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Research Article
Toxicity Assessment of Different Solvent Extracts of the Medicinal Plant, *Phragmanthera capitata* (Sprengel) Balle on Brine Shrimp (*Artemia salina*)

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Abstract

Background: There has been a surge in the use of medicinal plants in the past decades; hence, the screening of potential cytotoxic compounds is of utmost importance to guarantee their safe use in alternative medicine. Different solvent extracts of the leaves of *Phragmanthera capitata* (Sprengel) Balle, a parasitic mistletoe growing on rubber tree were evaluated for possible toxicity. **Methodology:** Brine shrimp (*Artemia salina*) hatchability (based on the Minimum Inhibitory Concentration (MIC_{50}) of the extracts to inhibit 50% hatching of the cysts) and lethality (based on the Lethal Concentration (LC_{50}) of the extracts to kill 50% of the hatched cysts (nauplii)) assays were employed to evaluate the possible toxicity of the species. **Results:** Lowest hatching percentage was recorded in the ethanolic extract (34.40%) with an MIC_{50} value of 0.14 mg mL^{-1} and the highest hatching success was observed in the aqueous extract (59.33%) with an MIC_{50} value of 0.59 mg mL^{-1}. All the extracts hatching success were significantly higher than the positive control (Amoxicillin) ($p<0.05$). The LC_{50} for the lethality assay in all the solvent extracts was greater than 1 mg mL^{-1}. **Conclusion:** Based on Meyer's toxicity index, $LC_{50}>1$ mg mL^{-1} were considered non-toxic hence, all the solvent extracts tested showed that they were not toxic and can be further explored for the development of plant-based pharmaceuticals drugs. Further *in vivo* and cell lines cytotoxicity test is recommended to substantiate these findings.

Key words: *Phragmanthera capitata*, toxicity, brine shrimp, hatchability, lethality, *Artemia salina*, nauplii, cyst, extracts

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There has been a surge in public interest in herbal therapies both in developing and developed countries with herbal remedies being available not only in drug stores, but now also in food stores and supermarkets. It is estimated that upto four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare. Medicinal plants have provided a source of inspiration for novel therapeutic drugs, as plant derived medicines have immensely contributed to the health and well being of humans[1]. The medicinal value of plants is chiefly due to the phytochemicals like alkaloids, essential oils, tannins, resins and many others that are present in them which produce a physiological action on the human body[2].

In spite of the positive perception of patients on the use of herbal medicines, their alleged satisfaction with therapeutic outcomes as well as their disappointment with conventional allopathic or orthodox medicines in terms of effectiveness and/or safety, the problem of safety of herbal remedies continues to remain a major issue of concern. The general perception that herbal remedies or drugs are very safe and devoid of adverse effects is not only untrue, but also misleading. Herbs have been reported to produce a wide range of undesirable or adverse reactions some of which could cause serious injuries, abortion of pregnancy, dizziness, vomiting, diarrhoea, abdominal pain, fast heart beat, ulcer, loss of appetite, life-threatening conditions and even death[1].

Much of the information on medicinal herbs made available to consumers is not backed by credible scientific data. For this reason, study is carried out to determine the toxicity of medicinal plants[3] and in recommendation from the World Health Organization (WHO)[4], in order to reduce adverse effects from the consumption of herbal medicines, there is need for a thorough scientific validation on the toxicity of these plants.

There had been a number of toxicity tests in which the responses have been measured in invertebrates. These tests have the advantages of being cost effective, reproducible, easy to experiment and environmentally relevant[5]. A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in plant crude extracts is the brine shrimp (*Artemia salina*) lethality assay. Brine shrimp is used as an indicator for the detection of anti-tumor, fungi toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticidal compounds and cytotoxicity of dental materials[2,6]. This assay has also been noted as a useful tool for the isolation of bioactive compounds from plants extracts[7]. Most study is based on Brine Shrimp Lethality Assay (BSLA) on the hatched cysts (nauplii) for toxicity analysis of natural products but a few other researchers have used the assay on the inhibition of hatching of the cysts[5,6,8].

Phragmanthera capitata (Spreng) Balle is an obligate hemi-parasitic plant which attaches to and penetrates the stems and branches of its host tree or shrub by a structure called the haustorium. It is a mistletoe in the Loranthaceae family. It is a woody parasitic shrub with pendent branches of about 2 m long. It has a yellow corolla with a pink-red tip and often associated with ant's nests. It grows on trees in secondary jungles, plantations and bush savannah areas; from Sierra Leone to Western Cameroons, Fernando Po and extending across the Congo basin to Zaïre, Nigeria, Gabon, Ivory Coast and Angola[9,10]. As with most mistletoe, it is a medicinal plant utilized in the treatment of a wide range of ailments across Africa and the world at large. The leaves, twig (stem) and/or combination of both parts have been used in different preparations and doses in folklore medicine to treat diverse ailments ranging from insomnia, diabetes, hypertension, infertility, gastrointestinal disorders, anxiety, bacteria/fungi infections, arthritis, epilepsy, cancer, etc.[10,11]. However, based on literatures, there had been no report on the possible toxicity of *P. capitata* despite its numerous folkloric applications.

