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Biological evaluation of ethanolic extract of *Aphanamixis polystachya* (Wall.) Parker leaf

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Keywords

Aphanamixis polystachya, antibacterial, cytotoxic, antidiarrheal

Abstract

The prime objective of present study is to evaluate *in vitro* antibacterial activity, cytotoxic activity and *in vivo* antidiarrheal activity of ethanolic extract of *Aphanamixis polystachya* (wall.) parker leaf. The crude leaf extract was found to show strong lethality against the brine shrimp nauplii in a dose dependent manner having LC50 value at a concentration of 40µg/ml. Antimicrobial activity of this extract was performed against both gram positive and gram negative bacteria using kanamycin as standard and the result showed moderate to good activity against *Sarcina lutea, Staphylococcous aureus, Pseudomonas, Hafnia, Shigella boydii, Shigella sonnie, Escherichia coli , Shigella dysenteriae,* and *Salmonella typhi.* Castor oil inducing antidiarrheal activity of ethanolic extract of *Aphanamixis polystachya* (wall.) parker leaf in Swiss Albino mice was evaluated using Loperamide as standard chemical but the extract didn't show remarkable antidiarrheal activity.

Introduction

Being the best creature of Almighty man is determined to discomfit disease, disorder, disability, discomfort and death to put foot print on earth using his intellectual ideas, potential activities as well as astute performance and has strong credence to be immortal from era to era. This is why; early man had to knock the door of natural Laboratory. Natural Laboratory is adorned with huge amount of botanical wealth. This botanical wealth is flourished by diverse types of plant parts (flowers, fruits, leaves, roots, rhizomes, woods and barks) day by day. Since the dawn of civilization these plants are regarded as a complete store house of remedies to cure all ailments of mankind. They are vital elements of natural Laboratory where a great number of chemical compounds are biosynthesized. Plants have provided the basis of thousand years old sophisticated traditional medicine systems and are supporting mankind with new remedies. The medicinal plants that have been used since ancient time, many have yielded most useful drugs that are very much in use in current medicine. Not only medicine but also for basic needs (food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours and fragrances) man's dependence on plant kingdom for the essentials of his existence has been of paramount importance (Albert F. Hill). Of all plant families the Meliaceae is more useful to man, chiefly for its high quality timbers and for the ease with which some species can be grown in plantations (White, C. T.1931 and

White, F. 1962). Profound availability of complicated structural secondary metabolites with significant bioactivities has attracted Meliaceae family an overwhelming attention in the field of natural products (Fang, 2011). *Aphanamixis polystachya* (wall.) parker belonging to Meliaceae family, a large evergreen tree found to grow in most of the hotter parts of India, as well as the lowlands and hill forests of Bangladesh, Malay and Ceylon,(Kritikar, 1999; Gupta, 2010 and Chopra, 1956) has renowned status on sub-continental ayurvedic treatment including Antioxidant, Laxative, Antineoplastic, Repellent, Antifeedant, Cytotoxicity, Hepatoprotective, Antimicrobial, Antiulcer activity, CNS depressant and analgesic activity, and spleen diseases because of its significant medicinal value and pharmacological activities as well (Chowdhury and Rashid, 2003; Jagetia, 2007; Rabi and Gupta, 1995; Talukder and Howse, 1995; Gole and Dasgupta, 2002; Shrivastava and Leelavathi, 2010; Krishnaraju, *et al*., 2009; Shinkar, 2007 and Hossain *et al*., 2009). High potential and the presence of elevate number of secondary compounds with their economical as well as medicinal values have brought *Aphanamixis polystachya* (wall.) parker into light for close supervision and scientific research. According to literature survey, different works have been done on different parts of *Aphanamixis polystachya* (wall.) parker using different solvent system but the motto of present study was to evaluate *in vitro* antibacterial, cytotoxic and *in vivo* antidiarrheal activity of ethanolic extract of Leaf of *Aphanamixis polystachya* (wall.) parker .

Materials and Methods

Plant material collection and identification

Fresh leaves of *Aphanamixis polystachya* (wall.) Parker was collected during summer in the month of May, 2014 from Paikgachha, Khulna, Bangladesh. The plant part was identified by the experts of Botany Department, University of Rajshahi.

