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Antimicrobial and Cytotoxic Activities of the Extracts of *Glochidion multiloculare*

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ABSTRACT

The current study was designed to investigate the antimicrobial and cytotoxic activities of methanol extract (MEGM), petroleum ether fraction (PEFGM), carbontetrachloride fraction (CTFGM), chloroform fraction (CFFGM) and aquous fraction (AQFGM) of stem bark of *Glochidion multiloculare* (Euphorbiaceae). Antimicrobial activity was evaluated by disc diffusion method and cytotoxic activity by brine shrimp lethality bioassay. In case of antimicrobial screening the CFFGM showed moderate inhibitory activity against *B. subtilis* having the zone size 12 mm and against *Escherichia coli and Escherichia coli* were 11 mm, while in the brine shrimp lethality bioassay, the petroleum ether soluble fraction revealed the highest cytotoxicity having LC_{50} of 3.11 μg/ml.

Keywords: *Glochidion multiloculare,* Euphorbiaceae, antimicrobial screening, brine shrimp lethality bioassay

INTRODUCTION

Glochidion was regarded as a genus of the family Euphorbiaceae, which consists of monoecious, rarely dioecious trees or shrubs. But molecular phylogenetic studies have shown that *Phyllanthus* is paraphyletic over *Glochidion*. A recent revision of the family Phyllanthaceae has subsumed *Glochidion* into *Phyllanthus* (Hoffmann *et al.*, 2006). *Glochidion multiloculare* (Roxb. ex Willd.) Muell.-Arg., Phyllanthaceae (synonym: *Phyllanthus multilocularis*), locally known as Aniatori, Keotomi, Keoura, Paniatori, Pannyaturi is an evergreen shrub or small tree. The plant is found in Bhutan, India, Myanmar, Nepal and Bangladesh. Traditionally many *Phyllanthus* species are used in

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haemorrhoids, diarrhoea, dysentery, anaemia, jaundice, dyspepsia, insomnia etc. and some of them can induce diuresis (Ghani, 1998). In Chinese traditional medicine *Glochidion puberum* is used in dysentery, jaundice, leukorrhagia, common cold, sore throat, toothache, carbuncle, furuncle, rheumatic arthralgia (Hu *et al*., 2004).

Biological investigations of *Phyllanthus* species revealed that many members of the genus possess anti-tumor promoting ability (Huang *et al*., 2006; Rajeshkumar *et al*., 2002; Tanaka *et al*., 2004), apoptosis inducing ability (Huang *et al*., 2004; Puapairoj *et al*., 2005), antiviral activity against hepatitis B virus (Lam *et al*., 2006; Venkateswaran *et al*., 1987), anti-angiogenic effect (Huang *et al*., 2006), analgesic effect (Santos *et al*., 1994, 2000), diuretic effect (Srividya and Periwal, 1995), lipid lowering activity (Khanna *et al*., 2002), hypocholesterolemic activity (Adeneye *et al*., 2006), antioxidative effect (Harish and Shivanandappa, 2006; Raphael *et al*., 2002; Sabir and Rocha, 2008), antidiabetic effect (Adeneye *et al*., 2006; Raphael *et al*., 2002; Srividya and Periwal, 1995), antiherpetic activity (Álvarez *et al*., 2009; Yang *et al*., 2007), hepatoprotective effect (Harish and Shivanandappa, 2006; Sabir and Rocha, 2008), anti-inflammatory action (Kassuya *et al*., 2006; Kiemer *et al*., 2003), antiatherogenic effect (Duan *et al*., 2005), anti-HIV activity (Notka *et al*., 2003, 2004; Ogata *et al*., 1992); antiplasmodial activity (Luyindula *et al*., 2004), antibacterial activity (Meléndez and Capriles, 2006), hypotensive activity (Leeya *et al*., 2010; Srividya and Periwal, 1995) etc.

