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Full Length Research Paper

Screening of *Acacia modesta* for antifungal, anti-termite, nitric oxide free radical scavenging assay and brine shrimp cytotoxic activities

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A variety of useful bioactive products are produced by the self generating machines, that is, plants. This make us interested in screening the crude methanolic extract and various fractions of *Acacia modesta* for antifungal, anti-termite, nitric oxide free radical scavenging and brine shrimp cytotoxic activities. The chloroform ($CHCl_3$) fraction exhibited low activity (15%) against *Fusarium oxysporum*. The crude methanolic extract exhibited good activity against *Heterotermes indicola* (termite). The $CHCl_3$ fraction showed moderate cytotoxic activity (40%) against brine shrimp. The results of nitric oxide free radical scavenging activity indicate that the crude methanolic extract, *n*- hexane and ethyl acetate (EtOAc) fractions of *A. modesta* have concentration dependent free radical scavenging activity.

Key words: *Acacia modesta*, antifungal, anti-termite, nitric oxide free radical scavenging activity, brine shrimp cytotoxic activity.

INTRODUCTION

Plant extracts were considered to be significant for various ailments by the ancient civilizations (Grabely and Thiericke, 1999). An estimate says that there are about 2 500 000 species of higher plants in the world and pharmacological activities of most of them are not studied (Jeevam et al., 2004). Natural products and their derivatives are counting for more than 50% of all the drugs in the world today; the higher plants being the major contributors, that is, about 25% (Cragg and Newman, 2005). Flowering plants are producing a variety of potent drugs, for example pilocarpine to treat glaucoma and dry mouth is derived from *Pilocarpus* spp. Reserpine and other hypertensive and tranquilizing alkaloids have been isolated from *Rauwolfia* spp (Newman et al., 2000). Members of the genus *Acacia*, family *Mimosaceae*, are commonly called *Acacias*, a large genus with 900 species (Hutchinson, 1964; Nasir et al., 1973), approximately 700 of which are natives to Australia. The tropical and sub-tropical regions of Africa, Asia and America are inhibiting remainder of the species. The wood of *Acacia* tree is in some cases very valuable, though very small in making railway carriages, wheels, handles, furniture and is the best for making charcoal (Gohl, 1975). The gums of genus *Acacia* are popular. The *acacia* trees of Pakistan that produces gums are *Acacia catechu, Acacia arabica, Acacia churnea, Acacia jacquemontii, Acacia farnesiana, Acacia leucophloae, A. modesta, Acacia auriculiformis* and *Acacia senegal* (National research council, 1979). Traditionally, gum Arabica is used for the treatment of low blood pressure caused by hemorrhage or surgical shock. In grafting of the destroyed peripheral nerves, in plastic surgery, 50% gum Arabic adhesive has been successfully used (Osol and Farrar, 1995). For the treatment of malaria, *Acacia concina* leaves are taken orally (Bora et al., 2007). The bark and leaves of *A. fernesiana* are crushed, boiled and is inhaled by the malarial patient (Bora et al., 2007). The EtOAc, *n*-hexane, water and ethanol extract of *Parkia bicolor A. Chev* exhibited a concentration dependent antimicrobial

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activity against *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida utilis* and *Aspergillus niger* (Ajaiyeoba and Edith, 2002). Against *Streptococcus viridans*, *S. aureus*, *E. coli*, *B. subtilis* and *Shigella sonnei*, the antimicrobial activity was determined using agar well diffusion method. The plant extract exhibited antimicrobial activity against all the test microorganisms. The minimum inhibitory concentration (MIC) of the stem bark ranged between 35 and 50 mg/ml (Banso, 2009). *A. modesta* (Phulai) or Palosa (Pushto), belonging to genus *Acacia,* Sub-family *Mimosaceae* of family *Leguminosae* is being used traditionally for the treatment of various ailments.

