

Full Length Research Paper

# Phytochemical screening and toxicity profiles of crude extracts of *Cissus quadrangularis* L. and *Solunum incanum* L. in mice

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Plant derived bioactive molecules are always in demand and are the main focus of research. Despite the growing market demand for herbal medicines, safety of medicinal plants is still a concern. The present work aimed at generating preliminary phytochemical and biosafety information of crude methanolic and chloroform extracts of *Cissus quadrangularis* L. (aerial parts) and *Solunum incanum* L. (fruit). Both plants are extensively used by traditional healers among the agro-pastoralist communities of Fafan Zone in Eastern Ethiopia. The investigation employed standard phytochemical screening procedures and acute (2000mg/kg single dose) and sub-acute (200 and 400mg/kg repeated doses) oral toxicity studies in Swiss albino mice. Changes in body weight, packed cell volume (PCV), Hb level and mortality were recorded to evaluate the toxicity profile of the crude extracts. The phytochemical study revealed the presence of several secondary metabolites in both plants. The acute toxicity study did not show extracts related mortality and body weight reduction at 2000 mg/kg of methanol and chloroform extracts of both plants. However, the sub-acute toxicity study exhibited that crude extract of fruit of *S. incanum*(400 mg/kg) showed relatively higher toxic effects of causing a more pronounced ( $p<0.05$ ) mortality, body weight loss, and reduction in PCV and Hb levels, compared to negative control. Extracts of *C. quadrangularis* revealed low mortality and a marginal increase of the hematological parameters. A detailed experimental analysis of these herbs extensively used by the agro-pastoralists of the area is essential to establish their therapeutic value and safety in use.

**Key words:** *Cissus quadrangularis*, *Solunum incanum*, phytochemical, toxicity.

## INTRODUCTION

Therapeutic use of plants dates back to human civilization and continuous efforts are being made

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towards its improvement. About 200,000 different types of natural products are known, which are plant origins. Moreover, many more are being identified from higher plants and microorganisms (Mosihuzzaman, 2012; Kinghorn et al., 2011). Some plant-based drugs have been used for centuries and for some such as cardiac glycosides, there is no alternative conventional medicine. Therefore, medicinal plants and their bioactive molecules are always in demand and are the main focus of research. This led to the recent (WHO, 2011) surge in the demand for herbal medicine.

To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. The medicinal importance of a plant is due to the presence of some endogenous substances like alkaloids, glycosides, resins, volatile oils, gums, tannins, and others in one or more parts of the plant (Himesh et al., 2011). Despite the growing market demand for herbal remedies, there are still concerns associated with not only their use, but also their safety. The herbal products which are standardized to identify their bioactive metabolites is less than 10% of herbal products available in the world market. Moreover, strict quality control measures are not always meticulously followed (Cragg et al., 2005).

In Fafan zone of Ethiopian Somali Regional State, two well-known medicinal plants called *Cissus quadrangularis* and *Solanum incanum* (solanaceae) are extensively utilized by traditional healers of the locality (Feyera et al., 2017). *C. quadrangularis*, which belongs to Vitaceae family, is a succulent perennial plant commonly found in tropical and subtropical xeric wood (Kavitha and Manimekalai, 2015). The plant is known by a local name in Somali called „Gaad“ and traditionally indicated for the treatment of different human and livestock ailments in the area. In humans, it is traditionally claimed to be effective in the management of helminthiasis, anorexia, skin diseases, hemorrhage, swellings, anemia, burns and wounds whereas in livestock, it was reported to have been used traditionally to treat tick infestation, lice infestation, wounds, leach infestation, bites of poisonous insects, skin sores/saddle sores (donkeys and camels), retained placenta and helminthiasis (Njoroge and Bussman, 2006; Rao and Merugu, 2013; Bharti et al., 2014).

*S. incanum* locally called „kiriir“, similarly serves several medicinal values in the studied area against both livestock and human health problems. It has been reported to be used for the treatment of such health problems in livestock as tick, mange and lice infestations, ringworm infection, and hyena or jackal bites, non-specific wounds, to manage infertility, swollen joints, retained placenta and other reproductive purposes.

