

## Toxicological Evaluation of Ethyl Acetate Extract of *Syzygium kanarensis* (Talbot) Raizada Leaves in Wistar Rats

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This study was designed to evaluate the oral acute toxicity of the ethyl acetate extract of *Syzygium kanarensis* leaves. Medicinal plants play an important role in ethnomedicine for the treatment of multiple diseases. *Syzygium* is a common fruit tree with different species under Myrtaceae family; *Syzygium cumini* and *Syzygium calophyllifolium* are known medicinal plants for the treatment of diabetes mellitus and various diseases. *Syzygium kanarensis* one of the unexplored plants tested for toxicity for the purpose of therapeutic value. The ethyl acetate extract of *Syzygium kanarensis* (Talbot) Raizada (Family: Myrtaceae) leaves was investigated for the study of cytotoxicity and oral acute toxicity in female Wistar albino rats according to OECD guideline 425. The preliminary phytochemical screening was carried out and confirmed the cytotoxicity by MTT assay using NIH/3T3 mouse embryonic fibroblast cell line with 5 concentrations (100 to 500 µg/ml) of the extract. For oral acute toxicity study female rats were divided into two equal groups. One group served as vehicle control (VC) while the other group received an orally single dose at the 2g/kg body weight (B.W) of *Syzygium kanarensis* leaves extract from ethyl acetate (SKLEE). Then both groups were observed carefully for 14 days. After blood samples were collected carefully by cardiac puncture, under anesthesia and were subjected to haematological and biochemical analyses. The vital organs of rats were preserved in 10% formalin for histopathological examination. The extract contains phenolics, terpenoids, tannins, saponins, steroids, volatile oil, glycosides, carbohydrates, and proteins. The result of cytotoxicity on NIH/3T3 cell line with  $IC_{50} = 425.07 \mu\text{g/ml}$  and the percentage of viable cells was calculated. In the acute toxicity study found no mortality, no significant changes in B.W, blood parameters and also with the study of histopathology. The data revealed that  $LD_{50}$  of the leaves extract was more than 2g/kg B.W. The study concluded that the extract presence of phytochemicals is useful for traditional medicine for various diseases because they were non-toxic when administered orally at the dose of 2g/kg B.W to rats. To the best of our knowledge, this is the first report of the extract from *Syzygium kanarensis* leaves.

**Keywords:** *Syzygium kanarensis* (Talbot), Raizada Leaves, Wistar Rats.

Medicinal plants have been used as therapeutic agents that take a holistic approach to treat various diseases. The immense variety of plants have played indispensable roles in the life of human beings and animals by the source of foods (fresh fruits, vegetables, grain, seeds and nuts), medicine (health care), aesthetic values and ecological monitors. Plant-derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparation that are currently recommended by medical practitioners and they are an important part of the health care system in the Western world<sup>1</sup> because drugs obtained from plants are frequently considered as less toxic and free from side effects than synthetic ones<sup>2</sup>. The usage of natural products have considerably improved and this increases order with the fact that the population believes that natural products do not present any toxic effects<sup>3,4</sup>. Based on the World Health Organization (WHO), 75-80% of the world's populations primarily depend on medicine obtained from plant materials and products<sup>5</sup>. Many benefits of using plant products for health-related issues are plenty because of the rich source plant-based like fibre, vitamin, carbohydrates, protein, calcium, magnesium, zinc, omega-3 and omega-6 fatty acids are amazing health benefits like keep body full energy to carry out day to day activities and against many diseases. Vital organs are most important in the body to play role in the regulation of physiological processes, so the organ damage is recovered, the risk of death can be reduced to a significant extent and frequency of a health problem can be minimized effectively from the plant products. Many unexplored plants are present in Western Ghats of India.

The rare plant *Syzygium (S.) kanarensis* described by Talbot and reported in 1897 from the green forests of the Gerusoppa ghat in North Kanara district of Karnataka, India. In 1986 Ahmedullah and Nayar reported this species to be endemic in Shimoga and North Kanara districts of Karnataka, India. In 1996 *S. kanarensis* was considered as critically endangered species and last seen by Talbot discounting the collection of R. Sundararaghavan. After a long gap of 47 years, this species rediscovered from the evergreen forests and it belongs to Myrtaceae family<sup>6</sup>. Total of 52 constituents was reported from the essential oil of *S. kanarensis*<sup>7</sup> and no data available in leaves extract

of *S. kanarensis*<sup>7</sup>. The phytochemical of alkaloids affect the different metabolic system in animals, and the toxic mechanism of action of alkaloids may vary considerably. Toxicity may arise by enzymatic alterations affecting physiological processes, inhibition of DNA synthesis and repair mechanisms by intercalating with nucleic acids, or affecting the nervous system. Several alkaloids may affect multiple functions<sup>8</sup>. To study the toxicity of plant extracts and compounds, the cytotoxicity test is one of the biological evaluations and screening tests that use tissue cells *in vitro* to observe toxicity by cell growth or not<sup>9</sup>. The aim of the present study is to access deals the analysis of phytochemicals, cytotoxicity, oral acute toxicity, hematological and biochemical parameters, histopathological in vital organs from SKLEE.