To relieve the body weakness of women after childbirth, the gum obtained from the bark is mixed with butter, almond and wheat flour and fed as a tonic. Z*huble sharbat*; One teaspoonful of gum dissolved in a glass of water, is used as a health tonic. It is used as a source of medicinal gum (27%), has commercial value (44%), cure of cough (11%) and used as tooth brush (locally called Miswak, 18%) (Hussain et al., 2006; Qureshi et al., 2007). The antibacterial efficacy of *A.modesta* extract against *Lactobacillus* (gram positive), strains of bacteria which cause dental carriers, has been established (Asghar et al., 2003). In normal rats fed on a diet containing powdered seeds of *A.modesta* and other *Acacia* species, the blood sugar level was lower than in rats fed with a standard semi-purified casein-glucose-starch diet (Singh et al., 1975). Gum is used as a sex tonic (Mahmood et al., 2004) and restorative (Qureshi et al., 2007).

The aim of the present study was to screen the crude methanolic extract and various fraction of *A. modesta* for antifungal, anti-termite, nitric oxide free radical scavenging and brine shrimp cytotoxic activities.

MATERIALS AND METHODS

Plant material

The plant *A. modesta* (aerial parts) was collected from the Northern region of Pakistan. The plant was identified by Prof. Dr. Abdur-Rashid, Department of Botany, University of Peshawar, Khyber PukhtoonKhwa, Pakistan.

Extraction

The collected plant material was dried in shade, chopped into small pieces and grinded to powder, using an electric grinder. The powder (8 kg) was soaked in commercial grade methanol for 15 days, twice, at room temperature, with occasional shaking. The collected materials were filtered and concentrated, at 40ºC, under vacuum; by rotary evaporator giving a blackish crude methanolic extract of 950 g.

Fractionation

The crude methanolic extract (950 g) was suspended in distilled water (500 mL) and partitioned with *n*-hexane (3 x 500 mL), $CHCl_3$ (3 x 500 mL) and EtOAc (3 x 500 mL) respectively to yield the *n*-hexane (250 g), $CHCl_3$ (190 g), EtOAc (55 g) and aqueous (360 g) fractions, respectively. 95 g of the crude methanolic extract was left for biological/pharmacological activities.

Antifungal activity

The antifungal activity of the crude methanolic extract and various fractions of *A. modesta* were carried out against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum*, *Fusarium oxysporum*, *Trichoderma harzianum* and *Rhizopus stolonifer* following (Bashir et al., 2009). The stock solutions of the test samples (24 mg / mL) were prepared in sterile dimethyl sulfoxide (DMSO). Sabouraud Dextrose Agar (SDA) was used to refresh the test organisms in Petri plates. 4 ml of SDA media was introduced into the test tubes to make slant. After autoclaving when the temperature is about 50°C, 66.6 µL from the stock solutions was introduced into respective test tubes. The seven days old fungal culture was introduced into the labeled test tubes and incubated at 25 ± 1ºC for seven days in growth chamber. Tubes supplemented with DMSO and Miconazole served as negative and positive control. The results were taken on day 7 by measuring the linear growth on the slanted test tubes in comparison with negative control.

Anti-termite activity

The anti-termite activity of the test samples was determined against *Heterotermes indicola* following (Salihah et al., 1993). The test samples (2 mg/ml) were prepared in respective solvents and introduced into the Petri plates, which contained blotting paper. The plates were left overnight to evaporate the solvent. 25 termites were then transferred to each Petri dish with the help of clean brush and observed after 24 h, till all the termites are dead. All the experiments were performed in triplicate and the average termites killed each day were noted.

Nitric oxide free radical scavenging assay

The nitric oxide free radical scavenging assay of the test samples was carried out following Bashir (2010). Stock solutions were prepared by dissolving 3 mg of the test samples in 1 ml of DMSO. Different concentrations of test samples- 0.3, 0.6, 0.9, 1.2 and 1.5 mg/ml of DMSO were prepared from stock solutions. 10 µl of each test sample was dissolved in 20 µl of Phosphate buffer; add 70 µl of sodium nitroprusside and incubate for 90 min. After incubation, shake it and add 50 µl of sulphanilic acid. Now take absorbance (pre-read) at 570 nm. Add 50 µl of [N-(1-Napthyl) Ethylenediaminedihydrochloride] and shake it. Now take end point at 570 nm. Vitamin C and DMSO were used as a positive control and blank respectively.