Different plant parts are also widely used in human for the treatment of skin problems, including skin infections, gonorrhoea, ringworm, burns, sores, rashes, wounds, bleeding and painful conditions (Tolossa et al., 2013;

Lulekal et al., 2008; Regassa, 2000; Sori et al., 2004).

However, despite the widespread usage of these herbs, there is gap of information regarding their phytochemical composition and safety profile. The present study was, thus, initiated in view of generating preliminary phytochemical and biosafety information of *C. quadrangularis* (aerial part) and *S. incanum* (fruit) extensively used by traditional healers among the agro-pastoralist communities of Fafan zone in Eastern Ethiopia.

## METHODOLOGY

### Plant extracts preparation

The fresh aerial part of *C. quadrangularis* and fruits of *S. incanum* were collected from their natural habitat, at the beginning of a rainy season in this specific livelihood zone (The zone geographically lies between 8° 44' N to 11° 00' N latitude and 40° 22' E to 44° 00' E longitude). Both plants were taxonomically identified at the National Herbarium, Department of Biology, College of Natural Sciences, Addis Ababa University. Herbarium specimen (collection number SA 001 for *C. quadrangularis* and SA 002 for *S. incanum*) was deposited. The plant materials were separately washed with distilled water, air dried, mechanically ground and coarsely pulverized using mortar and pestle. The powdered plant material were then subjected to cold maceration extraction using two solvents; methanol and chloroform separately, to obtain the crude methanolic and chloroform extracts, respectively. The selection of these solvents is based on their widespread use as a solvent for substances intended for human/animal contact and consumption, polarity, and to mimic the same or similar methods used by local people to obtain or prepare botanical products.

For the cold maceration technique, a similar step used by Tadesse et al. (2015) with slight modification was applied. A total of 250 g of the pulverized materials was soaked in each extraction solvents (1:10 ratio) followed by frequent agitation for three days and then filtered. The residue left after maceration was successively extracted twice. This is to make the solvents extract substantial quantities of the chemical constituents from the pounded plant materials. The resulting liquid was filtered using Whatman No. 3 filter paper (Whatman Ltd., England). Finally, the filtered extracts were dried in hot air oven at a temperature of 45 to 50°C. Labeled vials were used to keep the crude extract and stored in a refrigerator at 4°C until required for experimentation.

### Preliminary phytochemical screening

Chloroform and methanol extracts of the two plants were screened for the presence of active principles, such as alkaloids, anthraquinones, flavonoids, cardiac glycosides, polyphenols, saponins, phytosteroids, tannins, and terpenoids. A combination of several methods was used to identify the phytochemicals of the medicinal plants. Standard screening tests using conventional protocol, procedure and reagents, were conducted on both the methanolic and chloroform extracts using standard procedures to identify the constituents as stated by WHO (1978), Trease and Evans (1989) and Sofowara (1993).

### Toxicity studies

#### *Experimental animals*

For this biosafety test of the crude extracts, female Swiss albino

**Table 1.** Phytochemical constituents for the methanolic and chloroform crude extracts of aerial parts of *C. quadrangularis* and fruit of *S. incanum*.

Constituents	<i>C. quadrangularis</i>		<i>S. incanum</i>	
	Methanolic extract	Chloroform extract	Methanolic extract	Chloroform extract
Alkaloids	+	-	+	-
Flavonoids	+	-	+	-
Saponins	+	-	+	-
Tannins	-	-	+	-
Polyphenols	+	+	+	+
Terpenoids	-	+	-	+
Cardiac glycosides	+	-	+	+
Phytosteroids	-	+	-	+
Antraquinones	-	-	-	-

+, Present; -, absent.

mice (OECD, 2001), 8 to 12 weeks old and weighing 25 to 35 g, were obtained from the breeding colony of Akililu Lemma Institute of Pathobiology, Addis Ababa University. They were kept in clean polypropylene cages in a 12 h light/dark cycle with litter changed every week. The animals were provided with commercial pelleted ration (mice cubes) and clean water *ad libitum* and left under controlled conditions for one week to acclimatize before conducting any experimental procedure. The acute toxicity study was conducted in accordance with the guidelines on care and wellbeing of research animals (ILAR, 1996). The experimental procedure was approved by research and ethics committee of the Jigjiga University.

