted with the new environment of the laboratory, before being employed in any experiment. After one week of resting, the mice were suitable for conducting the experiments.

2.6 Anthelmintic Test

2.6.1 Juice Preparation

Fresh leaves (75g) of *C. curviflora*were blended into liquefactions in 250 ml of water. The blending was then centrifuged at 100 rpm. The supernatant was filtered through sterile paper in to conical flask. Thus, the concentration of the fresh leaves juice was 500mg/ml.

2.6.2 Anthelmintic Assay on Earthworms

Anthelmintics or antihelminthics are drugs that eliminate parasitic worms (helminthes) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They'll even be mentioned vermifuges (those that stun) or vermicides (those that kill). They're wont to treat people or animals that are infected by helminthes - a condition called helmintheasis. Parasites are of concern to the medical field for hundreds of years and therefore the helminthes still cause considerable problems for citizenry and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and therefore the treatment of those parasites, there are still no efficient products to regulate certain helminthes and therefore the indiscriminate use of some drugs has generated several cases of resistance[11]. Fresh leaves juice of *C. curviflora*was dissolved in minimum amount of DMF and therefore the volume was adjusted to 10ml with saline water. Drug and fresh juice at different concentrations were instantly prepared before starting the experiment6 earthworms were introduced into 10 mL of required formulations in each example as follows: Albendazole with fresh juice at a concentration of 5mg/ml, vehicle

(5 percent DMF in normal saline), Albendazole and fresh juice at a degree of 5mg/ml, 10mg/ml, 20mg/ml, 50mg/ml, and 100mg/ml respectively were added on them. Observations were captured for the time of paralysis and death of individual worm. Paralysis was said to occur when the worms weren't ready to move even in saline.

2.7 Nuropharmacological Activity

2.7.1Hole-Cross Test

The maximum compatible behavioral change may be a hyperemotional response to novel environmental stimuli. The purpose of this study was to distinguish the emotional behavior of mice using the hole-board test. the number of head-dips within the hole-board test in single-housed mice was significantly greater. Spontaneous movement of the animals through the opening from one chamber to the opposite was counted for five min during this test. The observations are made on 0, 30, 60, 90 and 120 min after administration of the test samples. There have been no effects of the test animals at 0 min. After 30 min observed that the mice began to sleep and thus little or no movement was observed. Even after 90 min of administration of the extract they were still sleeping[12].

2.7.2Light/Dark Box Test

The light/dark box experiment is considered on the congenital phobia of rodents to brightly illuminated areas and on the unprompted exploratory behavior of rodents in response to light stressors, that is, novel environment and light weight. The light/dark test could also be effective to portend anxiolytic-like or anxiogenic-like activity in mice. Transitions are reported to be an index of activity-exploration due to habituation over time, and therefore the time spent in each compartment to be a mirrored image of aversion. An open-topped rectangular box (46×27×30 cm high) was divided into a little (18×27 cm) area and an outsized (27×27 cm) area with a gap door (7.5×7.5 cm) located within the centre of the partition at floor level. The little compartment was painted black and lit by a weak red light (60 W; 4 lx), whereas the large compartment was painted white and strongly lit by a 60-W (400 lx) light. The compartments were equipped with infrared beam sensors (four within the white area, three within the black one). Each mouse was tested by placing it within the center of the white area, facing far away from the dark one and was allowed to explore the novel environment for five min and thereby enabling the detection of locomotion in each zone, time spent in each zone, latency of the primary crossing from one compartment to the opposite, and shuttle crossings between both compartments. the info for these four parameters were directly collected by observer. This test utilized the conflict between the animal’s tendency to pursue a replacement environment and its fear of bright light[13].

3. Statical Analysis

The experimental data was replicated three times,and the mean and standard deviation wereutilized to represent the results. Excel is alsoused for statistical studies.

4. RESULTS and DISCUSSIONS

4.1 Anticancer activity

The alcoholic extract of plant materials was standardized following established protocols, and its potential to inhibit cancer was evaluated using HeLa cell lines. The methanolic extract from the *C. curviflora* plant showed promising results (Table 2).