## MATERIALS AND METHODS

### Chemicals

Biochemical kits such as urea, creatinine, alkaline phosphatase, S.G.O.T (A.S.T), S.G.P.T (A.L.T), total protein kits were purchased from Gappe Hills, Pattimattom (PO), Dist. Ernakulam, Kerala - 683 562, India.

### Collection of plant material

The fresh leaves of *S. kanarensis* were collected from Pilikula Nisargadhama, Mudushedde (N 12.9299, E 74.8958), Mangalore, Dakshina Kannada district, Karnataka state, India (voucher specimen No. PND-800, flower: 2385 and plant fruiting: 2520).

### Preparation of crude extract

About 2 kg quantity of leaf material was thoroughly washed with clean water and spread on clean paper. Removed foreign particles with the help of cutter and then kept at room temperature for air drying for 20 days. The dried leaves were finely powdered mechanically and 150gm powder extracted first with hexane (500 ml) to remove overall lipid content, then the residue was extracted with ethyl acetate (500ml) using soxhlet apparatus and then filtered by Whatman no.1 filter paper. The excess solvent was removed by a rotary evaporator at 50°C. Then dried crude extract 18.6 gm (12.4% yield) was stored in the refrigerator (below 5°C) prior to use.

### Phytochemicals Screening

The phytochemicals screening of

SKLEE was carried out to reveal the presence or absence of chemical constituents using standard procedures<sup>10,11,12,13,14</sup>.

#### **Alkaloids test**

a. Mayer's test: About 2 ml of plant extract, 2 drops of Mayer's reagent were added along the sides of the test tube. The appearance of white creamy precipitate indicated the presence of alkaloids<sup>11</sup>.

b. Wagner's test: A few drops of Wagner's reagent were added to the plant extract along the sides of the test tube. A reddish-brown coloured precipitate indicated the presence of alkaloids<sup>11</sup>.

c. Hagner's test: The filtrate was treated with a few drops of Hagner's reagent. The Formation of yellow precipitate indicated the presence of alkaloids<sup>14</sup>.

#### **Test for phenolic compounds**

a. Ferric Chloride test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution was added. Dark green colour indicates the presence of phenolic compound<sup>11</sup>.

b. Gelatin test: The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds<sup>11</sup>.

c. Lead acetate test: The extract (50 mg) is dissolved in 5ml distilled water and to this 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds<sup>11</sup>.

#### **Test for Flavonoids**

a. Alkaline reagent test: Extract was treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids<sup>14</sup>.

b. Lead acetate Test: Extract was treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids<sup>13</sup>.

**Test for terpenoids:** 2mg crude extract was shaken with chloroform (2ml) followed by the addition of concentrated H<sub>2</sub>SO<sub>4</sub> (2 ml) along the side of the test tube, the reddish-brown colouration of the interface indicates the presence of terpenoid<sup>12</sup>.

**Test for tannins:** The 50 mg extract was stirred with distilled water (10 ml) and then filtered. A few drops of 5% ferric chloride were then added. Black

or blue-green colouration or precipitate was taken as a positive result for the presence of tannins<sup>12</sup>.

**Test for saponins:** The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A 2 cm layer of foam indicates the presence of saponins<sup>14</sup>.

**Test for steroids:** About 100 mg of dried extract of *S. kanarensis* was dissolved in 2ml of chloroform. H<sub>2</sub>SO<sub>4</sub> was carefully added to form a lower layer. The reddish-brown colour at the interface was indicative of the presence of the steroidal ring<sup>14</sup>.

**Test for glycosides:** The extract (50 mg) was hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests<sup>11</sup>.

a. Borntrager's test: To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, the chloroform layer was separated and 10% ammonia solution was added to it. A pink colour indicates the presence of glycosides.

b. Legal test: About 50 mg of extract was dissolved in pyridine sodium nitroprusside solution was added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

**Test for volatile oil:** For volatile oil estimation 50 mg of powdered material (crude drug) is taken and subjected to hydrodistillation. The distillate is collected in a graduated tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil<sup>11</sup>.

#### **Test for Carbohydrates**

a. Molish's test: To 2 ml of extract, two drops of alcoholic solution of  $\alpha$ -naphthol was added. The mixture was shaken well and a few drops of concentrated sulphuric acid were added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates<sup>11</sup>.

b. Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar<sup>11</sup>.