Several secondary metabolites were isolated from different *Phyllanthus* species, including flavonoids, lignans, alkaloids, triterpenes, phenols and tannins (Calixto *et al*., 1998; Chang *et al*., 2003; Ishimaru *et al*., 1992). Many secondary metabolites were isolated from *Glochidion* species, including tannins (Chen *et al*., 1995), glycosides (Otsuka *et al*., 2003), lignans (Otsuka *et al*., 2000), terpenoids (Hui and Li, 1976). Previous investigation of *Glochidion multiloculare* revealed glochidiol, glochilocudiol, glochidone and dimedone (Talapatra *et al*., 1973).

MATERIALS AND METHODS

Plants materials

The stem bark of *G. multiloculare* was collected from Modhupur, Tanghail in the month of April, 2009 and identified by Mr. Sarder Nasir Uddin, Scientific Officer, Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB-34200) representing this collection has been deposited.

Preparation of extract

The air dried powdered plant material (1000 g) was successively cold extracted with methanol (7 days) at room temperature with occasional shaking and stirring. The extractives were filtered through fresh cotton plug and followed by whatman no. 1 filter paper. The filtrate were then concentrated by a Buchii rotavapor at low temperature and pressure and afforded methanol (MEGM) extract (41.7398g). The cold methanol extract (10 g) was subjected to Solvent-Solvent partitioning using the protocol designed by Kupchan and modified by Wagene (19). The extract was portioned successively with petroleum ether (PEFGM), carbon tetrachloride (CTFGM) and chloroform (CFFGM).

Antimicrobial Activity Test

The antimicrobial activities of the crude extracts were determined by the disc diffusion method(Baur,A.W. *et al*., 1966; Gazi, H.R. *et al*., 2007; Nahar,K *et al*., 2008) against the bacterial strains listed in Table-1. These were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.Here Kanamycin (30 μg/disc) was used as the standard. The pet-ether, carbontetrachloride,chloroform, aqueous fractions and methanol extract were dissolved separately in chloroform and applied to sterile discs at a concentration of 400 μg/disc and carefully dried to evaporate the residual solvent.

Cytotoxic activity test

For cytotoxicity screening, DMSO solutions of the petroleum ether, carbontetrachloride, chloroform, methanol and aqueous soluble extracts were applied against *Artemia salina*[1] in a 1-day *in vivo* assay. Measured amount of each sample was dissolved in 100 μl of DMSO in a vial to get stock solution. Then 50 μl of solution was added to test tube each containing 5 ml of seawater and 10 shrimp nauplii. Thus, the final concentration of samples in the No.1 test tube was 400 μg/ml. Then a series of solutions of varying concentrations (every time half than previous) were prepared from stock solution by serial dilution method. In each case, fresh 50 μl DMSO was added to vial (total volume 100 μl; then shaking it) and from it, 50 μl of sample was taken to test tube.

RESULTS AND DISCUSSIONS

The methanolic extract of the stem bark (MeEGM) of *G. multiloculare* as well as its petroleum ether (PEFGM), carbon tetrachloride (CTFGM), chloroform (CFFGM) and aquous (AQFGM) soluble fractions were subjected to microbiological screening.

Table 1: Antimicrobial activity of test samples of *G. multiloculare*

Test microorganisms	Diameter of zone of inhibition (mm)					
	PEFGM	**CTFGM**	**CFFGM**	**MeEGM**	**AQFGM**	**KAN**
Gram Positive Bacteria						
Bacillus cereus	09	08	10	--	--	33
B. megaterium	09	08	10	--	--	33
B. subtilis	09	09	12	--	07	33
Sarcina lutea	09	08	10	--	--	33
Gram Negative Bacteria						
Escherichia coli	08	09	11	--	--	33
Pseud. aeruginosa	08	08	10	--	07	33
Escherichia coli	08	09	11	--	07	33
S. typhi	08	08	10	--	--	33
Shigella dysenteriae	08	08	10	--	07	33

Table 1: Antimicrobial activity of test samples of *G. multiloculare* (Conti.)