Brine-Shrimp cytotoxicity

The cytotoxic effect of the test samples was carried out against *Artemia salina* (brine-shrimp eggs) as per our reported procedure (Farrukh et al., 2009). Eggs were hatched in artificial sea water, prepared with a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, OH, USA) and double distilled water. After maturation, the nauplii were collected with the help of a Pasteur pipette. 20 mg of the test samples were dissolved in 2 mL of methanol, serving as stock solutions. From the stock solutions 5, 50 and 500 µL were transferred to vials (3 vials / concentration) with final concentration of 10, 100 and 1000 µg / mL, respectively. The vials were placed in the hood for half an hour or allowed overnight

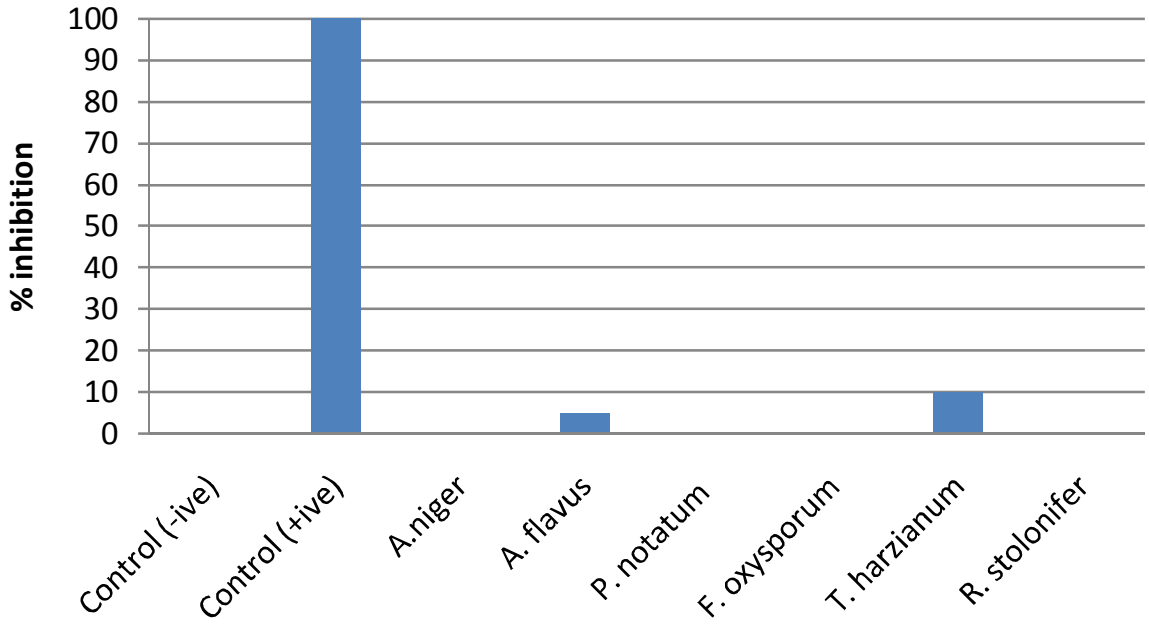

Figure 1. Antifungal activity (% inhibition) of the crude methanolic extract of *Acacia modesta.*