**Test for Proteins:** The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate was subjected to test for proteins<sup>11</sup>.

a. Millon's test: To 2 ml of filtrate few drops of Millon's reagent was added. A white precipitate indicates the presence of proteins.

b. Biuret test: Extract (2 ml) was filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by addition of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

#### **Cytotoxicity Assay**

Cell culture from the normal mouse embryonic fibroblast cells (NIH/3T3) was used for cytotoxicity screening of the SKLEE by MTT assay. NIH/3T3 the cell line was obtained from National Centre for Cell Sciences (NCCS), Pune, India.

#### **MTT assay**

To evaluate the cell viability MTT assay is a standard method for cytotoxicity<sup>15,16</sup>. The cell line of NIH/3T3 was seeded in 96-well plates at 200il density of suspension contained approximately 10,000 cells. Following 24-hrs incubation and attachment, the cell line was treated with different concentrations of extract from 100µg/ml to 500µg/ml of extract. The 96 well plates were incubated at 37°C with 5% CO<sub>2</sub> for 24 hrs. Then for the further incubation period, the media (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) was aspirated with carefully without disturbing the crystals of MTT at the wells bottom and 100µl of solubilisation solution of DMSO was added and then well plate was shaken gently in a shaker to solubilise the formazan. The microplate reader was used for the absorbance at the wavelength of 570nm was noted using Spectrophotometer<sup>17</sup>. The following formula was used to calculate the % viability.

$$\text{Cell viability \%} = \frac{\text{Mean absorbance of treated group}}{\text{Mean absorbance of control}} \times 100$$

#### **Approval from animal ethics committee**

The experiments were conducted in accordance with the guidelines cleared by the Animal Ethics Committee in Shri Sanganabasava Mahaswamiji College of Pharmacy and Research Centre, Vijayapur, Karnataka State, India (Registration number: BLDE/BPC/2019-2020/6).

#### **Evaluation of acute toxicity assay**

Accordance to OECD guidelines-425 (up and down procedure) dosing at 2g/kg, B.W experiment was performed on non-pregnant female

Wistar albino rats weighing 150-250gm were selected<sup>18</sup>. They were procured from Mohan Bhat, Laboratory Suppliers, B.E.M. School, Cross Road, Mangalore. The animals were kept under standard laboratory conditions maintained (12hr dark: 12hr light) at room temperature of 22-25°C. The rats were freely supplied with pellet diet and continuous supply of drinking water. Before dosing, animals were kept under the standard conditions for 5 days. The extract was homogeneously mixed with 1% Carboxymethyl cellulose (CMC) gel and administered orally using gavage needle at a single dose of 2g/kg B.W of rats (n=5), for VC group CMC gel administered the same volume as that of the treated group received. The acute toxicity was monitored with the extract treated rats and observed closely during the first 30 minutes to 4 hours. The behaviour of the animal was observed systematically for survival or death. The observation was made twice a day for up to 14 days. The clinical observations were made daily for the study of physical condition, food, water intake, skin, fur, eyes, mucous membrane, salivation, sleep, coma, diarrhoea, convulsion and any changes in the level of activity in both group of rats. At the end study of oral acute toxicity, the rats were weighed and blood samples were collected by puncture of cardiac under anesthesia using ketamine (50 mg/kg) and xylazine (5 mg/kg) intraperitoneal injection respectively then vital organs were excised and preserved in 10% formalin for the study of histopathological study in both groups.

#### **Collection of blood samples**

5 ml of blood samples was collected by puncturing the cardiac with syringe needles into labelled heparinised (ethylene diamine tetra acetate [EDTA]) tube for haematological evaluation and blood in the non-heparinised tube (without EDTA) allowed to coagulate, then centrifuged and the sera were separated for the study of biochemical analyses.

#### **Hematological analysis**

The blood indices like haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils, lymphocytes, monocytes, eosinophils, packed cell volume (PCV) were evaluated by automated

hematology analyzer (Yumizen H500 – Horiba).

**Biochemical analysis**

The blood sample was collected from animals to clean dry tubes and centrifuged at the speed of 3000 rpm at 25°C for 10 minutes. To obtain serum was stored at ×20°C. Serum analyzed for the determination of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, total protein (TP), albumin, globulins, urea, creatinine, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL),

low density lipoprotein (LDL) and very low density lipoprotein (VLDL) was performed by using automated biochemistry analyzer (Mindray BS-200).

**Histopathology study**

The vital organs were isolated from animal (liver, kidney and heart) and examined macroscopically. The morphology of internal organs was observed for the signs of toxicity. These organs were excised and fixed in 10% formalin bottles, then processed for histopathological study. The vital organs were embedded in paraffin