Table 1. Anticancer activity of MECC

Concentration (µm/mL)	Survival of the cell (%)	% of Inhibition
125	84.69	15.31
250	75.84	24.16
500	69.63	19.37
1000	60.84	29.16

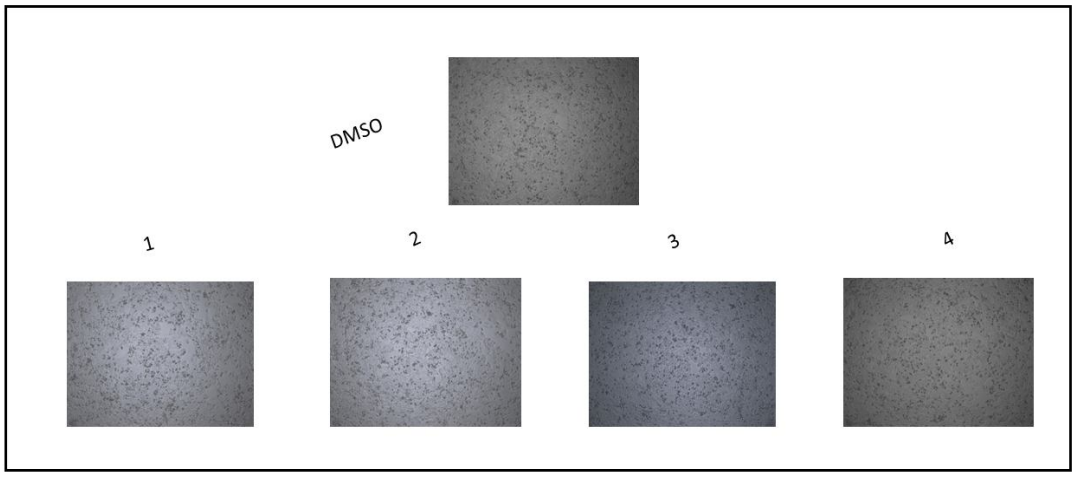

Figure 1: The Phase contrast image shows specific and significant morphological change. The serial1,2,3,4 refers the concentration of MEBD from 125 to 1000 µm/mL as shown in table 2.

4.2 Anthelmintic Activity

During previous research, phytochemical screening of methanol extract of *C. curviflora*leaves showed the presence of tannins, alkaloids, glycosides, carbohydrates and steroids.

Table-2: In vitro anthelmintic activity of *Chassaliacurviflora*

Test Samples	Conc. (mg/ml)	Time Taken for	Time Taken for Death

		Paralysis	
Fresh juice	5	38 min 21 s	1h 03 min 19 s
	10	34 min 40 s	40 min 25 s
	20	31 min 17 s	36 min 19 s
	50	11 min 25 s	22 min 31 s
	100	08 min 19 s	10 min 35 s
Albendazole	5	46 min 52 s	1h 15min 03 s
	10	32min 13 s	1h 11 min 24 s
	20	23 min 28 s	54 min 09 s
	50	21 min 37 s	22 min 36 s
	100	09 min 19 s	37 min 23 s

4.2 Neuropharmacological Activity

4.2.1 Hole-cross Model

The extract considerably reduced the locomotor activity as shown by the results of the hole-cross test. The locomotor activity lowering effect was evident for the both doses of 200mg / kg and 400mg / kg weight at the 2nd observation (30 min) and continued up to 3rd and 4th observation (60 and 90 min) period (Table-3). Moreover, the validation of hysteria was administered by measuring external signs, through hole-cross test.

Table-3: Effect of*Chassaliacurviflora*on Hole-Cross Test

Group	Route of Administration	Observation				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	Oral	14.2±0.078	9±0.032	10.6±0.81	10.8±0.21	5±0.67
Positive Control	Oral	8.2±0.37	11±0.247	6.2±0.036	4.6±0.048	3±0
Group I	Oral	7.8±0.17	6±0.447	3.4±0.089	2.8±0.045	1.6±0.07

Group II	Oral	1.4±1.3	1.8±0.047	2±0.031	2.2±0.017	1.4±0.069

Control: water, Positive control: Diazepam (2mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg). Values are expressed as Mean ±SEM (n=5).