From the foregoing therefore, the objectives of this study was to evaluate the potential toxicity of the acetone, methanol, ethanol and aqueous extracts of *P. capitata* using the brine shrimp hatchability and lethality assays (BSH and BSLA) in order to ascertain/validate its numerous ethno-pharmacological safe use and provide data base for the preliminary toxicity of *P. capitata* growing on rubber tree.

MATERIALS AND METHODS

Location and collection of sample: Leaves of mistletoe were collected from mature rubber plantations in Rubber Research Institute of Nigeria, Iyanomo, located on latitude 6°00'-6°15' N; longitude 5°30'-5°45'E and on altitude 27 ma.s.l., in Benin City, which lies on the wet lowland rainforest of Edo State, Nigeria.

Extraction procedure: Leaves were removed from the twigs, gently rinsed to remove dust and dirt, air-dried at room temperature (mean morning and night temperature of 24°C and mean noon temperature of 27°C) in a well aerated atmosphere and prevented from direct sunlight to avoid denaturation of vital phyto-constituents. Dried leaves were pulverized. The ground sample was put into separate conical

flasks containing acetone, methanol, ethanol and water, shaken in an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 24 h. The crude extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper. The acetone, methanol and ethanol extracts were further concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator (Strike 202 Steroglass, Italy) while the aqueous filtrate obtained was concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY). The acetone, methanol and ethanol extracts were reconstituted in their parent solvents to yield a 100 mg mL^{-1} stock solution while the aqueous extract was directly prepared in filtered sea water.

Preparation of the assay: The method described by Kibiti and Afolayan[12] was employed with little modifications. Five petri dishes containing 30 mL of the extracts were prepared in filtered sea water by first dissolving them in an infinitesimal amount of the parent solvents to yield a two-fold dilution series of concentrations (1, 0.5, 0.25, 0.125 and 0.0625 mg mL^{-1}). A positive control was also prepared by dissolving amoxicillin in sea water in the same concentrations as the plant extracts. Sea water only served as the negative control. The set up was allowed to stand for 30 min to allow the solvents to evaporate.

***Artemia salina* hatching assay:** This assay was evaluated as described by Kibiti and Afolayan[12]. A density of ten *A. salina* cysts was stocked in each of the petri dishes containing 30 mL of the prepared two-fold concentrations (1-0.0625 mg mL^{-1}) of the plant fractions and positive control. The petri dishes were partly covered, incubated at 30°C and under constant illumination for 72 h. The number of free nauplii in each petri dish was counted after every 12 h till end of 72 h. The percentage of hatchability was assessed by comparing the number of hatched nauplii with the total number of cysts stocked.

***Artemia salina* lethality assay:** *Artemia salina* cysts were hatched in sea water and 10 nauplii were pipetted into each petri dish containing the two-fold concentrations of the extracts and control as in the hatchability above. The petri dishes were then examined and the number of living nauplii (that exhibited movement during several seconds of observation) was counted after every 12 h and the set up was allowed to stand for 72 h under constant illumination. The percentage of mortality was calculated as:

$$\text{Mortality (\%)} = \frac{\text{Total nauplii-Alive nauplii}}{\text{Total nauplii}} \times 100$$

Data analysis: The percentage hatchability success and mortality data obtained from the 5 different concentrations of each fraction and control experiments were used to construct the dose-response curves. These were used to determine their corresponding MIC_{50} and LC_{50} values. The minimum inhibitory concentration 50 (MIC_{50}) was determined as the concentration of the plant extract/control drug that inhibited hatching of 50% of the cysts. The LC_{50} was taken as the concentration required for producing 50% mortality of the nauplii. The MIC_{50} and LC_{50} values were determined from the best-fit line obtained by regression analysis of the percentage hatchability and lethality versus the concentration. The statistical analysis was done on GENSTAT 8. A two-way analysis of variance (ANOVA) followed by Fischer's least significant different (for means separation) was used to test the effect of concentration and time of exposure of the plant extracts on the hatchability success of the cysts and mortality of the larvae, respectively.

RESULTS

Brine shrimp hatchability assay: The hatching success of *A. salina* incubated with different plant extracts and control is as shown in Fig. 1 with the aqueous extract having a significantly higher hatching success (59.33%) than the rest of the solvent extracts including both the positive control (amoxicillin) (23.47%) and sea water (46.67%) ($p<0.05$). The hatching success of the cysts in the acetone (37.87%) and methanol (35.47%) extracts showed non-significant difference from each other but the acetonic extract was significantly higher than the ethanolic extract (34.40%) which had non-significant difference from the methanol extract ($p>0.05$).