Preparation of extract

Thoroughly clean Leaves were shade dried and the dried Leaves were cut into small pieces and ground into a coarse powder with the help of a suitable Laboratory grinder. 200gm powder of *Aphanamixis polystachya* (wall.) Parker Leaves was macerated in 800 ml of 90% ethanol at 37°C for 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by clean, white cotton, followed by a filtration through Whatmann filter paper. The filtrate (ethanol extract) was then evaporated through rotary evaporator followed by desiccation to get the dried crude extract. The extract was stored in an airtight container and kept in a cool, dark and dry place in Phytochemistry Laboratory, Department of Pharmacy, and University of Rajshahi, Bangladesh for subsequent evaluation of biological activities.

Antibacterial activity

Microorganisms and media

The test microorganisms used in this study were both gram positive and gram negative bacterial strains including *Shigella boydii*, *Hafnia*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas*, *Shigella sonnie*, *Staphylococcous aureus*, *Sarcina lutea* and *Escherichia coli*. The microorganisms were collected as pure cultures from Microbiology Laboratory, Department of Pharmacy University of Rajshahi, Bangladesh. The bacterial isolates were first sub-cultured in a nutrient agar and incubated at 37°C for 18 h.

Disc diffusion method

The Antibacterial assay was performed by disc diffusion technique (Bauer *et al*., 1966; Reiner, 1980&1982). The sample solution of the extract to be tested was prepared by dissolving a definite amount of extract in appropriate solvent to attain a concentration of 50mg/ml. 10 µl of such solution was applied on sterile disc (5mm diameter, filter paper) and allowed to dry off the solvent in an aseptic hood. Thus the discs contain 500µg of crude extract. Standard antibiotic disc (Kanamycin 30µg/disc) and blank discs (impregnated with solvents) were used as a positive and negative control respectively. The test discs and standard disc were placed in a Petri dish seeded with particular bacteria and then left in a refrigerator at 4°C for 12-18 hrs in order to diffuse the material from the discs to the surrounded media in the Petri dishes. The Petri dishes were then incubated at 37°C for overnight to allow the bacterial growth. The Antibacterial activity of ethanolic extract of Leaves of *Aphanamixis polystachya* **(**wall.) parker was then determined by measuring respective zone of inhibition in mm.

Cytotoxic activity

Brine shrimp lethality bioassay

The Brine shrimp *(Artemia salina)* lethality bioassay was used to determine the cytotoxic activity of the Leaf extract. The test was performed by hatching 10mg of

eggs in 1000ml of artificial sea water (38g non-ionized sodium chloride; NaCl in one liter of sterilized distilled water having PH 8-9 using NaHCO3 solution) after incubation at 37°C for 48h with continuous oxygen's supply. The nauplii were allowed another 48h in sea water to ensure survival and maturity before use. Six doses of Leaf extract (5, 10, 20, 40, 80 and 160 µg/ml) in 5% DMSO was prepared. Each extract preparation was dispensed into clean test tube to make 10ml volumes. The concentration of DMSO was kept below 10µl/ml. For control, same volumes of DMSO (as in the sample test tubes) were taken in the test tube and same procedure was followed. After marking the test tubes properly, 10 living nauplii were added to each of the tubes with the help of the Pasteur pipette. After 24 hours incubation at 37°C test tubes were observed and the number of survived nauplii in each test tube was counted and the results were tabulated. From this table, the percentage of mortality was calculated at each concentration (Meyer, *et al*., 1982; McLaughlin, 1998 and Persoone, 1980).

Antidiarrheal Activity

Experimental animal

Young Swiss-albino mice aged 4-5 weeks, average weight 25-30 gm were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B) for the experiment. They were kept in standard environmental condition (RH 55% to 60%, room temperature 25± 2oC and 12 hour light/ dark cycle) for one week for adaptation after their purchase and fed ICDDR,B formulated rodent food and water.

Castor oil induced antidiarrheal activity

Castor oil induced diarrheal model was followed for this experiment. The employed mice were screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The test animals were selected randomly and divided into three groups having five mice in each group. The experimental animals were accurately weighed & properly marked. As group-I or the control received only distilled water containing 1% Tween-80 (25 ml/kg). Group-II or the positive control received standard anti-motility drug, Loperamide (50mg/kg) as oral suspension. The test group III was treated with suspension of Leaf extract of Aphanamixis *polystach* (wall.) parker at the oral dose of 500 mg/kg-body weight. Test samples, control and Loperamide were given orally by means of a feeding needle. The mice were fed with the samples, control and Loperamide 1 hour prior to the oral administration of castor oil at a dose of 0.5ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in four hours study after castor oil administration. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4-hours period and were noted for each mouse. The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones. During an observation period, the total number of stool output including diarrheic faces excreted by the animals was recorded. A numerical score based on stool consistency was assigned as follows: normal stool=1 and watery stool=2 (Jebunnessa, *et al*., 2009; Atta, *et al*., 2004 and Nwodo, *et al*., 1991).

