INTRODUCTION
The main physiological benefits of flaxseed oil are attributed to its high linoleic acid content that leads to its antioxidant potential [1] and hence was effective against several disorders such as diabetes, high blood pressure, cancer, inflammation and coronary artery diseases [2, 3]. Omega-3 fatty acids like α-linolenic acid (ALA), and its metabolites, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) inhibits different stages of mammary carcinogenesis [4-7]. Flaxseed oil and fish oil prevented lipid abnormalities in type-2 diabetes mellitus [8]. Pumpkin seeds are excellent sources of proteins, vitamins and oil [9-11], particularly ω-6 fatty acids, which possess a number of biological properties including antioxidant, anti-inflammatory and hypolipidemic activities [12]. Previous studies have shown that a combination of flaxseed and pumpkin seed mixture supplemented to diet was helpful in preventing diabetic complications in adult rats [13].

S. platensis, is a filamentous multicellular cyanobacterium belonging to the algae of the class Cyanophyta has a unique blend of nutrients. It is gaining importance as a potent anti-viral [14, 15], anticancer agent [16, 17] and in health improvement [18] as a nutraceutical and as a source of potential pharmaceutical agent.

Diabetes mellitus, a metabolic disorder, is turning as a major health problem. Although there are a number of drugs available for the treatment, prolong use of these drugs are found to cause adverse effects. Hence, various studies are in progress to find an alternative drug from the natural sources, which are effective in bringing down the complications of diabetes. Recently a large number of reports on S. platensis have proven beneficial effects on blood glucose levels and the lipid profile of type 2 diabetes mellitus subjects [19].

Inflammation is caused due to infection, oxidative stress and allergies during which the molecules like histamine, prostaglandins, bradykinin, and cytokines are released from the cells and tissues causing deleterious effects [20]. The flaxseed oil, S. platensis oil & sunflower oil have exhibited anti-inflammatory activities in different models [21-23]. However, no study has been reported about the effects of the combination of S. platensis oil and flaxseed oil mixture on their biological properties. Therefore, in the present investigation, the anticancer, antidiabetic and anti-inflammatory properties of flaxseed oil and S. platensis oil either alone or in combination were evaluated.

MATERIALS AND METHODS
Materials
The flaxseeds (Linum usitatissimum) were procured from the local market and the S. platensis was purchased from Avantha Holdings Limited, Nanjangud, Mysore, Karnataka, India.

Authenticity was confirmed by the Department of Studies in Botany, Manasagangotri, Mysore. It was stored at 4 °C and used for the experiments.

Sample preparation
The mixtures of oil samples were prepared by using 10% DMSO and following samples were used for the experiments: S1-[Sunflower oil (SFO)], S2-[Flaxseed oil (FSO)], S3-[S. platensis oil (SPO)], S4-[FSO: SPO (75: 25)], S5-[FSO: SPO (50: 50)] and S6-[FSO: SPO (25: 75)]

Experimental animals
The adult male albino rats (120-150 g) were approved by the Institutional Animal Ethics Committee (IAEC) of the University of Mysore, [Letter no. UOM/IAEC/19/2012]. All the animals were placed in laboratory cages in an animal house maintained at temperature 23±2 °C in 24 h standard light/dark cycles. All the animals had free access to standard food pellets and filtered water.

Chemicals and reagents
Insulin was procured from Torrent pharmaceuticals LTD under license from Novo Nordisk India, GOD-P0D reagent was procured from Aspen Laboratories, India, HBSS (Hank’s Balanced Salt Solution) with sodium bicarbonate was procured from Himedia,
India. Bovine serum albumin, tris buffer, linoleic acid, lipoxidase were purchased from SRL, India. Diclofenac sodium was purchased from Cipla pharmaceuticals, India. All solvents and other chemicals used in the studies were of analytical grade and purchased from SD Fine Chemicals Ltd., Mumbai, India.