The effect of different solvent concentrations on the hatching success of the cyst was also evaluated and the result is depicted in Fig. 2a and b. Figure 2a shows the activities of the different plant extracts/positive control at varying concentrations to the hatching success of the cysts. The percentage hatching success of cysts incubated with the acetone extract showed significant differences at varying concentrations. The lowest concentration (0.0625 mg mL^{-1}) had the highest hatching percentage (67.33%) and it was not significantly different from the cysts incubated at 0.25 mg mL^{-1} with a hatching success of 59.33%. There was zero percent (0%) hatchability at the highest

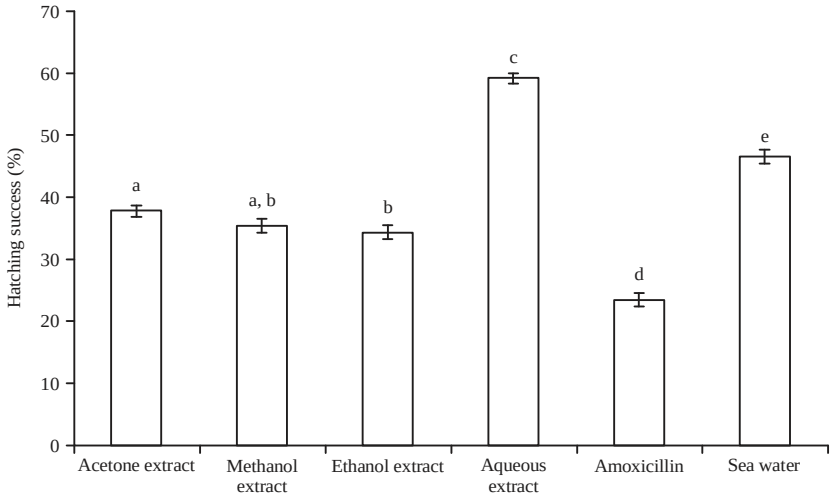

Fig. 1: Percentage hatching success of *Artemia salina* cysts incubated in different solvent extracts and controls. The values are means of five concentrations for each plant extract/control±SD of three replicates. Bars with different letters are significantly different ($p<0.05$)

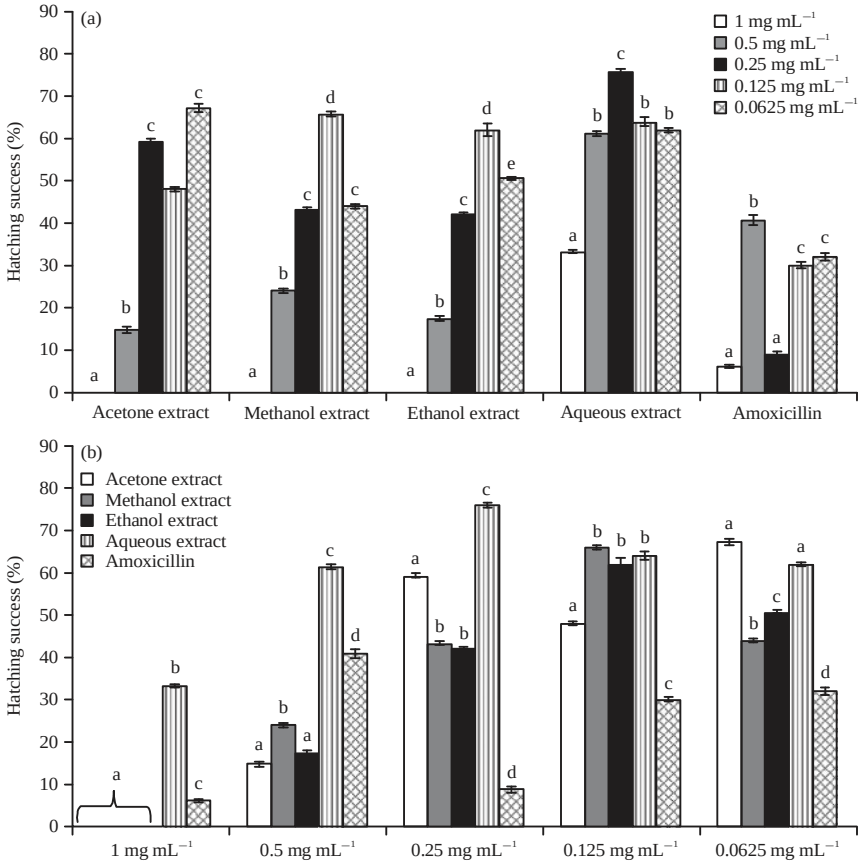

Fig. 2(a-b): Percentage hatching success of *Artemia salina* cysts incubated in different concentrations of the plant extracts and control. The values are means of the replicates (at different hours) for the concentrations for each plant extract/control±SD of three replicates. Set of bars with different letters is significantly different ($p<0.05$), (a) Set of bars represents the effect of the solvent extracts/positive control at varying concentrations on the hatching success of the cysts and (b) Set of bars represents the effect of the solvent extracts/positive control to a particular concentration on the hatching success of the cysts