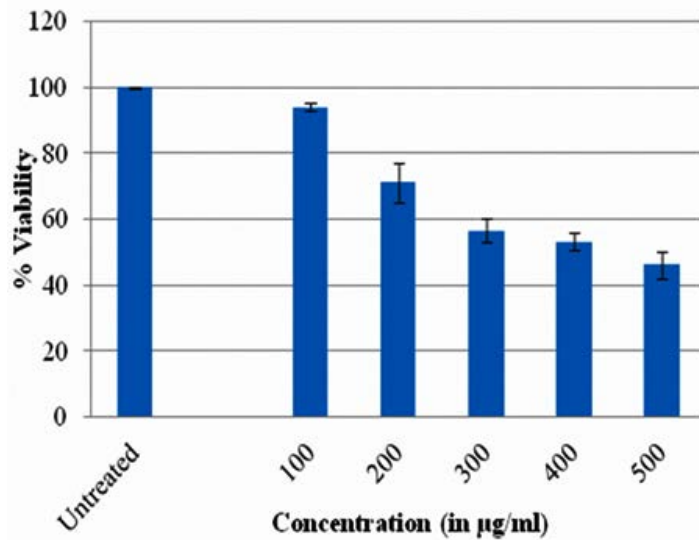


Fig. 1. Cell viability percentage of the SKLEE on the NIH/3T3 cell line after 24 hr cell seeded on culture plate

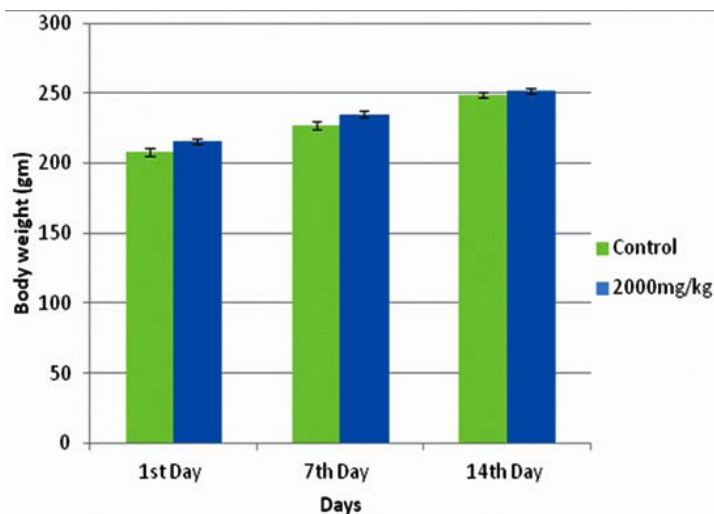
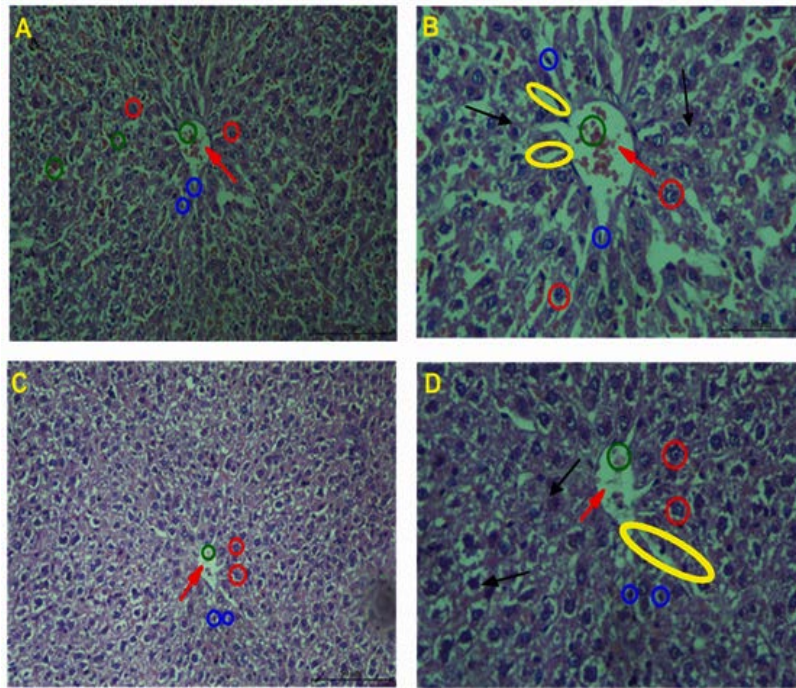
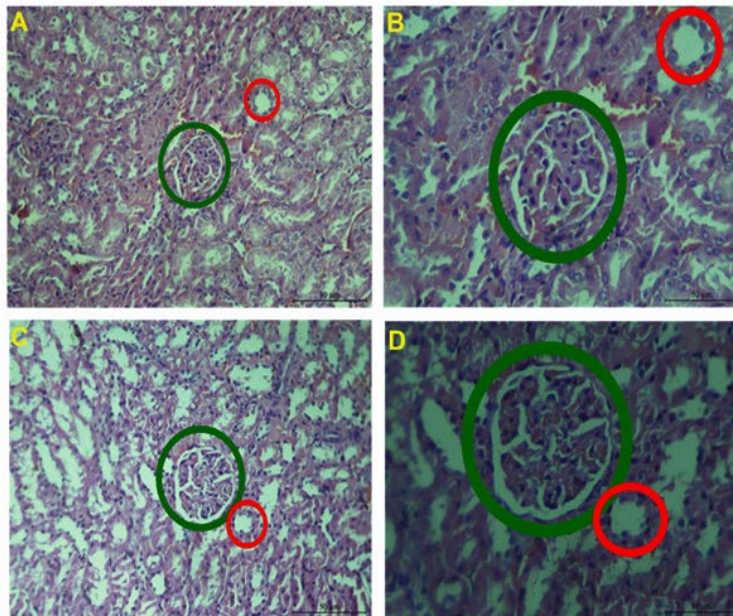