#### Acute oral toxicity study

Acute toxicity study protocols were done using the limit test dose of 2000 mg/kg (OECD, 2001). Six randomly selected female Swiss albino mice were used for each crude extract. For each extract, two groups of mice were required, one treatment and one control group. Before the administration of a single dose of the extract, the mice were fasted for 2 h (water allowed) (CDER, 1996; OECD, 2001). The treatment and the control groups respectively received a single dose of crude extract (2000 mg/kg) and normal saline (10 ml/kg). The mice were observed continuously for 1 h after administration of the extracts intermittently for 4 h, over a period of 24 h and for 14 days. During this period, the mice were observed for behavioral, neurological, autonomic or physical changes such as alertness, motor activity, restlessness, convulsions, hair erection, coma, diarrhea and lacrimation and other signs of toxicity manifestation (OECD, 2001). Body weight was monitored on days 0, 7 and 14.

#### Sub-acute oral toxicity test

The sub-acute toxicity study on plant extracts was performed as per the OECD guidelines 407 (OECD, 2008). The animals were divided into three groups. Groups 1 (n=6) and 2 (n=6) received extract doses of 200 and 400 mg/kg, respectively. The doses were selected based on the acute toxicity study finding. Group 3 (n=6) received 10 ml/kg body weight of normal saline and served as control. After extract treatments, mortality, food and water consumption as well as observation for any abnormal clinical signs of the animals were evaluated daily for 28 days, whereas body weight was recorded once in a week throughout the study period.

At the end of 14 days observation period, the animals were anaesthetized and their blood samples were collected through cardiac puncture.

#### Hematological parameters

All experimental animals were humanely sacrificed at the end of the experiment by diethyl ether in desiccators. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes. The blood samples were analyzed for haemoglobin (Hb) content using standard techniques (Dacie and Lewis, 1984). Packed cell volume (PCV) was determined using micro-haematocrit centrifuge and microhaematocrit tube reader according to Ekaidem et al. (2006).

#### Data analysis

For the toxicity studies, differences in haematological parameters and body weights for all treated and control mice were determined using a One-Way Analysis of Variance (ANOVA). A P values less than 0.05 were considered significant. All data were expressed as mean  $\pm$  standard error of the mean.

## RESULTS AND DISCUSSION

### Phytochemical screening

The methanolic and chloroform extract yields of the plant materials were 12.4 and 5.8% for *C. quadrangularis* and 11.6 and 4.2% for *S. incanum*, respectively. In both plants, better yields were obtained from methanolic extraction. The preliminary phytochemical screening of the plant materials showed the presences of different secondary metabolites which are of medicinal importance (Table 1). Alkaloids, flavonoids, tannins, polyphenols, and cardiac glycosides are present in the methanolic extracts of both plants. Chloroform extract of the plant materials exhibited positive result for terpenoids and phytosteroids

The active principles usually remain concentrated in the storage organs of the plants (Himesh et al., 2011).

Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in traditional practice (Das et al., 2010).

The preliminary phytochemical screening tests may be useful to identify bioactive principles present in the plant and encourages novel drug discovery and development from plant origin. These tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds as well (Usha and Karpagam, 2017). The phytochemical screening in the present study has revealed the presence of alkaloids, flavonoids, tannins, polyphenols, cardiac glycosides terpenoids, and phytosteroids in both herbs. Furthermore, fruit of *S. incanum* presented anthraquinones in its chloroform extract. The presence of these and other different phytoconstituents in the crude extracts of these medicinal plants may be responsible for their claimed therapeutic properties in human and veterinary diseases.

Plant phenolics (Flavonoids and tannins) are a major group of compounds that act as primary antioxidants. Flavonoids enhance the effects of vitamin C and function as a free radical scavenger. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina and Afanas'ev, 1997). Since these compounds were found to be present in the extracts, it might be responsible for the antioxidant capacity of both plants. Saponins also cause hemolysis of red blood cells (Winter et al., 1993).

### Toxicity studies

Only a few of the traditional herbal medicines have been verified by clinical trials. Moreover, their efficacy and safety are still questioned by users (Cheng et al., 2009). Exposure to plant based chemicals can be hazardous and results to adverse effects on human being and animals. Hence, evaluation of toxic properties of natural products is crucial when considering human and animal protection. In practice, the evaluation typically includes acute, sub-acute, sub-chronic, chronic, genotoxic and teratogenic effects (Asante-Duah, 2002). The present investigation assessed the safety profile of crude extracts of *C. quadrangularis* aerial part and *S. incanum* fruits using acute and sub-acute mice models.