concentration of 1 mg mL^{-1}. The methanol, ethanol and aqueous extracts had significant higher hatching percentage of the cysts at 0.125 mg mL^{-1} (66, 62 and 64%, respectively). There was also 0% hatchability at the highest concentration of 1 mg mL^{-1} in the methanol and ethanolic extracts. While, there was non-significant difference at 0.25 and 0.0625 mg mL^{-1} in the methanolic extract but the same was not true in the ethanolic extract that showed significant difference at all concentrations tested ($p<0.05$). The aqueous extract had non-significant difference at 0.5, 0.125 and 0.0625 mg mL^{-1}. There was a dose-dependent response in the positive control with an anomalous significantly higher hatchability at 0.5 mg mL^{-1}.

Figure 2b is an expression of the percentage hatchability of the solvent extracts in response to particular concentrations. At 1 mg mL^{-1}, acetone, methanol and ethanol extracts had 0% hatchability while the aqueous extract had a significant higher percentage hatchability of 33.33%, all the extracts were significantly different from the positive control. The aqueous extract also had significant higher hatching success (61.33 and 76%, respectively) at 0.5 and 0.25 mg mL^{-1}. There was non-significant hatchability success between acetone and ethanol extracts at 0.5 mg mL^{-1} while at 0.25 mg mL^{-1}, methanol and ethanol was not different significantly from each other. At 0.125 mg mL^{-1}, methanol, ethanol and aqueous extracts showed non-significant difference and at 0.0625 mg mL^{-1}, acetone and aqueous extracts were not significantly different from each other ($p<0.05$).

The effect of exposure time on the hatching success on *A. salina* is shown in Fig. 3a and b. Figure 3a showed the response of the cysts in each solvent extract to varying time of exposure. The same trend was observed in all the extracts and controls tested. There was a lower significant hatching success at 24 h in all the extracts and controls. There were no

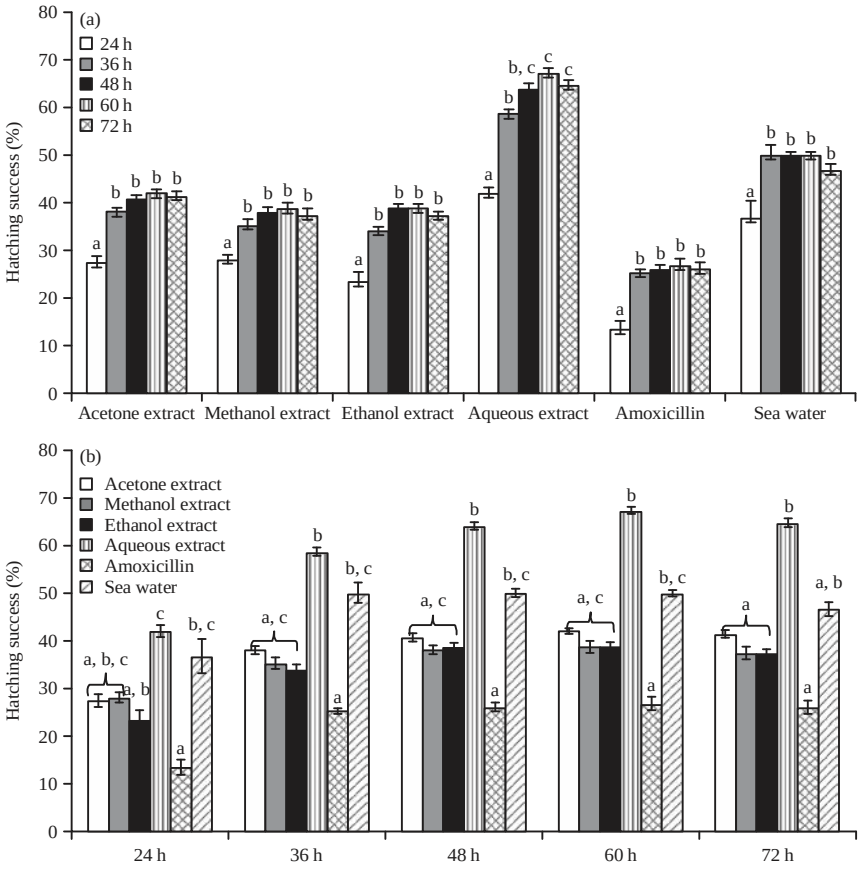

Fig. 3(a-b): Percentage hatching success of *Artemia salina* cysts incubated at different durations in the plant extracts/controls. The values are means of replicates (of all the concentrations) for each plant extract/control±SD of three replicates. Set of bars with different letters are significantly different ($p<0.05$), (a) Set of bars represents the effect of the solvent extracts/positive control at varying time of exposure on the hatching success of the cysts and (b) Set of bars represents the effect of the solvent extracts/positive control to a particular time of exposure on the hatching success of the cysts