Fig. 2. Effect of SKLEE on B.W in rats



**Fig. 3.** Photomicrograph of liver sections (H & E). (A) Vehicle control (20x) and (B) Vehicle control (40x). (C) SKLEE treated (20x) and (D) SKLEE treated (40x). The red arrow indicates central vein, black arrow indicates hepatocytes, green circle indicates red blood cells, red circle indicates N, blue circle indicates K cells and yellow circle indicates blood sinusoids



**Fig. 4.** Photomicrograph of kidney sections (H & E). (A) Vehicle control (20x) and (B) Vehicle control (40x). (C) SKLEE treated (20x) and (D) SKLEE treated (40x). The green circle indicates G, red circle indicates T

wax; serial section (3- $\mu$ m thickness) obtained by cutting the embedded tissue with a microtome and stained with haematoxylin and eosin (H & E). The slides were observed under Nikon phase contrast microscope (Y-TV55 ECLIPSE) and captured the magnified (X20 and X40) images of tissue structures for further study.

#### Statistical analysis

Animal study of all data was expressed as mean  $\pm$  SEM and one way analysis of variance in ANOVA followed by Dunnett T3 test for multiple comparisons. It was used to analyze the significance for the effect of SKLEE group with compared to a vehicle control group with the help of SPSS software, version 20; values are considered statistically significant when  $P < 0.05$ . All data points show the mean of the standard error of means (SEM).

## RESULTS

#### Phytochemical screening

SKLEE appeared in the green colour and good smell. The phytochemical studied of extract contains phenolics, terpenoids, tannins, saponins, steroids, volatile oil, glycosides, carbohydrates and proteins (Table 1).

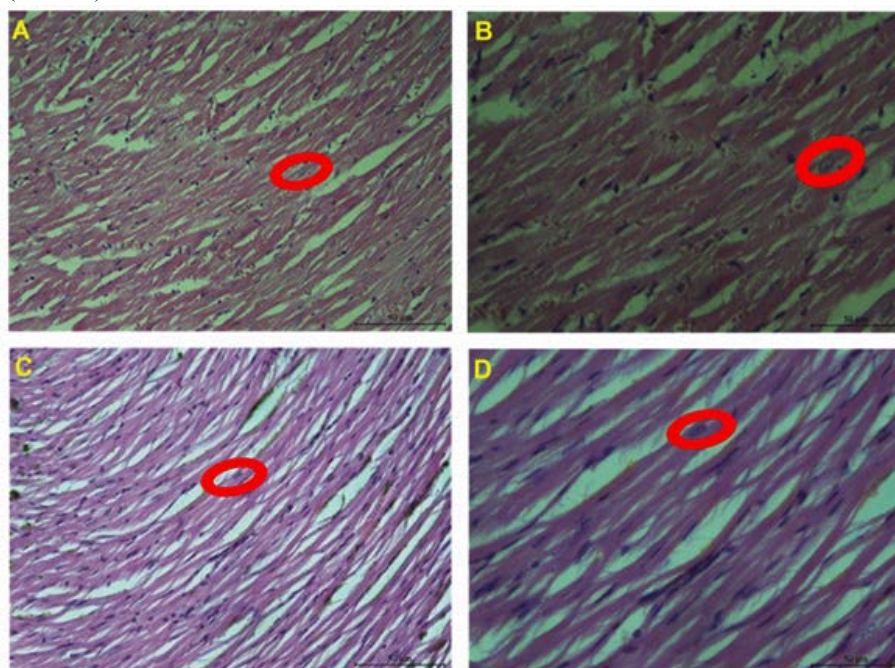
#### Cytotoxicity study of SKLEE on NIH/3T3 cell line

To determine the cytotoxic effect of SKLEE by MTT assay on mouse fibroblast cell line (NIH/3T3) the viable cells were assessed in different concentration. The cell viability assay was used for cytotoxic effects on SKLEE and it was expressed as  $IC_{50}$  value (Table 2). Dose-dependent cell viability reduction after 24 hr was observed in extract treated on NIH/3T3 cell line. For cell growth, dose dependent exposure to SKLEE showed the viability of cell more than 50% decrease at the dosage of 500  $\mu$ g/ml. Graphs were plotted using % of cell viability Y-axis and concentration of SKLEE X-axis (Figure 1). From the plotted graph the SKLEE concentration required for 50% of cell viability ( $IC_{50}$ ) was calculated as 425.07  $\mu$ g/ml.