### Acute oral toxicity study

The acute toxicity test indicated a similar safety profile in all the crude extracts tested. For *C. quadrangularis*, both the methanolic and chloroform crude extracts caused no visible signs of acute toxicity at the maximum dose

administered (2000 mg/kg). The test animals did not display any visible signs of acute toxicity such as lacrimation, restlessness, loss of appetite, tremors, hair erection, salivation, diarrhoea, convulsions and coma when compared with the control during the 14 days observation period. The mice were physically active and death was not observed.

For *S. incanum*, amongst the Swiss albino mice treated with methanolic fruit extract, there was no mortality or any signs of toxicity or side effects recorded. However, some of the animals which received its chloroform extract showed mild signs of toxicity. Toxicity signs observed in all the cases were initial excitement, restlessness, and difficulty in breathing, loss of appetite, general weakness and depression in the first 4 h. All these signs were reversed on the second day and the animals remained normal thereafter. With continues monitoring, none of the experimental animals were dead in the first 24 h and throughout the period of experiment.

Generally, test substance related mortality was not recorded at 2000 mg/kg. Therefore, the approximate medium acute toxicity lethal value (LD<sub>50</sub>) of experimental plants was determined to be higher than 2000 mg/kg and as such could be generally regarded as safe (GRAS). This finding is in concordance with those of Clarke and Clarke (1967), who reported that any compound or drug with oral LD<sub>50</sub> estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. Thus, it can be said that the crude extracts of both plants are not acutely toxic because there was no mortality recorded even at 2000 mg/kg bodyweight, thus indicating the safety of the extracts. This is in line with OECD guideline for testing of chemicals using Swiss albino mice (OECD, 2001).

Similarly, a study done by Ilavarasan et al. (2005) using methanolic bark extract of *Cassia fistula* showed that the plant did not cause any mortality up to 2000 mg/kg and was thus considered as safe. Another study done by Sangetha (2008) also exhibited similar results for a single dose (2000 mg/kg) administration of *Cassia spectabilis* leaf extracts that was revealed to be non-lethal to the tested mice. Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index of drugs and xenobiotics (Rang et al., 2001).

*In vivo* acute toxicity studies in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that a toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. However, these studies are not able to detect effects on vital functions like the cardiovascular, central nervous and respiratory systems which are not usually assessed during the study. Such effects of natural products should be evaluated prior to their therapeutic use (Syahmi et al., 2010). In principle, the limit test serves as a suggestion for classifying crude extracts based on the expected outcome at which dose

**Table 2.** Effect of administering 2000 mg/kg of *C. quadrangularis* and *S. incanum* methanolic and chloroform crude extracts on body weight of mice over a period of four weeks.

Group	Treatment	D <sub>0</sub>	D <sub>7</sub>	D <sub>14</sub>
<i>C. quadrangularis</i>	ME	26.00±0.82	26.75±0.66	27.00±0.82
	CE	26.91±1.01	26.75±0.87	28.00±0.82
<i>S. incanum</i>	ME	26.50±0.99	26.75±0.98	27.75±0.12
	CE	26.58±0.82	26.91±0.74	27.83±0.87
Control	Normal saline	26.75±0.85	27.33±0.96	28.50±0.92

Values are mean±SEM; n= 6; D, Day; D<sub>0</sub>, day treatment commenced; D<sub>7</sub>, 7th day after treatment; D<sub>14</sub>, 14th day after treatment; ME, methanol extract; CE: chloroform extract.

level the animals are able to survive (Jothy, 2011).

The effects of crude extracts on the percentage change in body weight of the control and treated mice are shown in Table 2. Normal body weight increment was observed in all the experimental animals without any strong difference between control and extract treated groups.