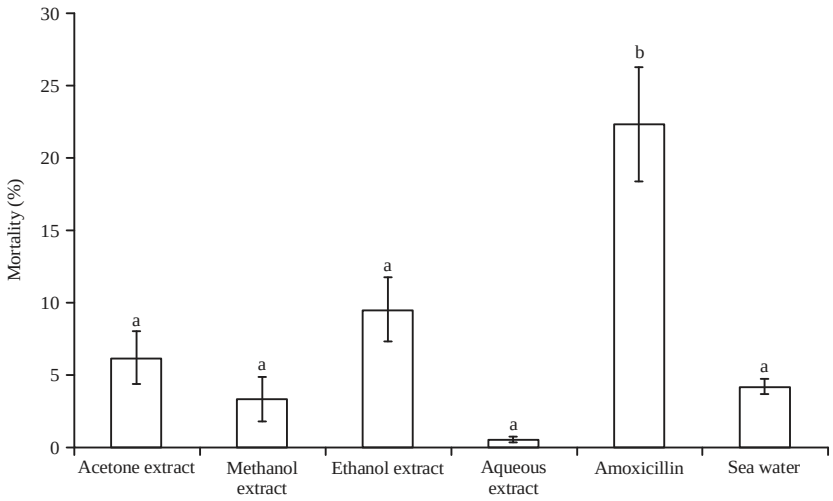

Fig. 4: Percentage mortality of *Artemia salina* nauplii incubated in different plant extracts and controls. Means are values of five concentrations for each plant fraction/control±SD of three replicates. Bars with different letters are significantly different ($p<0.05$)

Table 1: Hatchability and Lethality of *Artemia salina* in different plant extracts as shown by their MIC_{50} and LC_{50} values, respectively

	Hatchability assay		Lethality assay	
	MIC_{50} (mg mL^{-1})	R^2	LC_{50} (mg mL^{-1})	R^2
Acetone extract	0.15	0.8314	>1	0.9398
Methanol extract	0.21	0.9985	>1	0.9398
Ethanol extract	0.14	0.8995	>1	0.9778
Aqueous extract	0.59	0.8325	>1	0.8068
Amoxicillin	<0.0625	0.7971	0.89	0.9604

MIC_{50} and LC_{50} are defined as the concentration (mg mL^{-1}) of the plant extracts and positive control (Amoxicillin) sufficient to obtain 50% of hatching inhibition of the cysts and nauplii mortality of *Artemia salina*, respectively. The R^2 is the coefficient of determination from the regression equation

significant differences in hatching success from 36-72 h in most of the extracts ($p>0.05$) and controls except in the aqueous extract where there was a lower significant difference at 36 h from both 60 and 72 h ($p<0.05$).

In Fig. 3b, the response of the cyst in different solvent extract to a particular time exposure was shown. The aqueous extract had a higher hatching success at all levels of exposure but was not significantly different from sea water at all levels of exposure ($p>0.05$). The acetone, methanol and ethanol extracts showed non-significant difference from one another at all levels of exposure ($p>0.05$). Though the test drug had the lowest hatching success in all the levels of exposure time, the same was not significantly different from the acetone, methanol and ethanol extracts ($p>0.05$). The test drug was significantly lower than the aqueous extract and sea water at all the levels of exposure to time ($p<0.05$) except at 72 h where it was not significantly different from the sea water hatching success ($p>0.05$).

The inhibitory effects of the different solvent extracts and positive control on the hatchability success were expressed as MIC_{50} (Table 1) which represents the potential of the extracts to inhibit hatching of the cysts by 50% (that is 50% hatching success). The positive control exhibited more potent inhibitory activity (76.53%) with a MIC_{50} value of <0.0625 mg mL^{-1} while the extracts had inhibitory effect in the other: Ethanol>acetone>methanol>aqueous.

Brine shrimp lethality assay (BSLA): The percentage lethality/mortality of *A. salina* larvae (nauplii) incubated in different solvent extracts of *P. capitata* and controls are shown in Fig. 4. There was a significantly higher mortality percentage (22.33%) of the nauplii incubated with the test drug than the extracts and sea water ($p<0.05$). Although, there was non-significant difference ($p>0.05$) between the extracts and the sea water, the aqueous extract had the least mortality of 0.50% while the ethanolic extract had the highest mortality of 9.50%.