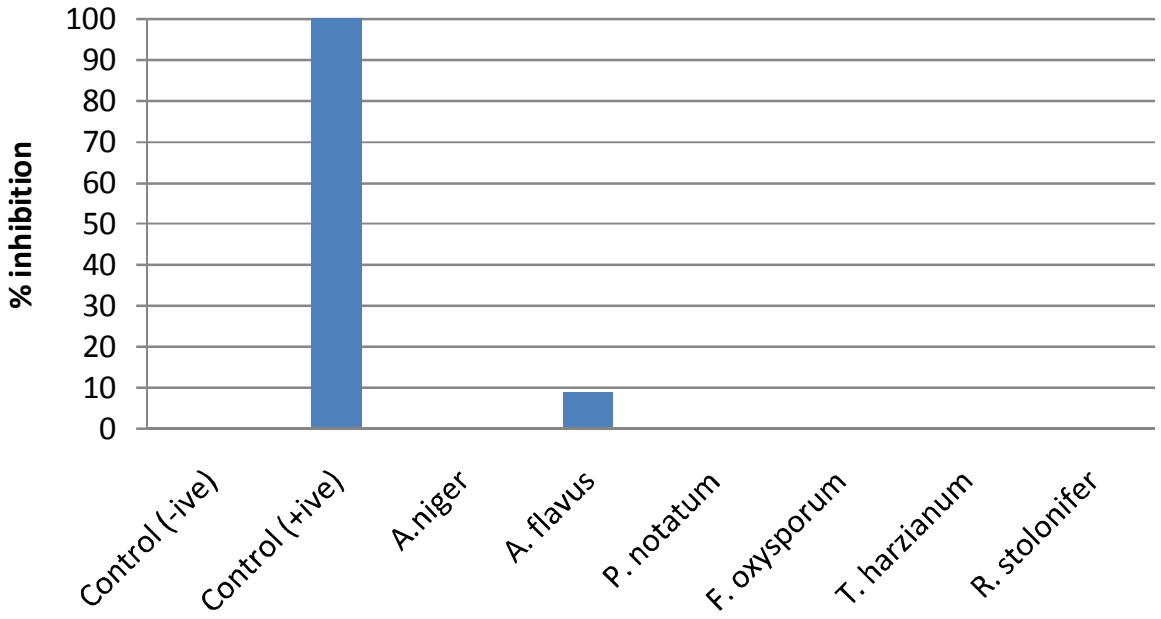

Figure 2. Antifungal activity (% inhibition) of *n*-hexane fraction of *Acacia modesta.*

so that methanol evaporates completely. To each vial 1 mL of sea water and 10 larvae were added. The final volume of each vial was adjusted to 5 ml with sea water. The vials were incubated under illumination at 26 ± 1ºC for 24 h. Methanol was used as negative and reference cytotoxic drug (Etoposide) as positive control. After incubation period brine shrimps that survived were counted using a magnifying glass. The data were analyzed with a Finney computer program (Probit analysis) to determine LD_{50} values with 95% confidence interval.

RESULT AND DISCUSSION

The results of the antifungal activity are depicted in (Figures 1 to 5). All the test samples were inactive against *A. niger*, *P. notatum* and *R. stolonifer.* All the fractions except aqueous fraction showed very low activity against *A.flavus*. The $CHCl_3$ and EtOAc fractions exhibited low activity against *F. oxysporum,* while rest of

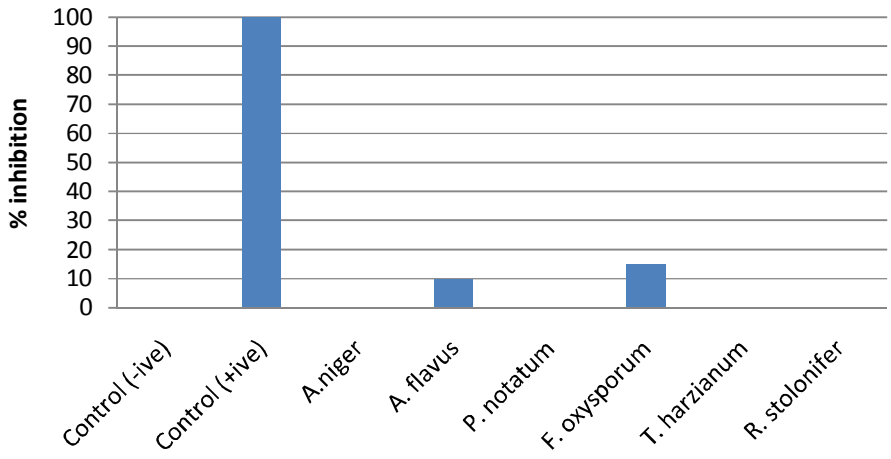

Figure 3. Antifungal activity (% inhibition) of $CHCl_3$ fraction of *Acacia modesta.*