#### Oral acute toxicity study

##### Behavioral and Body weight

Acute toxicity studied in female Wistar rats treated orally with a single dose of 2 g/kg B.W. The B.W of SKLEE treated group compared to the vehicle control group showed no significant changes. The B.W tested in both VC and treated group with SKLEE (2 g/kg) were increased



**Fig. 5.** Photomicrograph of heart sections (H & E). (A) Vehicle control (20x) and (B) Vehicle control (40x). (C) SKLEE treated (20x) and (D) SKLEE treated (40x). Red circle indicates N

progressively and results were expressed in Table 4 and Figure 2. The behaviour of VC and treated rats after dosing effects were noted. After 24hr no mortality and no signs were observed in treated rats were noted carefully after dosage. No observable signs of toxicity were detected during the experimental period up to 14 days and all behavioural observation results of rats are illustrated in Table 3.

#### Organs weight of the rats in an oral acute toxicity study

Isolated organs were found to be free of any lesions and no significant changes occurred in

the respective organs weights of the rats shown in Table 5. Organ weight is an important indication of the pathological and physiological status of animals<sup>19</sup>.

#### Effects of extract on haematological parameters in the acute toxicity study

The haematological parameters were tested in both groups by estimating the blood count is shown in Table 6. There was no significant difference in the parameters of Hb, total RBC, total WBC, MCHC, MCV, MCH, Neutrophils, Lymphocytes, Monocytes, Eosinophils and PCV count between the groups.

#### Effects of extract on biochemical parameters in acute toxicity study

The results in the extract treated group showed that the renal function test of urea and creatinine were not shown any significant

**Table 1.** Phytochemicals in SKLEE were presence of phenolics, terpenoids, tannins, saponins steroids, volatile oil, glycosides, carbohydrates and proteins

Phytochemical tests	Extract (SKLEE)
Alkaloids	-ve
Phenolic compounds	+ve
Flavonoids	-ve
Terpenoids	+ve
Tannins	+ve
Saponins	+ve
Steroids	+ve
Volatile oil	+ve
Glycosides	+ve
Carbohydrates	+ve
Protein	+ve

(+) = Present) (×) = Absent

**Table 2.** Study on the Cytotoxicity of SKLEE by using NIH/3T3 cell line

Concentration (µg/ml)	Mean ± standard error	% Cell Viability
100	1.428 ± 0.015	94.04 ± 1.02
200	1.080 ± 0.092	71.09 ± 6.05
300	0.859 ± 0.057	56.55 ± 3.75
400	0.808 ± 0.040	53.19 ± 2.63
500	0.702 ± 0.063	46.21 ± 4.14
Untreated	1.519 ± 0.003	100 ± 0.19
IC <sub>50</sub> = 425.07 µg/ml		R <sup>2</sup> = 0.894

**Table 3.** Observation for the SKLEE at 2g/kg B.W of an animal

Observation	30 Min.		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Week	
	C	E	C	E	C	E	C	E	C	E	C	E
Skin and Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convulsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

C= Control, E= Extract, N= Normal



differences (Table 7). In the liver function test parameters AST (SGOT), ALT (SGPT), globulins, albumin, total Proteins, total Bilirubin and alkaline Phosphatase showed no significant among the groups (Table 8) and Total Cholesterol, triglycerides, H D L, L D L and V L D L among the groups showed no significant variations of the report showed (Table 9). Totally the biochemical parameters showed that when the extract treated group at 2 g/kg was compared with vehicle control group report was not significant differences.

#### Histopathology study of oral acute toxicity

The liver, kidney and heart the tissue in SKLEE (2 g/kg B.W) treated rat revealed characteristic of normal structure and no morphological changes detected at the cellular level compared with VC treated group.

In histopathological study of liver sections was observed in treated SKLEE rat after 14 days (Figure 3, C and D) at the dose of 2 g/kg B.W The SKLEE treated rat organs of liver section comparison with the VC treated rat liver section

**Table 4.** Effects of extract on B.W of rats in acute toxicity study

Groups	Body weight (gm)		
	1 <sup>st</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
Vehicle Control Group (1% CMC gel)	208.00±2.54	227.00±2.54	249.00±1.87
Extract (SKLEE) Group (2g/kg B.W)	216.00±1.87	235.00±2.23	252.00±2.00

*n*=5, the values are expressed as mean ± SEM, \**P* < 0.05 (Anova/Dunnett T3 test) when compared to VC and SKLEE treated group.

**Table 5.** Effects of extract on organ weight of rats in acute toxicity study

Groups	Organs Weight (Gram)		
	Kidney	Heart	Liver
Vehicle Control Group (1% CMC gel)	2.07 ± 0.07	0.93 ± 0.04	9.34 ± 0.13
Extract (SKLEE) Group (2g/kg B.W)	1.75 ± 0.15	0.97 ± 0.05	9.43 ± 0.25

*n*=5, the values are expressed as mean ± SEM, \**P* < 0.05 (Anova/Dunnett T3 test) when compared to VC group from SKLEE treated group.