Sh. boydii	09	07	10	--	--	33
Vibrio mimicus	09	07	09	--	07	32
V. parahemolyticus	10	08	10	--	--	33
Fungi						
Candida albicans	10	08	09	--	--	32
Aspergillus niger	10	09	09	--	07	33
Sacharomyces cerevaceae	10	09	10	--	08	33

KAN: standard kanamycin disc (30 µg/disc); a diameter of zone of inhibition less than 8 mm was considered inactive; *Pseud.* = *Pseudomonas*

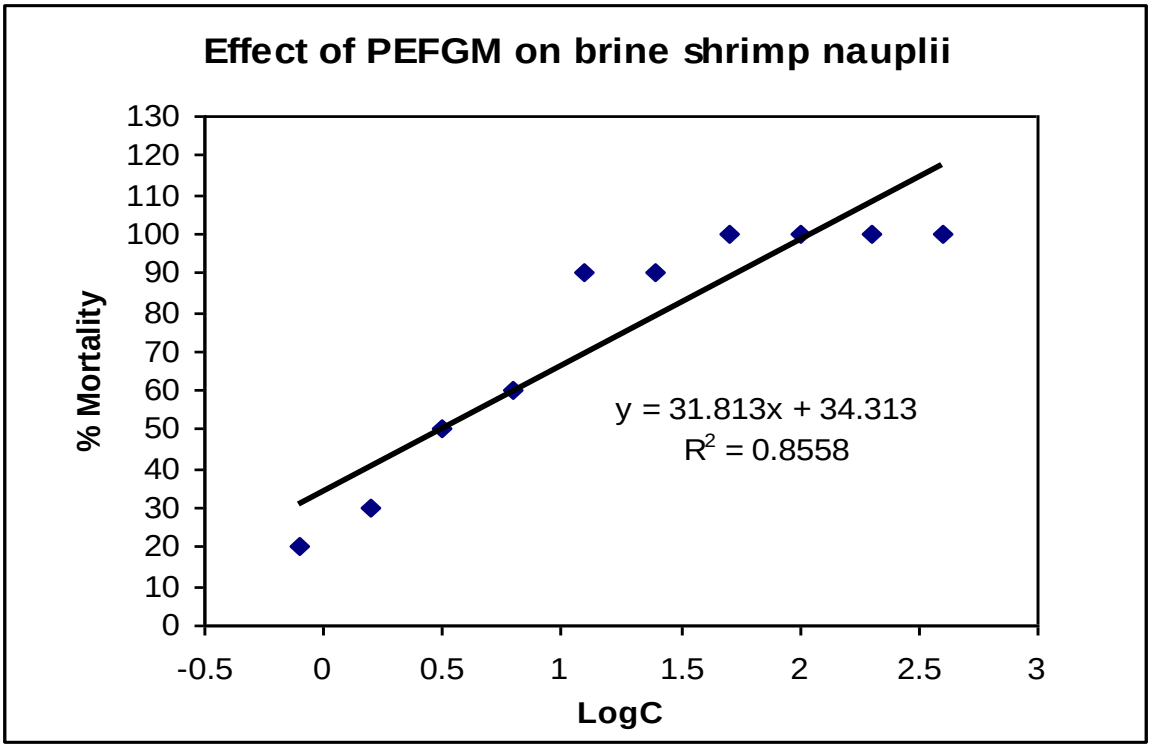

Fig 1: Effect of Petroleum ether soluble fraction of *G. multiloculare* on brine shrimp nauplii

In this test, the CFFGM of the methanolic extract appeared to be moderate in terms of both zone of inhibition and spectrum of activity. In this study, the zones of inhibition produced by the CFFGM, PEFGM and CTFGM were ranged from 9-12 mm, 8-10 mm and 7-9 mm respectively (Table 1).