Results

Man is familiar to different types of disease and disorder since human race begins and his efforts to survive against all odds using natural elements are highly appreciated. Researchers are always trying to find out new and comparatively easier and cheaper way to come round from the drastic and epedemic situation of fatal disease and never ending disorders. This study is a part of such efforts to find out a reliable source of antibacterial, cytotoxic and antidiarrheal agent**.** In the present study, ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaf was examined for in *vitro* Antibacterial activity, cytotoxic activity and *in vivo* Antidiarrheal activity**.**

Antibacterial activity

This study was performed to find out antibacterial effect of *Aphanamixis polystachya* (wall.) Parker leaf extract. Antibacterial activity of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker leaf was evaluated using Kanamycin (30µg /disc) as standard by measuring the zone of inhibition in mm. Table-1 exhibits that the ethanolic extract at a dose of 500µg/disc showed moderate to good Antibacterial activity in comparison with standard Kanamycin (30µg /disc) against *Sarcina lutea, Staphylococcous aureus, Pseudomonas, Hafnia, Shigella boydii, Shigella sonnie, Escherichia coli , Shigella dysenteriae,* and *Salmonella typhi* with the zone of inhibition ranging from 8 to 20 mm. The highest zone of inhibition was observed against *Salmonella typhi* (20mm) and the lowest zone of inhibition was observed against *Sarcina lutea* (8mm). The blank discs (30 µl /disc) didn't show any zone of

inhibition. Antibacterial activity of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker leaf against different bacterial strains is tabulated in Table-1 in terms of diameter of zone of inhibition in mm.

Table-1: *In vitro* antibacterial activity of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaf

Bacterial strains	Diameter of zone of inhibition in mm		
	Kanamycin(30µg/disc)	Ethanolic extract (500µg/disc)	Blank(30 µl /disc)
Shigella boydii	26	11	0
Hafnia	25	10	0
Shigella dysenteriae	25	13	0
Escherichia coli	26	12	0
Pseudomonas	26	10	0
Salmonella typhi	28	20	0
Shigella sonnie	27	12	0
Staphylococcous aureus	27	9	0
Sarcina lutea	22	8	0

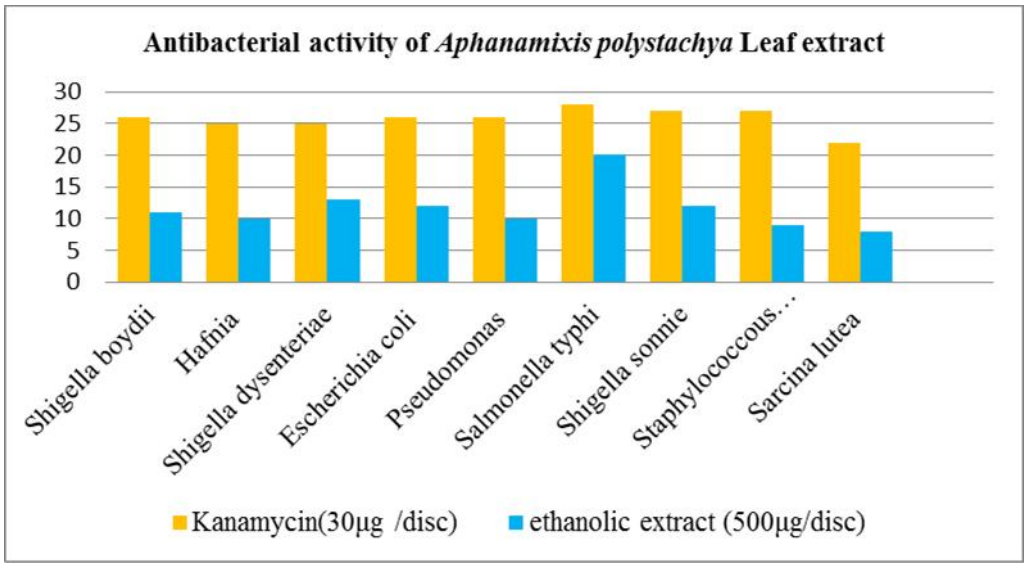

Figure-1: *In vitro* Antibacterial activity of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker leaf