The effect of varying concentrations of the plant fractions on the mortality of larvae is shown in Fig. 5a and b. The degree of mortality of nauplii was in a concentration dependent fashion. The highest mortality was observed in all the extracts at 1 mg mL^{-1} while the control had a maximum mortality (100%) at 1 mg mL^{-1}. There was 0% mortality of the nauplii at concentrations of 0.0625-0.5 mg mL^{-1} in the methanolic and aqueous extracts. There was also 0% mortality at concentrations of 0.125 and 0.0625 mg mL^{-1} in the acetone extract while the ethanolic extract had 0% only at 0.0625 mg mL^{-1} (Fig. 5a).

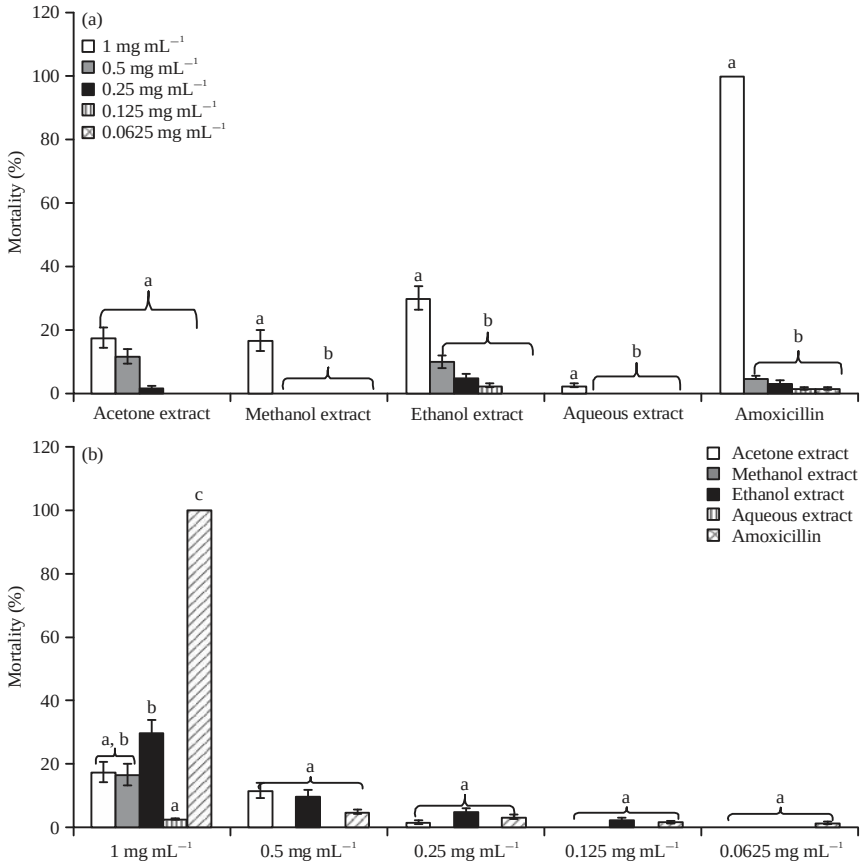

Fig. 5(a-b): Percentage mortality of *Artemia salina* cysts incubated in different concentrations of the plant extracts and control. The values are means of the replicates (at different hours) for the concentrations for each plant extract/control±SD of three replicates. Set of bars with different letters are significantly different ($p<0.05$), (a) Set of bars represents the effect of the solvent extracts/positive control at varying concentrations on the mortality of the nauplii and (b) Set of bars represents the effect of the solvent extracts/positive control to a particular concentration on the mortality of the nauplii

Figure 5b illustrates the relationship of the solvent extracts and control in respect of a particular concentration. At 1 mg mL^{-1}, the positive control had a higher significant mortality (100%) from the extracts ($p<0.05$). Though the ethanolic extract had the highest mortality of the nauplii (30%) but it was not significantly different from the acetone (6.17%) and methanolic (3.33%) extracts ($p>0.05$) but significantly higher than the aqueous extract (2.5%) ($p<0.05$). At 0.5-0.0625 mg mL^{-1}, all the plant extracts and control had no significant difference from one another ($p>0.05$).

The percentage mortality due to exposure time is captured in Fig. 6a and b. The result showed that the percentage mortality was time dependent as the longer the nauplii were exposed to the plant extracts, the higher the mortality (Fig. 6a). Exposure of the nauplii from 12-48 h in the acetone and methanol extracts showed no mortality but further exposure gave a non-significant mortality at 60 h and significant mortality at 72 h (Fig. 6a). Significant mortality in the ethanol extract was only recorded after 72 h while the aqueous extract had non-significant mortality although, ($p<0.05$). The test drugs showed a higher significant mortality of the nauplii at 12-60 h ($p<0.05$). The extracts gave non-significant different mortality from one another and sea water at 12-60 h ($p<0.05$) (Fig. 6b).