Table 2: LC_{50} data of test samples of *G. multiloculare*

Test samples	Regression line	R^2	LC_{50} (µg/ml)
VS	y = 33.623x + 66.812	0.9548	0.32±0.12
PEFGM	y = 31.813x + 34.313	0.8558	3.11±0.11
CTFGM	y = 34.431x + 26.048	0.9144	4.96±0.66
CFFGM	y = 35.236x + 19.043	0.9089	7.56±0.11
MeEGM	y = 35.437x + 15.792	0.9387	9.23±0.33
AQFGM	y = 28.793x + 15.081	0.9852	16.32±0.40

The values of LC_{50} are expressed as mean±SD (n=3)

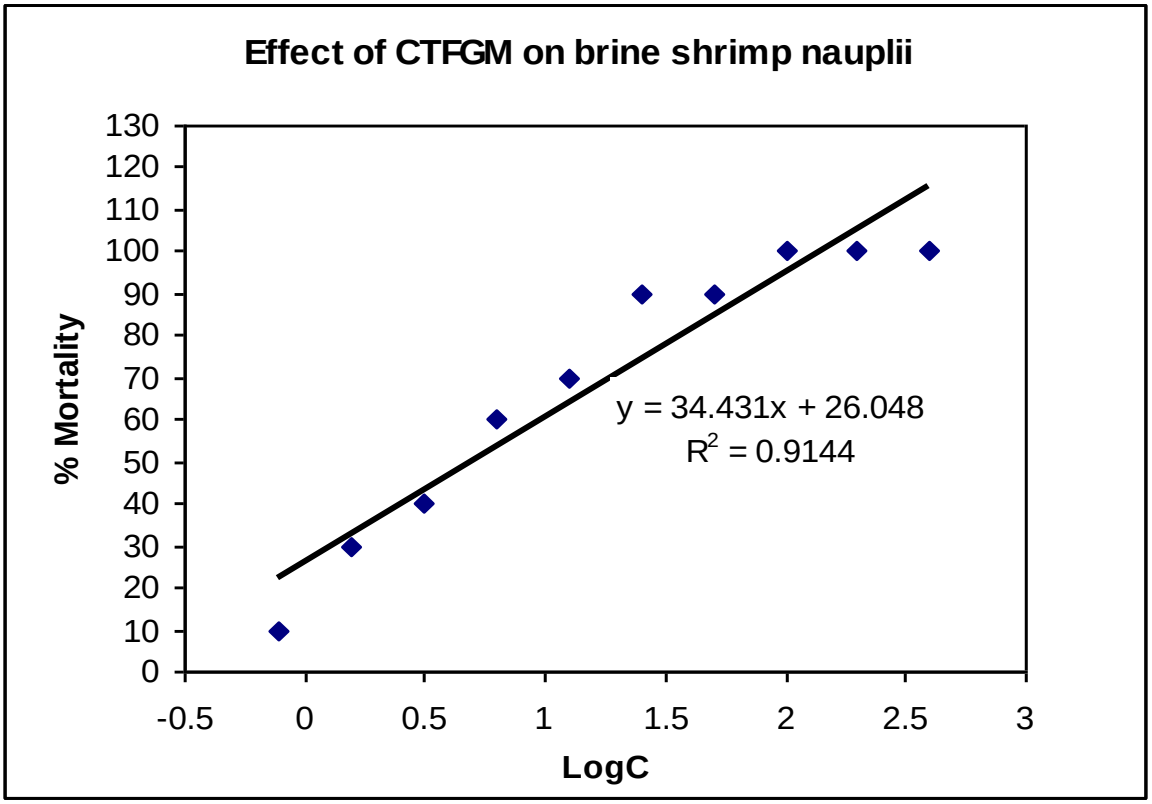

Fig 2: Effect of Carbontetrachloride soluble fraction of *G. multiloculare* on brine shrimp nauplii