One of the indicators for drugs' toxic effect is change in body weight. The adverse effect will be significant if the body weight loss occurred in animals is more than 10% of their initial weight (Raza et al., 2002). During the 14 days of acute toxicity evaluation, all animals which were orally treated with crude extracts (methanol and chloroform) of both plants at single dose of 2000 mg/kg exhibited body weight increment and did not show significant changes in behavior. Apart from that, the physically observed features such as skin, fur and eyes were found to be normal. This indicates that the administration of single dose (2000 mg/kg) of the crude extracts had insignificant level of toxicity on the growth of the animals. Besides, evaluation of mice feeding and water consumption is important in the acute toxicity study of a product with therapeutic purpose (Iversen, 2003). In this study, the food intake and water consumption also was not affected by the administration of all extracts of the plant materials and none of the extracts induced appetite suppression and caused no deleterious effects. Thus, it can be speculated that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen, 2001).

### Sub-acute oral toxicity test

Administration of different sub-acute doses of crude extracts of both plants caused a variable loss in body weight of the treated mice with a more pronounced loss which was recorded at higher dose (400 mg/kg), while the control group gained weight after 28 days of experimental observation. Particularly, chloroform extract of *S. incanum* (400 mg/kg) and methanol extract of *C. quadrangularis* (400 mg/kg) caused a statistically significant ( $p<0.05$ ) body weight loss as compared to the

control group. Similarly, in mice administered with high doses (400 mg/kg) of *S. incanum* chloroform extract and *C. quadrangularis* methanolic extract, a statistically significant ( $p<0.05$ ) steady drop in the body weight was observed as against the control and pre-treatment value. The effects of the crude extracts on the change in mean body weight and mortality of the control and treated mice are shown in Table 3.

Phytochemicals may have a useful or harmful effect on animals. Tannins and anthraquinones are reported to have both pro-oxidant as well as antioxidant effects which causes tissue damage and protection on the body, respectively. The observed weight changes in animals treated with higher doses of the extracts indicate the presence of tannins and other phenolics which are responsible for altered absorption of nutrients and food intake (Kumar and Singh, 1984). According to this study, even though the animals were fed with adequate diet, the repeated chloroform extract at higher doses probably caused interference with absorption of nutrient such as proteins, resulting in weight loss.

In the present study, the significant decrease in body weight was observed in the groups repeatedly treated with chloroform extract of *S. incanum* (400 mg), methanolic extract of *C. quadrangularis* (Meth400) and other dose could be attributed to the suppression of the animals' appetite by the extracts leading to reduced food intake (Ogbonnia et al., 2010).

The assessment on the hematological parameters is important as it can point directly to the systemic effects caused by the administered extract (Pariyani et al., 2015). The effect of sub-acute administration of the crude extracts on hematological parameters in the animals fed with the extracts for 28 days is presented in Table 4.

This study recorded significant increase in hemoglobin and PCV in all the groups treated with both doses of methanolic extracts of *C. quadrangularis* when compared with the control group and those treated with methanolic extracts of *S. incanum* ( $p<0.05$ ). However, the observation of these parameters in groups treated with both doses of methanolic extracts of *S. incanum* showed slightly

**Table 3.** Potential lethal toxic effects (number of deaths) and body weight changes caused by crude extracts of *C. quadrangularis* and *S. incanum* in mice over four weeks.

Treatment	number of deaths	number survived	Body weight	
			D <sub>0</sub>	D <sub>28</sub>
CQ-Meth200	0/6	6/6	28.00±0.73	28.67±0.76
CQ-Meth400	1/6	5/6	27.17±0.70	22.25±0.86 <sup>ab</sup>
CQ-Chlor200	0/6	6/6	27.00±0.97	27.50±0.88 <sup>a</sup>
CQ-Chlor400	0/6	6/6	26.50±0.99	24.25±1.17 <sup>a</sup>
SI-Meth200	0/6	6/6	27.00±0.73	27.83±0.75
SI-Meth400	1/6	4/6	27.33±0.7	24.58±0.55 <sup>a</sup>
SI-Chlor200	0/6	6/6	26.67±1.02	24.41±0.88 <sup>a</sup>
SI-Chlor400	3/6	3/6	27.17±.95	23.25±.0.73 <sup>ab</sup>
Control	0/6	6/6	26.57±0.64	30.08±0.33

Values are mean ± SEM; n = 6; D, Day; D<sub>0</sub>, day treatment commenced; SEM, standard error of mean; SI, *S. incanum*; CQ, *C. quadrangularis*; Chlor, chloroform; Meth, methanol; all superscripts indicate significance at p < 0.05 (<sup>a</sup>compared to negative control; <sup>b</sup>compared to SI-Meth200 and CQ-Chlor200)

**Table 4.** Packed cell volume (PCV) and hemoglobin [Hb] changes of experimental mice following sub-acute administration of crude extracts of *C. quadrangularis* and *S. incanum*.