Cytotoxic activity

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay indicates Cytotoxicity of Leaf extract. The extract was found to show lethal activity against Brine shrimp nauplii and LC50 was found at 40µg/ml. The tabulated values of percent of mortality (Table 2) were analyzed to find out whether *Aphanamixis polystachya* (wall.) Parker Leaf extract has potential Cytotoxic activity or not. The percent of mortality of brine shrimp nauplii was determined in a dose dependent manner. All the nauplii remained alive in control group and 100% of mortality was found at a concentration of 160µg/ml. However, increasing the concentration of *Aphanamixis polystachya* (wall.) Parker leaf extract increases the percent mortality rate significantly.

Table-2: Result of Brine shrimp lethality bioassay of 90% ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaves

Test sample	Conc. («g/ml)	No. of shrimp taken	No. of alive shrimp	No. of died shrimp	%mortality	LC_{50} («g/ml)
90% ethanolic extract	5	10	10	0	0	40
	10	10	9	1	10	
	20	10	8	2	20	
	40	10	5	5	50	
	80	10	2	8	80	
	160	10	0	10	100	

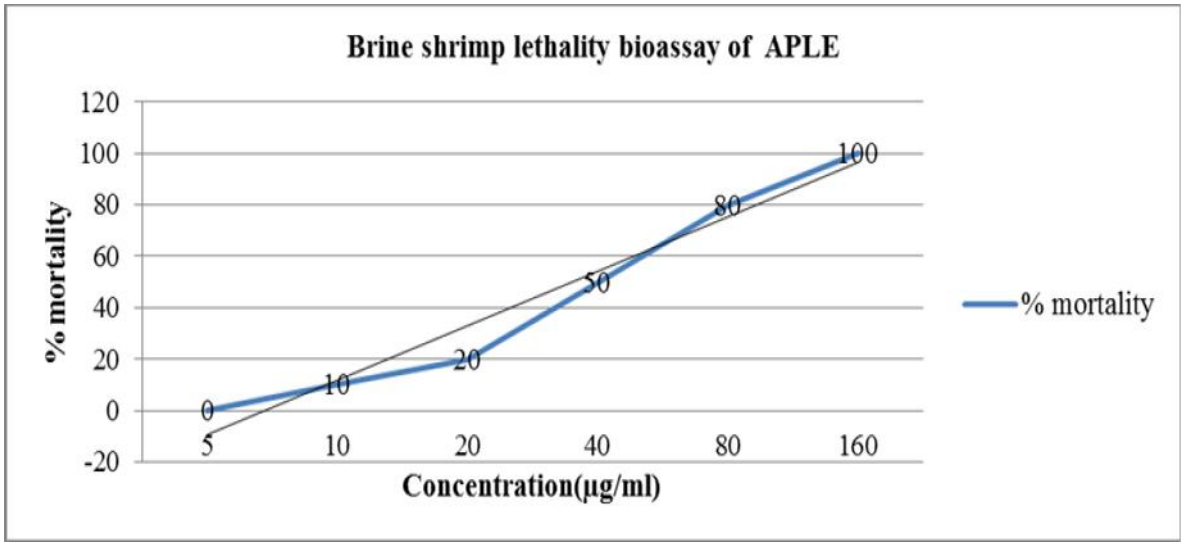

Figure-2: LC50 of *Aphanamixis polystachya* (wall.) Parker Leaf extract
APLE= *Aphanamixis polystachya* Leaf extract

Antidiarrheal activity

At the dose of 500mg/kg-body weight, the ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaf compared to the control group, offered about 0.74 hr of the mean latent period where as standard Loperamide provided 1.65 hr of the mean latent period for diarrheal episode. Table -4 indicates that mean number of stool of *Aphanamixis polystachya* (wall.) parker leaf extracts induced mice increases with time in comparison with standard Loperamide and control group. Antidiarrheal activity of *Aphanamixis polystachya* (wall.) Parker leaf in castor oil induced mice at the dose of 500 mg/kg as compared to the standard antidiarrheal agent Loperamide didn't show marked effect i.e. neither delayed the onset of diarrheal episode nor decreased the frequency of defecation. The antidiarrheal activity of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker leaf is showed in the following tables.