**Table 6.** Effects of extract and vehicle control treated groups on haematological parameters

Parameters	Vehicle Control Group (1% CMC gel)	Extract (SKLEE) Group (2g/kg B.W)
Hb (g/dl)	12.62 ± 0.12	12.64 ± 0.12
Total RBC (×10 <sup>6</sup> /μl)	6.79 ± 0.16	6.64 ± 0.17
Total W.B.C (×10 <sup>3</sup> /μl)	8.16 ± 0.45	8.80 ± 0.63
MCHC (g/dl)	29.08 ± 0.18	29.32 ± 0.30
MCV (fl)	63.54 ± 1.65	64.82 ± 1.35
MCH (Pg)	18.24 ± 0.34	18.96 ± 0.10
Neutrophils (%)	30.40 ± 1.36	32.80 ± 1.77
Lymphocytes (%)	67.60 ± 1.91	64.20 ± 2.90
Monocytes (%)	1.40 ± 0.24	1.40 ± 0.24
Eosinophils (%)	1.60 ± 0.24	1.60 ± 0.24
PCV (%)	43.36 ± 0.48	42.84 ± 0.55

*n*=5, the values are expressed as mean ± SEM, \**P* < 0.05 (Anova/Dunnett T3 test) when compared to VC group.

(Figure 3, A and B) showed central vein normally, nucleus (N), kupffer (K) cells and contained RBC (Red Blood Cells). In kidney histopathological study of VC rat (Figure 7, A and B) and SKLEE treated (2 g/kg B.W) rat (Figure 4, C and D) were showing the same structure in both of the glomerulus (G) and Tubules (T) (Figure 4, A and B). In heart tissue sections were showing normal structure in both of VC and SKLEE treated groups (Figure 5, A, B, C and D). Liver, kidney and

heart histopathological study confirmed a normal structure in SKLEE treated group.

## DISCUSSION

Before the study of pharmacological and development of the pharmaceutical product from the medicinal plant, the acute toxicity studies provide valuable information for medicines<sup>20,21</sup>. Medicinal plants presumed to be safer side without

**Table 7.** Effects of the extract and vehicle control treated groups for renal function test of rats

Parameters	Vehicle Control Group (1% CMC gel)	Extract (SKLEE) Group (2g/kg B.W)
Urea (mg/dl)	17.8 ± 1.01	18.20 ± 1.20
Creatinine (mg/dl)	0.72 ± 0.037	0.76 ± 0.05

*n*=5, the values are expressed as mean ± SEM, \**P* < 0.05 (Anova /Dunnett T3 test) when compared to VC group from SKLEE treated groups

**Table 8.** Effects of extract and vehicle control treated groups for liver function test in female rats

Parameters	Vehicle Control Group (1% CMC gel)	Extract (SKLEE) Group (2g/kg B.W)
S.G.O.T (A.S.T) (U/L)	29.42 ± 1.93	26.66 ± 2.83
S.G.P.T (A.L.T) (U/L)	26.80 ± 1.46	26.60 ± 1.56
Alkaline Phosphatase (IU/L)	43.43 ± 1.68	42.92 ± 1.58
Total Bilirubin (mg/dl)	0.23 ± 0.01	0.22 ± 0.02
Total Protein (g/dl)	7.3 ± 0.11	7.1 ± 0.19
Albumin (g/dl)	3.4 ± 0.10	3.4 ± 0.08
Globulins (g/dl)	3.8 ± 0.16	3.9 ± 0.16

*n*=5, the values are expressed as mean ± SEM, \**P* < 0.05 (Anova /Dunnett T3 test) when compared to VC group from SKLEE treated groups.

**Table 9.** Effects of extract on the serum lipid profile of vehicle control treated and SKLEE treated groups

Parameters	Vehicle Control Group (1% CMC gel)	Extract (SKLEE) Group (2g/kg B.W)
Total Cholesterol (mg/dl)	118.00 ± 5.94	127.00 ± 4.03
Triglycerides (mg/dl)	99.60 ± 5.00	107.40 ± 3.05
H.D.L (Cholesterol) (mg/dl)	38.40 ± 1.93	41.00 ± 1.30
L.D.L (Cholesterol) (mg/dl)	59.68 ± 3.00	64.52 ± 2.14
V.L.D.L (Cholesterol) (mg/dl)	19.92 ± 1.00	21.48 ± 0.61

*n*=5, the values are expressed as mean ± SEM, \**P* < 0.05 (Anova /Dunnett T3 test) when compared to VC group.