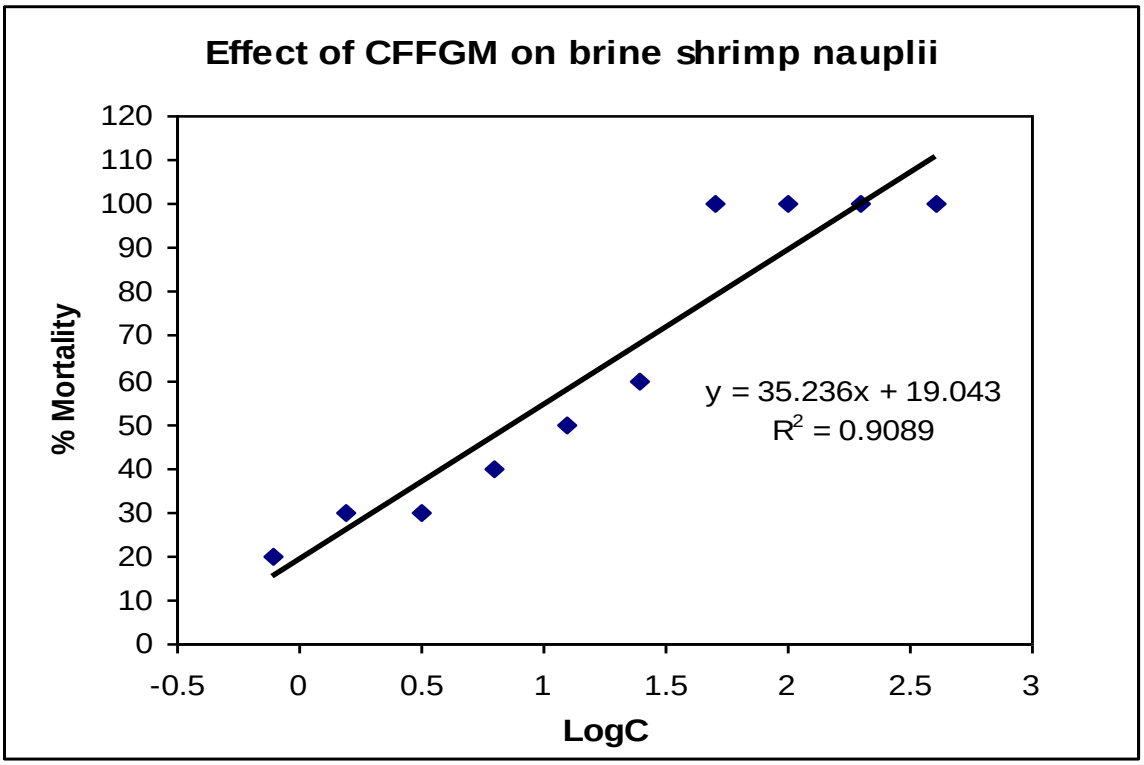

Fig 3: Effect of Chloroform soluble fraction of *G. multiloculare* on brine shrimp nauplii