Table -3: Effect of *Aphanamixis polystachya* Leaf extract on latent period of castor oil induced diarrheal episode in mice.

Group (dose)	Numbering of mice	Latent period(hr)	Mean of latent period (hr)	Standard Deviation (SD)	Standard Error(SE)	t-test (P-value)
Control	M1	0.77	0.80	0.117	0.058	--
	M2	0.68				
	M3	0.96				
	M4	0.89				
	M5	0.72				
Loperamide (50mg/kg)	M1	1.82	1.65	0.304	0.152	5.2 P<0.01
	M2	1.36				
	M3	1.54				
	M4	1.44				
	M5	2.10				
APLE(500 mg/kg)	M1	0.88	0.74	0.100	0.053	0.865 P<0.1
	M2	0.73				
	M3	0.78				
	M4	0.67				
	M5	0.62				

Values are t-test. (n=5); p< 0.1, vs. control, Student's t-test
APLE= *Aphanamixis polystachya* Leaf extract

Table -4: Effect of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaf on stool of castor oil induced diarrheal episode in mice.

Group	Number of Stool calculation							
	Number of hour	Number of mice					Mean no. of stools	SD
		M1	M2	M3	M4	M5		
Control	1h	3	2	2	3	2	2.4	0.54
	2h	7	6	5	6	5	5.8	0.83
	3h	4	5	3	4	4	4	0.70
	4h	3	4	2	3	3	3	0.70
Positive Control	1h	1	1	0	1	0	.6	0.55
	2h	2	2	1	2	1	1.6	0.54
	3h	2	3	2	2	2	2.2	0.44
	4h	2	2	2	1	2	1.8	0.45
APLE	1h	3	4	4	3	3	3.4	0.54
	2h	7	8	6	7	5	6.6	1.14
	3h	6	6	4	5	6	5.4	0.89
	4h	6	6	5	4	5	5.2	0.84

Control (25 ml/kg).= distilled water +1% Tween-80; **Positive Control (50mg/kg) =** Loperamide
APLE (500 mg/kg)= *Aphanamixis polystachya* Leaf extract

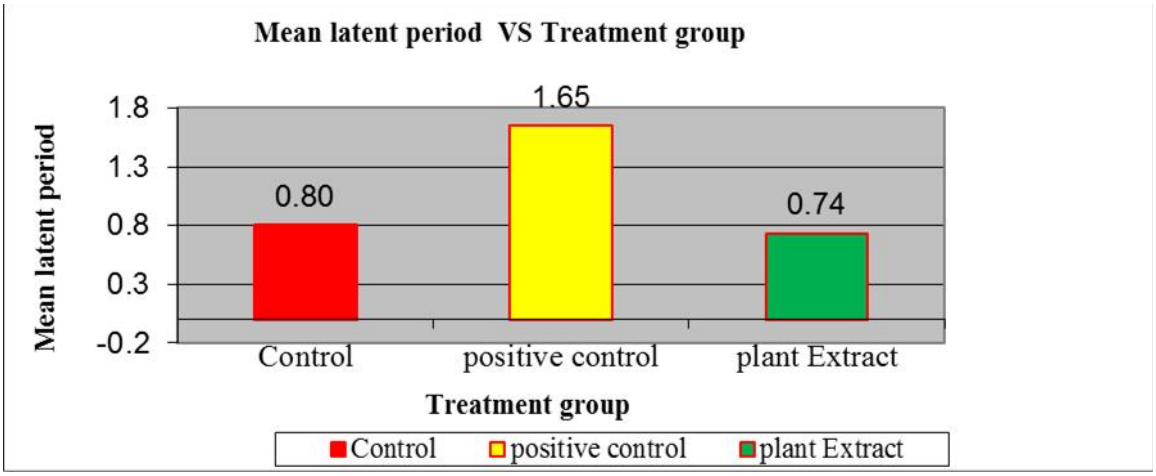

Figure-3: Effect of ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaf on latent period of castor oil induced Diarrheal episode in mice.