any compromising side effects on health<sup>22,23</sup> but in many studies reported the harmful effects of improper use of medicinal plants<sup>24</sup>. Therefore, the evaluation of toxicological effects from the extract of any medicinal plant intended to be used in the animal model is a crucial part of its assessment for potential toxic effects. *S. kanarensis* is a medicinal plant and more than 50 compounds have been identified in aerial parts<sup>7</sup>. Medicinal properties of SKLEE are due to the presence of phytoconstituents. The presences of phytochemicals in SKLEE are phenolics, terpenoids, tannins, saponins steroids, volatile oil, glycosides, carbohydrates, and proteins (Table 1). These constituents may be responding to hyperglycemia. No data available on the studies of toxicity effect in *S. kanarensis* leaves extracts. Many studies exist on the oral acute toxicity of plant extracts using rat models<sup>22,25</sup>. The determine cytotoxicity activity by MTT assay on mouse embryonic fibroblast cell line (NIH/3T3) was used for cytotoxic effects of the extract were expressed as IC<sub>50</sub> values (Table 2). The MTT assay indicated that SKLEE on NIH/3T3 cell line showed toxicity more than 50% cytotoxic effects in the concentration of 500µg/ml.

Determination of acute toxicity is one of the major prerequisites for the development of newly synthesized drugs. Evaluation of acute toxicity of any extract plays an important role in the designing of drugs and their toxicological effects. So, the present reports in acute toxicological study dose treatment to Wistar rats at 2 g/kg B.W concentration of SKLEE showed no mortality, clinical signs, change in the skin, eye colour change, general physique, diarrhoea, and coma were recorded (Table 3) up to 2 weeks. Throughout the 14 day periods, all animals were found to be healthy with no changes. Therefore, this SKLEE oral LD<sub>50</sub> value should be greater than 2g/kg B.W. In rat's exposure to potentially any toxic substances, there will be a reduction in B.W gain<sup>26</sup>. In our acute toxicity studies, all rats continued to gain weight throughout the experimental periods (Figure 2). This suggests that the administration of the SKLEE at 2g/kg B.W did not affect the B.W of the rats. The relative organ weights of the rats in the extract treated group did not show any significant changes in comparison with that of the VC treated group. It indicated that the SKLEE did not affect the appetite and no deleterious effect

on the growth of animals. No significant changes were observed in rat's physiological as metabolic activity in comparison to VC treated group. If any changes in some blood parameters indicate the toxic effects of extract in rats blood either at a physiological or pathological level. Therefore, to study focus on the liver and kidney functions test as biochemical parameters. The enzymes of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are mainly found in the liver and kidneys. An increased level of ALT, ALP and total protein in blood indicates impaired liver and kidney function<sup>27</sup>. Creatinine and urea levels rise in the blood due to poor clearance by the kidneys<sup>28</sup>. The analysis of biochemical parameters studied in particular ALT, ALP, creatinine, urea, and total protein were tested and showed there were no significant differences in the treated group rats compared to VC group rats. The report indicates in haematological, biochemical parameters and lipid profile showed good results in both groups of rats. The body has essential vital organs for proper functions that are liver and kidney. The organs functions of the liver to the metabolism of intake<sup>29</sup> and kidney for excretion of the waste<sup>30</sup>. To assess the toxicity of any new extract, it is very essential to know the status of these vital organs, which can be checked through the liver and renal function test in female rat's blood. The histopathological images of liver, kidney, and heart also confirmed that the SKLEE treated group at 2g/kg B.W showed no changes compared with VC treated group observed under the microscope. The obtained results showed that SKLEE is practically non-toxic after acute administration at 2g/kg B.W on an animal model of Wistar rats.

## CONCLUSION

This is the first report describing the screening of phytochemicals, cytotoxicity by MTT assay on NIH/3T3 mouse embryonic cell line, oral acute toxicity on Wistar rats and histopathological from ethyl acetate extract of *S. kanarensis* leaves. Leaves extract of *S. kanarensis* containing phytochemicals such as phenolics, terpenoids, tannins, saponins steroids, volatile oil, glycosides, carbohydrates, and proteins were present. Several studies from other species of the same genus confirmed the presence of these phytochemicals

contributes medicinal as well as physiological properties of the plants studied in the treatment of different ailments. Therefore, extracts from one of the rare and rediscovered plant after 47 years of *S.kanarensis* could be seen as a good source for useful drugs. Cytotoxicity by MTT assay method on mouse fibroblast NIH/3T3 cell line showed IC<sub>50</sub> value 425.07µg/ml range. The oral acute toxicity reports confirmed the oral dose up to 2g/kg B.W and no related sign of toxicity or mortality in female Wistar rats. In B.W, hematology, biochemical serum analysis and histopathology study of vital organs did not show toxicity in SKLEE treated group comparisons with the vehicle control group. To our best of our knowledge, this is the first report from the application of *S. kanarensis* plant extract. Further studies are under process for the isolation of bioactive compounds for anti-diabetic study on an animal model. A focus of the study in this direction certainly would bring awareness in availing of the health benefits of the natural plant.

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