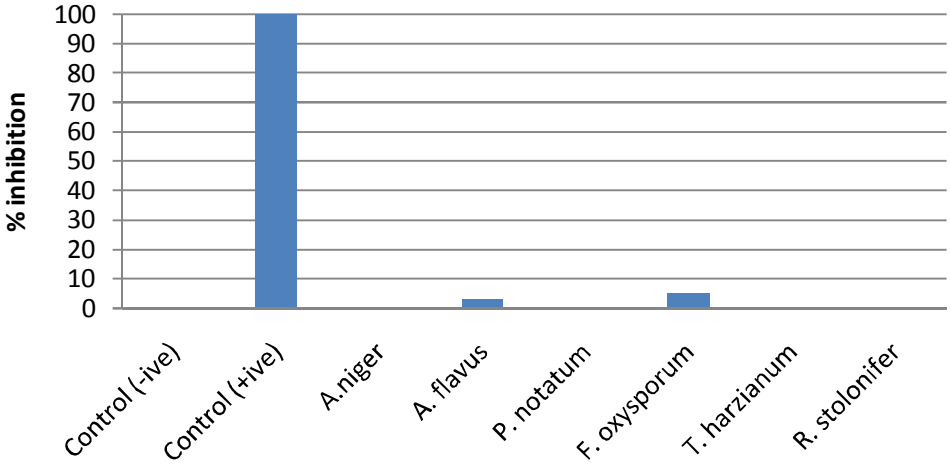

Figure 4. Antifungal activity (% inhibition) of EtOAc fraction of *Acacia modesta.*

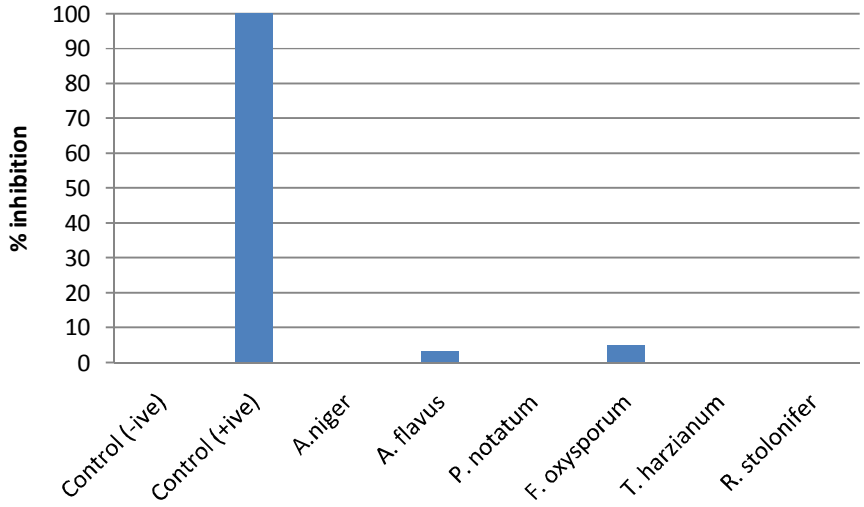

Figure 5. Antifungal activity (% inhibition) of Aqueous fraction of *Acacia modesta.*

Table 1. Anti-termite activity of the crude methanolic extract, $CHCl_3$ and aqueous fraction of *Acacia modesta.*

Sample	No. of termites	Day	Average termites killed	Average	S. D
Crude methanolic extract	25	1	22	23.5	2.12
		2	25		
$CHCl_3$		1	13	16.5	6.027
		2	20		
		3	25		
Aqueous		1	7	18	9.64
		2	22		
		3	25		

the fractions was inactive against it. All the fractions were inactive against *T. harzianum* except the crude methanolic extract. The above results indicate the *A. modesta* has no antifungal agents because neither the crude methanolic extract nor any of its fractions used in the current research, showed significant antifungal activity against the tested fungi.

Against the *H. indicola*, the anti-termite activity of crude methanolic extract, $CHCl_3$ and aqueous fractions of *A. modesta* were carried out at "Termites lab at Nuclear Institute of Food and Agriculture (NIFA), Peshawar, Khyber PukhtoonKhwa, Pakistan. All the experiments were performed in triplicates. The results are given in (Table 1).