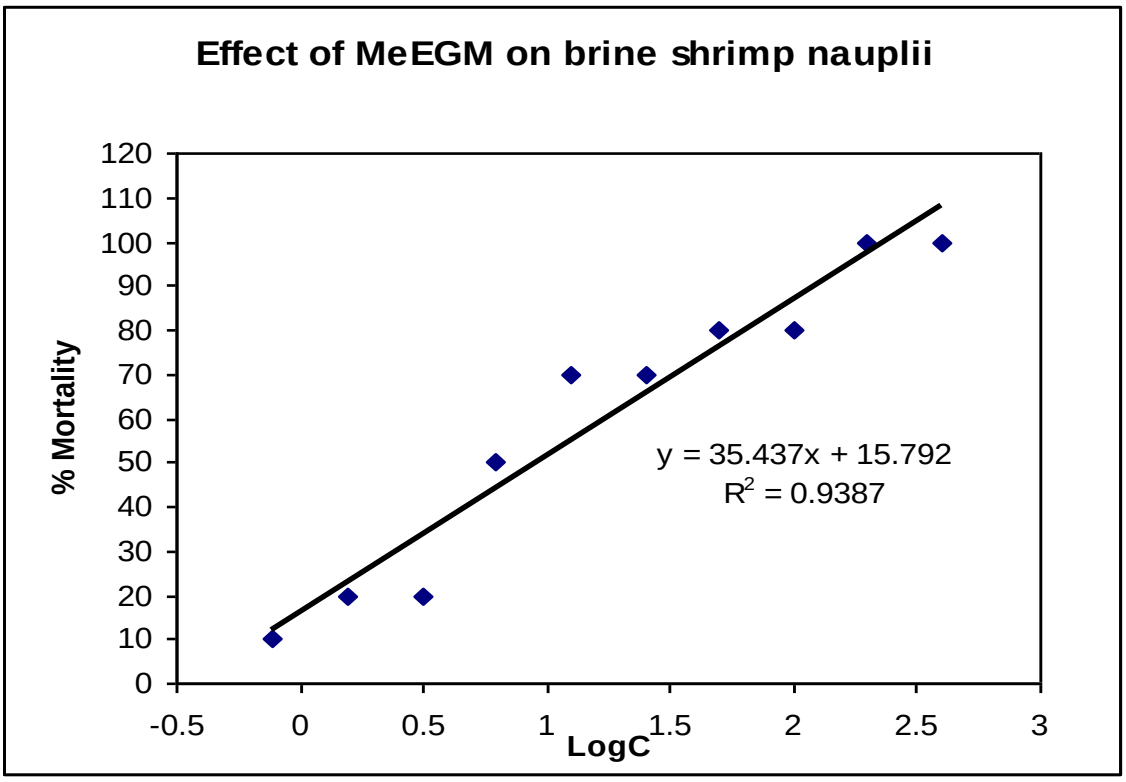

Fig-4: Effect of Methanol soluble extract of *G. multiloculare* on brine shrimp nauplii

The CFFGM showed moderate inhibitory activity against *B. subtilis having* the zone size 12 mm and against *Escherichia coli and Escherichia coli* were 11 mm. This fraction also showed mild inhibitory activity against *Bacillus cereus, B. megaterium, Sarcina lutea, Pseudomonas aeruginosa, S. typhi, Shigella dysenteriae,* Sh. boydii, *V. parahemolyticus,* Sacharomyces cerevaceae (10 mm each). At the same time, the petether soluble fraction demonstrated mild inhibitory activity against *V. parahemolyticus, Candida albicans, Aspergillus niger, Sacharomyces cerevaceae* (10 mm each). The CTFGM and AQFGM of *G. multiloculare* exhibit the least activity against any microbe. The MeEGM showed no activity.