Discussion

Antibacterial activity

Various Scientific researchers have already been performed to treat various infections for exploration of new natural bioactive compounds as an alternative therapy to so called antibacterial agents with a view to avoiding Antibacterial resistance plight. Based on the present study, we can consider the leaves of *Aphanamixis polystachya* (wall.) Parker to be an important source of antimicrobial property. The effective inhibitory potency observed with the leaves; proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates. However, Kishore *et al* reported that the presence of alkaloids, proteins, glycosides, saponins, flavonoids and tannins of crude extract might be responsible for antimicrobial activity. *Aphanamixis polystachya* (wall.) parker leaf contains huge quantity of glycosides, alkaloids saponin, and flavonoids (Shadid Hossain, *et al*., 2016) which are accountable for antibacterial activities.

Antibacterial activities using fruits (Apu, *et al*., 2013) and stem bark (Shaikh, *et al*., 2012) of *Aphanamixis polystachya* (wall.) Parker were previously reported mentioning significance of this plant for potential antibacterial activity. But this study represents moderate to good antibacterial activity of leaf extract of *Aphanamixis polystachya* (wall.) Parker against both gram positive and gram negative bacteria indicating that this plant is going to be a gigantic source of Antibacterial agent for upcoming decades due to the presence of diverse chemical group.

Cytotoxic activity

Plant species contain huge amount of chemical compounds which may be poisonous, medicinal and nutritional. So researchers need to know about all the compounds of plants responsible for pharmacological effect. In order to identify toxic property of plant extract Brine shrimp lethality bioassay is widely used. It is considered to be a yardstick for Cytotoxic activity because of detecting broad spectrum of bioactivity present in crude extracts of medicinal plants as well as indicating cytotoxicity and wide range of pharmacological activities of the compounds. . The Cytotoxic activity is assumed to be the output of availability of different secondary metabolites and chemical groups such as glycosides, alkaloids saponin, flavonoids and tannin (Peteros, 2010, Mazumder, *et al*., 2010, Shadid Hossain, *et al*., 2016). These compounds are known to be free radical scavanger, reactive species quencher, detoxification inducer, tumor production and proliferation cell inhibitor and apoptosis inducer (Krishnaraju, 2009). These bioactive compounds are present in *Aphanamixis polystachya* (wall.) parker leaf extracts and accountable for cytotoxic activity because the biological activities of plants may be due to the presence of this diverse group of chemical compounds (Mohammad Sekendar Ali, 2013). Extracts are regarded as non-toxic if its LC50 is greater 100µg/ml in brine shrimp lethality assay (Gupta, 1996). However, Apu *et al*.,(2013) established that fruit extracts of *Aphanamixis polystachya* (wall.) parker provide LC50 value at < 100 µg/ml whereas this study showed that leaf extract of *Aphanamixis polystachya* (wall.) Parker gave LC50

value at 40µg/ml remarking profound cytotoxic activity of this plant. So, ethanolic extract of leaves of *Aphanamixis polystachya* (wall.) parker has potential cytotoxicity. According to the literature, compounds that present Brine shrimp (*Artemia salina*) toxicity, in general also have Cytotoxic properties against cells of solid tumors found in humans (McLughilin, 1991).

Antidiarrheal activity

Ricinoleic acid is produced from triglyceride after mixing of orally administered castor oil with bile and pancreatic enzymes which is absorbed partially from the gastrointestinal tract and metabolized like any other fatty acid but most remains in the intestine where it occurs its antiabsorptive or secretory effect through producing soap or surfactant like ricinoleate salts with Sodium and Potassium in the lumen of the intestine. Literature survey so far revealed provides laxative properties of *Aphanamixis polystachya* (wall.) parker. Laxative properties of *Aphanamixis polystachya* bark was established few years ago (Chowdhury and Rashid, 2003). No much work has been done on antidiarrheal activity due to rapid onset of diarrheal episode and increased frequency of defecation of plant extract induced mice. This study showed that there was no decreased frequency of defecation and increased mean latent period of test group than that of positive control group. It claimed that ethanolic extract of *Aphanamixis polystachya* (wall.) Parker Leaf possesses no Antidiarrheal activity. It demands further investigation to find out medicinally active chemical compounds responsible for this type of mechanism of action.

Conclusions

Plant is regarded as men's best friend due to abundance of significant bioactive constituents and their proper use in different physical eliments. *Aphanamixis polystachya* (wall.) Parker is highlighting for several bioactive natural metabolites providing an incredible medicinal value. According to above discussion, we can consider the Leaf extract of *Aphanamixis polystachya* (wall.) Parker to be a good source of Antimicrobial and Cytotoxic property. Based on the present study, further investigations are required to explore the bioactive molecules which are responsible for the extracts' activities as well as their mechanisms of action.

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