In recycling of woody and other plant material, termites play an important role. The tunneling efforts of termites help to aerate soil. Termites are important members of the community of decomposers. They are able to decompose cellulose, the main component of wood. They are abundant in tropical and subtropical environments, where they help in breaking down and recycling one third of the annual production of dead wood. But when they start destroying wood and wooden products of human homes, building materials, forests, and other commercial products, they become economic pests causing a great economic loss (Suszkiw, 1998).

The crude methanolic extract showed significant activity against *H. indicola*. The experiment extended only for two days. On day 1.22 termites were dead on an average and on the second day, no termite survived. The average and standard deviation (SD) was 23.5 and 2.12, respectively. The experiment for the aqueous fraction extended for three days. On average, 7 termites were killed on day 1. On the second day of the experiment, 22 termites were dead on an average and on the third day, no termite survived. The average and SD was 18 and 9.64, respectively. The experiment for the anti-termite activity of the $CHCl_3$ fraction extended for three days. Day 1 results indicated that 13 termite, on average, was dead. On the next days, 20 and on the last day of the experiment no termite was alive. The average and SD was 16.5 and 6.02, respectively.

An important messenger molecule involved in many physiological and pathological processes within the mammalian body is nitric oxide (NO). It has both beneficial and detrimental effect on the human health (Hou et al., 1999). When NO is produced in appropriate form, it helps in the protection of an organ such as liver from ischemic damage. When NO is produced in higher levels, it is toxic to tissue and contribute to the vascular collapse associated with septic shock and is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Taylor et al., 1997). Keeping in view the importance of NO the crude methanolic extract and various fractions of *A. modesta* were screened for NO radical scavenging activity at different concentrations as shown in (Table 2). At the concentration of 0.3 mg/ml, the $CHCl_3$ fraction exhibited moderate activity of 31.84% while rest of the fractions exhibited low activity at this concentration. Results of the assay at the concentration of 0.6 mg/ml revealed that the crude methanolic extract, *n*- hexane and $CHCl_3$ have moderate NO radical scavenging activity- 33.56, 30.13 and 40.58%, respectively. The EtOAc and aqueous fractions showed low activity (16.09 and 11.47%) at this concentration. Looking at (Table 2), the crude methanolic extract, *n*-hexane and $CHCl_3$ have moderate NO radical scavenging activity- 43.83, 38.69 and 41.95% respectively at 0.9 mg/ml, while the other two fractions exhibited low activity at 0.9 mg/ml. At the concentration of 1.2 mg/ml, the results recorded showed that all the fractions except aqueous fractions (20.03%) have moderate NO radical scavenging activity. Moving to the concentration of 1.5 mg/ ml all the test samples exhibited moderate NO radical scavenging activity that is crude methanolic extract (56.50%), *n*-hexane (53.25%), $CHCl_3$ (52.22%), EtOAc (42.29%) and aqueous fractions (33.90%), respectively.

The above results indicate that the crude methanolic extract, *n*-hexane and EtOAc of *A. modesta* have concentration free radical scavenging activity. Therefore

Table 2. Nitric oxide free radical scavenging activity of the crude methanolic extract and various fractions of *Acacia modesta.*

Concentration of sample (mg/ ml)	Crude Met. Ext	*n*-hexane	$CHCl_3$	EtOAc	Aqueous
0.3	29.62	20.54	31.84	14.04	6.16
0.6	33.56	30.13	40.58	16.09	11.47
0.9	43.83	38.69	41.95	23.28	18.23
1.2	51.19	41.78	43.49	32.70	20.03
1.5	56.50	53.25	52.22	42.29	33.90

Standard: Vitamin C was used as a standard at concentration of 47.87 µg/ml.