4.3.3 Light/Dark Box Test Results

The mice of Group I (200mg/kg) spent maximum time in dark area and therefore the mice of Group II (400mg/kg) also spent maximum time in dark area. Number of entries in dark area is smaller than light area of both Group I and Group II. The extract significantly decreased the CNS activity as shown by the results of the light/dark test (Table- 4). The CNS activity lowering effect was evident for the both doses of 200mg / kg and 400mg / kg weight at the 2nd observation (5 min after 30) and continued up to 3rd and 4th observation (5 min after 30) period (Table-4). Moreover, the validation of hysteria was administered by measuring external signs, through light/dark test.

Table-4: **The light/dark box test results of leaf extract of *Chassaliacurviflora***

Group	**Route of Administration**	**No. of entry of Light area**	**Time in Light area (seconds)**	**No. of entry in Dark area**	**Time in Light area (seconds)**
Control	Oral	2.8	52	3.8	248.2
Positive Control	I.P.	5.8	68.2	6.4	231.8
Group I	Oral	1.8	34	2.8	266
Group II	Oral	1.6	22	2.6	278

Control: water, Positive control: Diazepam (2mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg). Values are expressed as Mean ±SEM (n=5).

5.DISCUSSION

Chassaliacurviflora is one of the flowering plant species belonging to the family Rubiaceae, which has made researchers extract potential anticancer properties from it. The phytochemicals, which are biologically active compounds in plants, occur in plenty in this plant whose origin is in the tropical and subtropical regions of Africa and Asia[2].

Phytochemical screening has revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and saponins in *Chassaliacurviflora*[14]. Of these phytochemicals, alkaloids and flavonoids were found to be important for their anti-cancer properties [15]. According to a study, these compounds tend to perturb the growth and proliferation of cancer cells, through mechanisms including, but not limited to, the induction of apoptosis, inhibition of angiogenesis, and interference with DNA replication [16].

In another research it was found that the methanolic extract of *Chassaliacurviflora* showed some cytotoxic effects against human colorectal carcinoma (HCT116) cells. The possible mechanism for the anticancer activity was thought to be due to its high phenolic content, which may exert an oxidative stress in cancer cells and cause cell death[17].

The fresh juice of *C. curviflora*leaf contains of these chemical compounds. It's evident from experimental data that the plant which contains flavanoids, alkaloids, tannins etc showed significant anthelmintic property. That's why, the fresh juice of the leaf *C. curviflora*showed significant anthelmintic activity (Table-1). Results were comparable with standard drug, Albendazole[18], [19].

The neuropharmacological effect of MECC was determined using hole cross method and light/dark box test. The effectiveness of traditional remedies may depend on a combination of different components. Several studies have identified certain compounds that exhibit both anxiety-inducing and calming effects. The sedative effects of tannin may be attributed to its non-specific CNS depression [20], [21]. The effects of flavonoid, steroid, and protein kinase C (PKC) activation on the body are a result of genetic expression of transcriptional factors. They were also noted for their ability to shield neurons from various metabolic and oxidant challenges [22]. Through extensive studies, the photochemical measurements of the *C. curviflora* extracts unveiled a wide range of compounds including alkaloids, flavonoids, saponins, tannins, steroids, gums, and cardiac glycosides. Multiple studies have demonstrated the powerful anti-anxiety and anti-epileptic properties of alkaloids, glycosides, and flavonoids [23][24].Multiple laboratory research studies have demonstrated that plant extracts containing these secondary metabolites possess anxiolytic and sedative effects by interacting with the GABAergic complex system [24]. The results indicated that extracts of *C. curviflora* have a notable impact on the locomotive activity of mice. The analysis also supports the typical treatment of epilepsy and anxiety using the plant [25]. Thus, the leaf extracts exhibit neuropharmacological activity.

6.CONCLUTION

The fresh leaves juice and methanol extract of the leaves of *C. curviflora*possesses significant anthelmintic, and neuropharmacological activity. The results of phytochemical screening stated that, methanolic extract of *C. curviflora* contain tannins, alkaloids,

glycosides, carbohydrates and steroids. From the literature review of the plant, it had been observed that the agent which has anthelmintic effect may show the CNS effect. The results of open field hole-cross and light/ dark box tests justified the statement. It's also identified that higher dose of the plant extract is simpler than the lower dose.

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