The expected dose/concentration expected to kill 50% nauplii was calculated and presented in Table 1. The test drug had the highest lethal dose of 0.89 mg mL^{-1} while, all the plant extracts had lethal doses >1 mg mL^{-1}.

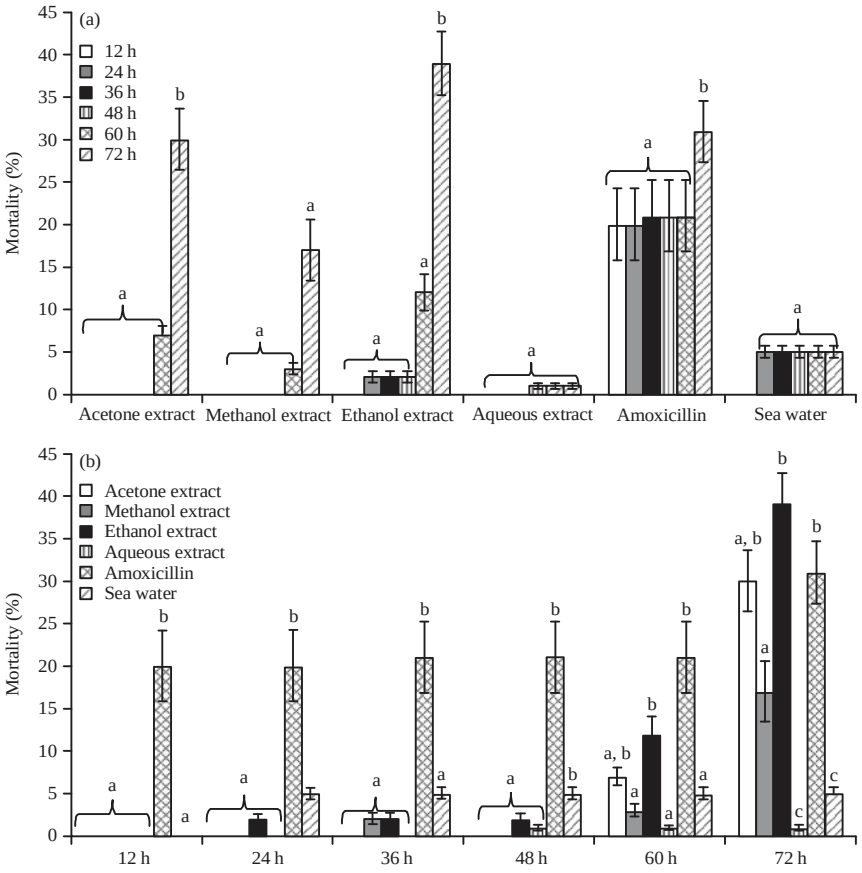

Fig. 6(a-b): Percentage mortality of *Artemia salina* cysts incubated in different time durations in the plant extracts/controls. The values are means of replicates (of all the concentrations) for each plant extract/control±SD. Set of bars with different letters are significantly different ($p<0.05$), (a) Set of bars represents the effect of the solvent extracts/positive control at varying time of exposure on the mortality of the nauplii and (b) Set of bars represents the effect of the solvent extracts/positive control to a particular time of exposure on the mortality of the nauplii

DISCUSSION

For the past 30 years, *A. salina* (brine shrimp) nauplii have been used as the first line of preliminary evaluation of the general toxicity of herbal remedies[6]. In this study, the toxicity of *P. capitata* was evaluated using both the inhibition of hatching of the cysts and mortality of the hatched cysts (nauplii) in different concentrations of plant extracts and controls. The hatching success of *A. salina* cysts incubated with aqueous extracts had the highest hatching success which means it had the least inhibitory hatching activity (40.67%) and presumably less toxic than the rest solvent extracts (Fig. 1). This could explain why most traditional herbal medicines are prepared using water as a solvent because it is not or less toxic[8].

There was an increase in hatching success of the cysts incubated in the plant extracts as the concentration decreases, but a maximum hatching success was achieved at 0.25 mg mL^{-1} and a further decrease in concentration resulted in decrease in hatching success (Fig. 2a, b). *Artemia salina* has a resistant cyst stage which is tolerant of a wide range of salinity from saturated saline to almost fresh water[5,13] and until the dormancy is broken, hatching will not occur hence, at 0.25 mg mL^{-1}, the plant extracts exhibited an optimum breaking of the dormancy of the cysts and further increase or decrease in the concentration exhibited an inhibitory action on the cysts. From the results, it is observed that the extracts at 1 mg mL^{-1} exhibited a toxic/inhibitory effect therefore preventing the cysts from hatching.

