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Letter to the Editor

Brine shrimp cytotoxicity potential of alpha-mangostin and dulxanthone D from *Garcinia mangostana*

Sir,

Garcinia mangostana L (Guttiferae family) is a tropical evergreen tree mainly distributed in Southeast Asia, India and Sri Lanka. Fruit is widely known as "queen of fruits" for its sweetness and juiciness as well as its importance in enhancing person's health and often used in traditional medicines for treatment of abdominal pain, dysentery, chronic diarrhea, suppuration, skin infection, wound, leucorrhoea, chronic ulcer and gonorrhoea (Sasikumar and Ghosh, 2017). Rind of mangosteen was widely used as a 'Svayathuhara' in Ayurvedic medicine for inflammatory diseases and was documented in the classification of herbs and foods in Ayurveda comprehensive text called "Charaka Samhita" (Mohan et al., 2018).

Brine shrimp lethality bioassay is a simple, rapid, inexpensive and high throughput cytotoxicity test of bioactive metabolites based on the killing ability of brine shrimp (*Artemia salina*). This bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts and is a preliminary toxicity screen for further experiment on mammalian animal model (Sarah, 2017).

G. mangostana were purchased from Ooty in Nilgiris District, Tamil Nadu, India. The pulverized mangosteen pericarp (50 g) was macerated in methanol and the extract was collected, dried with rotator evaporator. Chloroform soluble fraction obtained from methanol extract was dried and subjected for modified solvent-solvent fractionation which led to obtain two metabolites 1 (α -mangostin) and 2 (dulxanthone D) chloroform residue obtained (Sasikumar and Ghosh, 2017).

Xanthone metabolites 1 and 2 were evaluated for the cytotoxicity potential using brine shrimp bioassay. Fresh cysts were produced from Marina Labs, Chennai, India. The cysts were hatched in a hatching tank containing artificial seawater made through dissolving a commercial marine salt (2%) in RO water (mineral water). The tank was well aerated with the aid of an air pump and proper light source (1000-4000 lux). The nauplii were hatched within 24-36 hours at 30-35°C

(Caldwell et al., 2003). The toxicity of prenylated compounds were tested at various concentrations viz. 2, 4, 6, 8 and 10 mg/mL. Ten brine shrimps were introduced into each container containing 4 mL of the artificial seawater. The sample (1.0 mL) was added to each containers and final volume was 5 mL per container. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours. The percentage mortality was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100 (Apu et al., 2010).

The results revealed, percentage of death of larvae increased in a dose-dependent manner and highest mortality found in alpha-mangostin is 100% whereas in dulxanthone D showed 60% at 2 mg/mL respectively (Table I). The control samples with solvents (seawater and DMSO) did not yield significant brine shrimp mortality. Previously reported cytotoxicity was

Table I

Brine shrimp cytotoxicity assay of xanthone metabolites		
Concentration (mg/mL)	Percentage of mortality	
	Alpha-mangostin	Standard
2	100	60
4	100	80
6	100	100
8	100	100
10	100	100

depended on the concentration of alpha mangostin in the solvent extract of *G. mangostana* using solvent, hexane ($LC_{50} = 30 \mu\text{g/mL}$) and methanol ($LC_{50} = 72 \mu\text{g/mL}$). However, water extract did not show any adverse effect on viability (Manasathien, 2017). α -Mangostin also showed strong cytotoxic effect in human keratinocyte cells ($LC_{50} = 0.94 \mu\text{g/mL}$). In another report, the methanolic and ethyl acetate extract of α -mangosteen pericarp exhibited significant brine shrimp cytotoxicity with LC_{50} values of 153.88 and 161.75 $\mu\text{g/mL}$ respectively after 24 hours (Ngawhirunpat et al., 2010). The dichloromethane fraction of mangosteen displayed a high toxicity ($IC_{50} = 24.89 \mu\text{g/mL}$), whereas the ethyl acetate fraction displayed a lower cytotoxicity ($IC_{50} =$



129.0 µg/mL). The compounds with IC₅₀ <100 µg/mL in the brine shrimp lethality assay are considered active and highly potential cytotoxic against tumor cell lines (McLaughlin, 1991).

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