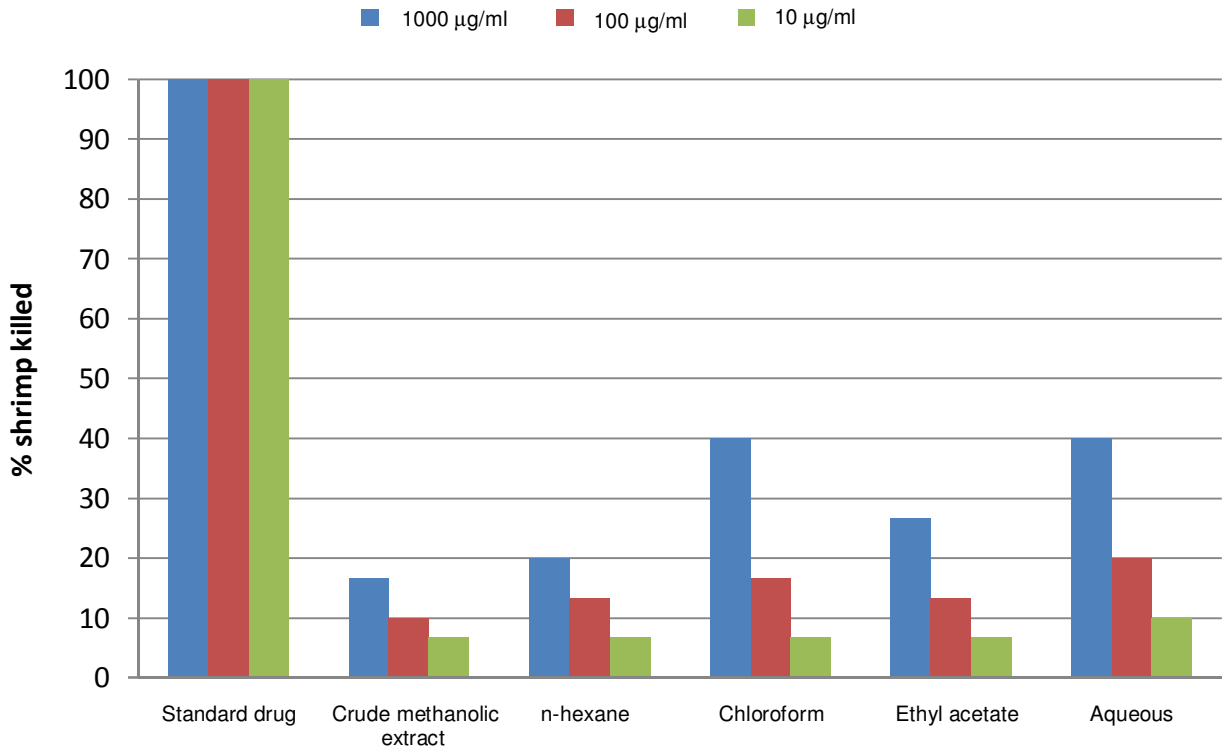

Figure 6. Brine shrimp cytotoxicity of crude methanolic extract and various fractions of *Acacia modesta*. *Etoposide was used as a standard drug.

this plant can be searched for free radical scavenging compounds. To check the cytotoxic effect of the crude methanolic extract and various fractions of *A. modesta*, brine shrimp (*Artemia salina*) lethality bioassay was employed (Farrukh et al., 2009). The results of the assay are mentioned in (Figure 6). The results indicated that the crude methanolic extract showed low cytotoxicity (16.66%) at 1000 µg/ml. The LD_{50} value was 4251653.0. The upper and lower limits were 0.000 and 5791.19, respectively while the G value was 2.6687. The *n*-hexane fraction showed a toxicity of 20% at 1000 µg/ml and 13.33 and 6.66% at the concentration of 100 and 10 µg/ml. The LD_{50} value recorded was 377166.8. The values of the upper limit, lower limit and G were 377166.8, 3362.08 and 1.8072, respectively for this fraction. The $CHCl_3$ fraction showed lethality of 40% at 1000 µg/ml. It showed lethality of 16.66 and 6.66% at 100 and 10 µg/ml. Upper and lower limit values were 486675.7 and 675.38. The G value for the $CHCl_3$ fraction was 0.4205. The EtOAc fraction showed 26.66, 133.33 and 6.66% mortality rate at 1000, 100 and 10 µg/ml respectively. The G value was 0.8962, while the upper and lower limits were 1.701412 and 1746.599, respectively. The results of the aqueous fraction revealed 40% mortality rate at 1000 µg/ml; 20% at 100 µg/ml and 10% at the concentration of 10 µg/ml. The upper and

lower limits recorded were 236150 and 636.32 respectively. The G value was 0.5449.

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