Treatment	Packed cell volume (%)		Hb (g/dl)
	D <sub>0</sub>	D <sub>28</sub>	D <sub>28</sub>
CQ-Meth200	45.00±1.00	50.83±0.70 <sup>abc</sup>	19.67±0.71 <sup>ad</sup>
CQ-Meth400	46.00±0.58	51.00±.58 <sup>abc</sup>	19.83±0.74 <sup>ad</sup>
CQ-Chlor200	46.00±0.26	47.50±0.62	16.67±0.48 <sup>b</sup>
CQ-Chlor400	46.33±0.61	48.17±0.47	16.67±0.33 <sup>b</sup>
SI-Meth200	46.67±0.71	44.83±0.65	13.17±.60
SI-Meth400	46.67±0.99	44.12±0.70	14.83±0.79
SI-Chlor200	48.33±0.91	48.00±0.68	15.17±0.79
SI-Chlor400	47.67±1.14	47.00±1.12	15.00±.57
Control	46.67±0.88	47.16±1.04	16.17±0.93

Values are mean ± SEM; n = 6; D, Day; D<sub>0</sub>, day treatment commenced; SEM, standard error of mean; SI, *S. incanum*; CQ, *C. quadrangularis*; Chlor, chloroform; Meth, methanol; all superscripts indicate significance at p < 0.05 (<sup>a</sup>compared to negative control; <sup>b</sup>compared to SI-Meth200; <sup>c</sup>compared to SI-Meth400; <sup>d</sup>compared to all extracts of *S. incanum*).

decreased values. This decrement in hematological parameters are not significantly (p>0.05) different from the control. Consumption of the different doses of the chloroform extracts of both plants did not cause significant changes in the PCV and Hb when compared with the control. Only higher dose (400 mg/kg) of chloroform extract of *C. quadrangularis* seemed to show a marginal increase of both parameters. These increases were not significantly different between the two extracts and with the control at p < 0.05.

It was clearly noted that crude extracts of *C. quadrangularis* positively affected the level of Hb and PCV more than *S. incanum* treated mice. This was particularly significant for the methanolic extract of *C. quadrangularis*. The observed significant increment in hemoglobin concentration and PCV suggests that this crude methanolic extract may have properties that

stimulate erythropoiesis in the bone marrow when orally administered and may be very useful in the treatment of anemia. The phyto-constituents such as phenols (Ofokansi et al., 2005) and flavonoid (anti-oxidant and free radical scavenger) (Salahdeen and Yemitan, 2006) are highly implicated in such phenomenon.

Some of the biological functions of flavonoids, for example, include protection against allergies, free radicals, platelet aggregation microorganisms, ulcers, hepatotoxins and tumors (Okwu, 2004). Tannins and saponins were not present in *C. quadrangularis*. It is noteworthy that the presence of tannins and saponins in the methanolic extract of *S. incanum* may be a contributing factor to the slight marginal decrease in the haematological parameters. Saponins, for instance, have the properties of precipitation of proteins, cholesterol-binding and haemolysis. Other phyto-components such

as alkaloids and glycosides found in these plants also do not have properties relating to increased haematopoiesis or hemolysis (Vadivel and Janardhanan, 2000). Thus, *C. quadrangularis* appeared to be more effective than *S. incanum* in modulating haematopoiesis and protecting hemolysis.

## Conclusion

The phytochemical screening indicated the presence of different secondary metabolites in both plants, which are responsible for bioactivity of the plants. The results show that methanol and chloroform extracts of *C. quadrangularis* and *S. incanum* did not cause any clear acute toxicity in an animal model. The sub-acute toxicity on the other hand revealed that crude extract of fruit of *S. incanum* showed toxic effects and mortality at high doses and thus prolonged use should be discouraged and low doses are recommended. However, an advanced experimental analysis of chronic toxicity of both plants is essential to establish therapeutic value and the safety in use.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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