**Proximate analysis**

The moisture content was determined by drying 2±0.1 g of sample (flaxseed/Spirulina) at 105±1 °C for 40 h and the total ash was determined by incineration of the samples weighing 2±0.1 g at 550 °C for 48 h [24]. The crude fibre content was determined by drying 2±0.1 g of the sample [25]. Analysis of total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) was carried out [26]. Total lipids of flaxseed and *S. platensis* samples were determined [27, 28]. Protein content was determined by Kjeldahl protein units and the protein was calculated as nitrogen (% × 6.25) [29]. Carbohydrate was determined by the difference between 100 and the sum of the percentages of all the other components. All proximate determinations were done in triplicates.

The fatty acid methyl esters (FAMEs) were prepared from the extracted lipids by the esterification reaction according to the method described by Chee [30]. Gas chromatography was performed using GC-2010 (Gas Chromatography, Shimadzu, Japan) [30]. Each fatty acid component was expressed as mass of fatty acid in 100 g of oil.

**Cell lines and culture**

Human chronic myelogenous cell lines (CM cells) were purchased from National Centre for Cell Science, Pune, India. Cells were grown in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml of penicillin and 100 µg of streptomycin/ml and incubated at 37 °C in a humidified atmosphere with 5% CO₂.

**MTT assay**

The cytotoxic effects of the different oil samples (S₁-S₆) were assessed against the CM leukemic cells (5×10⁴ cells) using 3-(4,5-dimethyl-2-y1)-2,5 diphenyl tetrazolium bromide (MTT) assay [31]. The test samples are dissolved in DMSO and treated with different concentrations of oil samples (20, 40 and 80 mg/ml respectively). Cells in the control wells received the same volume of medium containing DMSO. After 48 h treatment, cells were harvested and incubated with MTT (0.5 µg/ml) for 4 h at 37 °C in 96 well plate. The blue MTT formazan precipitate formed in the viable cells is solubilized by the addition of 70 µl DMSO. The suspension is placed in microinovator for 5 min and absorbance was measured at 540 nm using multimode reader (Varioskan Flash Multimode, Thermo scientific, USA). The experiment was performed in triplicate and repeated at least for three times.

**Trypan blue dye exclusion assay**

To study the growth suppressive effects of the oil samples (S₁-S₆), 0.5×10⁴ CM leukemic cells/ml were plated in a 24 well plate (Corning, USA) in 1 ml of complete medium [31]. The cells were treated with various concentrations of the oil samples (20, 40 and 80 mg/ml respectively). DMSO treated cells were used as control. Cells were harvested after 48 h and stained with 0.4% trypan blue and calculated using a hemocytometer for viable cells. Experiments were done in triplicates and the percentage of growth inhibition by different samples at different concentrations was plotted against time (48 h).

**Antidiabetic studies**

**Glucose uptake assay by porcine diaphragm**

Glucose uptake assay was performed using a porcine diaphragm [32] with the few modifications. Porcine diaphragm was obtained from a slaughter house and washed many times using ice cold saline to remove the blood stains. Then the diaphragm was used to investigate the inhibitory effects on the glucose uptake process by oil samples (S₁-S₆) at 20 and 40 mg/ml concentrations. Diaphragm weighing around 100-150 mg was suspended in a 24 well culture plate containing saline. 0.2% glucose was added to each well to initiate the reaction. DMSO treated plates served as control. 0.4 units of insulin were used in each well to enhance the glucose uptake by the diaphragm and the volume was made up to 2 ml with saline. Plates were incubated for 30 min at 37 °C in an atmosphere of 100% O₂ with shaking. The quantity of glucose formed in the culture plate was assayed using the GOD-POD method.