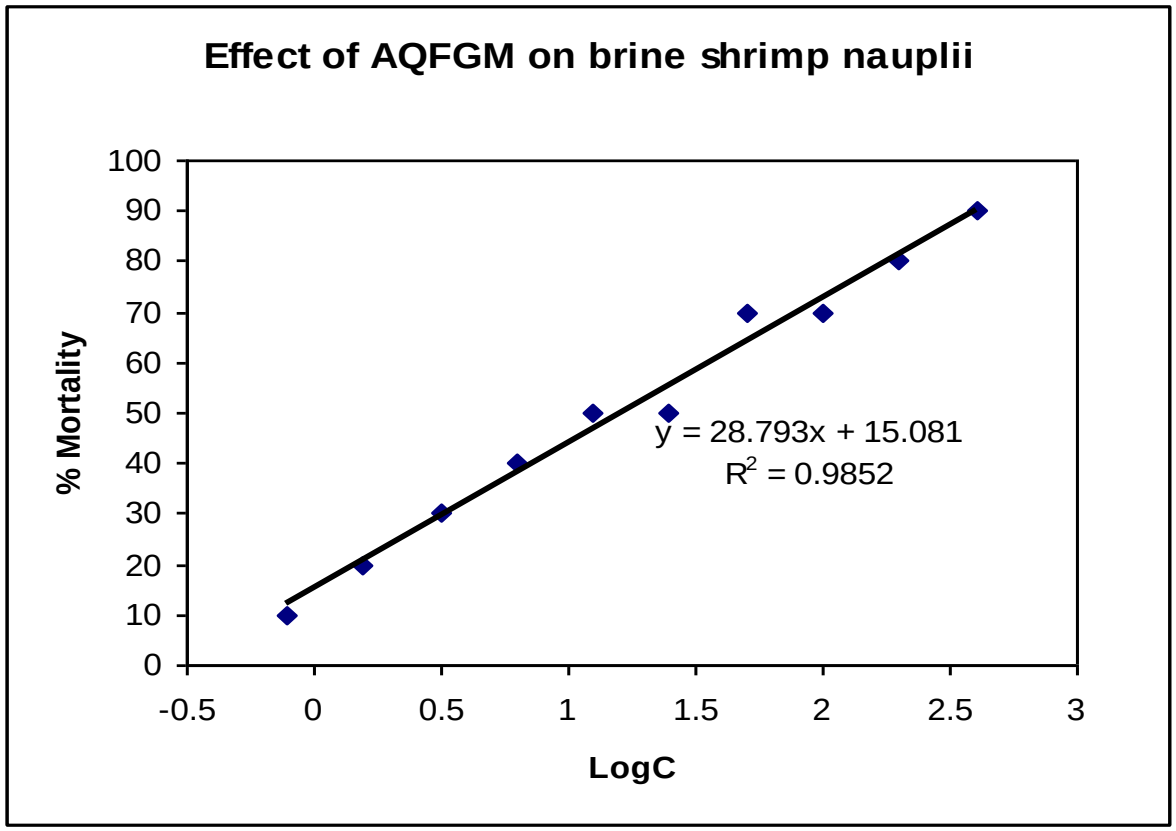

Fig-5: Effect of Aquous soluble fraction of *G. multiloculare* on brine shrimp nauplii

Following the procedure of Meyer *et al.* 1982, the lethality of the methanol soluble extract (MeEGM) of G. *multiloculare* as well as its petroleum ether, carbon tetrachloride, chloroform and aquous soluble fractions to brine shrimps were determined and the result are summarized in Table2 & 3.

In the brine shrimp lethality bioassay, the petroleum ether soluble fraction revealed the highest cytotoxicity having LC_{50} of 3.11 μg/ml. The degree of lethality was directly proportional to the concentration of the extract ranging from the lowest concentration (0.20 μg/ml) to the highest concentration (100 μg/ml). Maximum mortality took place at a concentration of 100 μg/ml, whereas least mortality was observed at 0.20 μg/ml.

In comparison with positive control (vincristine sulphate), the cytotoxicity exhibited by the extractives was promising. These bioactivities exhibited by the plant extractives substantiate the folk uses of the plant in various diseases.

Table-3: Effect of methanol extract, petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions of *G. multiloculare* on brine shrimp nauplii

Glochidion multiloculare											
Conc.(C) (µg/ml)	Log C	% Mortality					LC_{50} (µg/ml)				
		PEFGM	**CTFGM**	**CFFGM**	**MeEGM**	**AQFGM**	**PEFGM**	**CTFGM**	**CFFGM**	**MeEGM**	**AQFGM**
400	2.6020	100	100	100	100	90	3.11±0.11	4.96±0.66	7.56±0.11	9.23±0.33	16.32±0.40
200	2.3010	100	100	100	100	80					
100	2.0000	100	100	100	80	70					
50	1.6989	100	90	100	80	70					
25	1.3979	90	90	60	70	50					
12.5	1.0969	90	70	50	70	50					
6.25	0.7958	60	60	40	50	40					
3.125	0.4948	50	40	30	20	30					
1.5625	0.1938	30	30	30	20	20					
0.78125	-0.1075	20	10	20	10	10					

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