The hatching success of the cysts in response to exposure time as shown in Fig. 3a suggests that after 36 h of incubation in the various plant extracts, no further significant hatching success was observed ($p<0.05$) suggesting that 36-48 h is the best hatching time for brine shrimp which is also in agreement with the reports of Meyer *et al.*[14]. There was a fairly low hatching success of the plant extracts on the cysts except for

the aqueous extract which had a hatching success of over 50% at 36 h and above. The low hatchability success observed with this plant species could be attributed to the presence of chemical metabolites which probably may cause the eggs to further encyst in response to the chemical toxins.

According to Otang *et al.*[5] the resistance of the brine shrimp cysts to unfavourable environmental conditions makes the hatchability assay less desirable hence the lethality assay is a more appropriate test for the preliminary screening of herbal toxicity because it has the advantage of circumventing the toxin tolerance of the cyst stage as the nauplii that are very sensitive to toxins are used[13].

In accordance to Meyer *et al.*[14] and Bastos *et al.*[15] with respect to brine shrimp lethality test, the criterion of toxicity for plant remedies is as follows; the plant extract showing LC_{50} values greater than 1000 μg mL^{-1} (1 mg mL^{-1}) are considered non-toxic, LC_{50} values equal/greater than 500 μg mL^{-1} (0.5 mg mL^{-1}) but not greater than 1000 μg mL^{-1} are considered to have weak toxicity while those having LC_{50} values less than 500 μg mL^{-1} are considered toxic. The BSLA result of all the solvent extracts of *P. capitata* leaf showed that the extracts were not toxic with LC_{50}>1 mg mL^{-1} (Table 1) hence, these extracts may be considered safe for consumption as a herbal medicine. On the other hand, this non-toxic result could be discouraging as an alternative for the treatment and management of cancer/tumor, as brine shrimp lethality test is usually an indicator for the preliminary screening of bioactivity including for anticancer[16]. This result is in agreement with the results obtained from Indonesian mistletoe, *Dendrophthoe pentandra*, growing on different host plants and *Macrosolen cochinchinensis* growing on *Artocarpus heterophyllus*[16] which had LC_{50} values >1000 μg mL^{-1}.

There was a proportionate relationship of the concentration to the degree of lethality of the nauplii (Fig. 5a, b). The degree of mortality increased with increase in concentration as the maximum mortality of 17.5, 16.7, 30 and 2.5% occurred at the highest concentration of 1 mg mL^{-1} incubations of acetone, ethanol, ethanol and aqueous extracts, respectively while the test drug had a 100% mortality of the nauplii. Conversely, least mortality of 0% was observed in all the solvent extracts at the least concentration of 0.0625 mg mL^{-1} (Fig. 5a, b). The percentage mortality of the nauplii in all the extracts at concentration range from 0.0625-0.5 mg mL^{-1} was non-significant ($p>0.05$) except at 1 mg mL^{-1} where there was significance between the aqueous extract (2.5%) and the ethanolic extract (30%) (Fig. 5b). The administration of different concentration range was to set a baseline between safe and lethal limits in order to prevent the effect of acute overdose in future *in vivo* trials as simple zoological invertebrates are used for convenient toxicological screening system of medicinal plants[5].

The effect of the plant extracts on the nauplii over duration of time was done to ascertain the maximum sensitivity of nauplii on the toxic metabolites/chemical compounds present in the different solvent extracts. Mortality of the nauplii was first observed at 60 h of exposure to the extracts and an exponential significant lethality at 72 h ($p<0.05$) in the extracts except for the aqueous extract. Lewis[17] sited by Carballo *et al.*[6] reported that maximum sensitivity of the nauplii to test compounds is reached at the second and third instar stages and this is after 48 h of incubation, this is in agreement with this present findings that mortality was hardly noticed before 48 h in the plant extracts. According to Otang *et al.*[5], toxic effects of plant toxins can be delayed and thus suggesting that long exposure time is advisable for the evaluation of toxicological risks of plant extracts with brine shrimp lethality assay. The delayed mortality effect observed at 72 h suggests that the different plant extracts may have some nutritive values that may have acted as food and also the presence of less toxic compounds to brine shrimp.

CONCLUSION

This study showed that the different solvent extracts of *P. capitata* growing on rubber tree are not toxic (LC_{50}>1 mg mL^{-1}) in the brine shrimp lethality assay hence, it may be considered safe for use in traditional/alternative medicine. However, further *in vivo*, *in vitro* and cancer cell lines toxicity tests are required to further substantiate these claims and also to ascertain if it has anticancer potentials because there had been a report on *Macrosolen cochinchinensis* (a mistletoe) which had no toxicity on brine shrimp but showed cytotoxicity in cell lines. The indication being that the cytotoxic compounds in the extracts might be selectively toxic on cancer cells and hopefully less toxic to normal cells[16].

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