**In-vitro gluconeogenesis assay in isolated rat liver slices**

The antidiabetic properties of the different oil samples (S₁-S₆) were studied by the in-vitro gluconeogenesis assay [33]. Adult male albino rats were fasted for overnight and were killed by cervical dislocation. The liver was excised and washed in ice cold saline and stored on ice. Oil samples (S₁-S₆) were dissolved in DMSO at different concentrations (5-20 mg/ml) and were transferred to different wells in a 24 well plates containing Hank’s Balanced Salt Solution (HBSS). Sodium pyruvate (10 mmol/l) prepared in HBSS was added to the 24 well plates. Liver slices were cut as described [34] with the few modifications. The slices were weighed using a digital balance. The weights of tissue slices were between 100 and 150 mg and are added to plates containing HBSS medium and pyruvate with oil samples at different concentrations. DMSO treated plates served as control and insulin (1 mmol/l) was taken as the standard. The culture plates were incubated at ambient temperature (27 °C) for up to 60 min. Aliquots were taken from the plates at 0, 30 and 60 min. The percentage of glucose formed in the culture plate was assayed using the GOD-POD method as identified under.

**Glucose estimation by GOD-POD method**

Glucose in the culture plates was assayed by the GOD-POD assay kit protocol. Briefly, 50 µl of the incubated medium was transferred to a 96 well ELISA plate. The GOD-POD color reagent (200 µl) was added to each well. The color was developed in the dark at 37 °C for 30 min and then the optical density was evaluated at 505 nm and the percentage production of glucose was estimated by applying the convention:

\[
\% \text{ production of glucose} = \left( \frac{\text{glucose in DMSO control} - \text{glucose in sample}}{\text{glucose in DMSO control}} \right) \times 100
\]

**In-vitro anti-inflammatory activities**

**HRBC (Human red blood cell) membrane stabilization assay**

The HRBC membrane stabilization assay was carried out [35] with slight modifications. The blood was collected from a healthy donor, who was not taken NSAIDS for 2 w prior to the experiment. The collection of blood was carried out as per the ICMR guidelines Govt. of India (vide O. M. No 19015/53/1997-II P). The equal volume of Alkaver solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) was added to the blood sample and centrifuged at 2500rpm. The obtained packed cells were washed with isosalone and 10% suspension was taken using the isosalone. Diclofenac (50µg/ml and 100 µg/ml) was used as a positive control. The different concentrations of oil samples S₁-S₆ (10-40 mg/ml) are taken and to each concentration, 1 ml of phosphate buffer, 2 ml of hypo saline & 0.5 ml of packed cells were added. Then the test tubes were incubated for 20 min at 37 °C and centrifuged at 3000 rpm for 15 min. The supernatant containing hemoglobin was estimated at 560 nm spectrophotometrically. The percentage of hemolytic or anti-denaturation activity was calculated by using the following formula:

\[
\text{Percent inhibition} = \left( \frac{\text{control} - \text{test}}{\text{control}} \right) \times 100
\]
Percent inhibition = \( \frac{(\text{control} - \text{test})}{\text{control}} \times 100 \)

**Statistical analysis**

The data obtained were analyzed using GraphPad software Prism 5.1 and Excel software. The data were expressed as mean ± standard deviation and all experiments were compared with control and performed in triplicates.

**RESULTS AND DISCUSSION**

The results of the proximate analysis are provided in the table 1 and 2. Chemical composition of the flaxseed and *S. platensis* such as moisture, total ash, total dietary fibre, crude fibre and total lipids is depicted in the table 1. The fatty acid profile of the flaxseed and *S. platensis* oils is presented in percentages and are detailed in the table 2.

**Table 1: Chemical composition of ground flaxseed and *S. platensis* powder**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>% composition</th>
<th>Ground flaxseed</th>
<th><em>S. platensis</em> powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture</td>
<td>6.85±0.0575</td>
<td>11.94±0.0809</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Total ash</td>
<td>4.39±0.0010</td>
<td>6.60±0.8019</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Total dietary fibre</td>
<td>14.77±0.5299</td>
<td>0.72±0.0349</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Crude fibre</td>
<td>6.42±0.1335</td>
<td>0.33±0.0424</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Total lipid</td>
<td>27.39±2.4534</td>
<td>4.51±0.3112</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Crude protein</td>
<td>15.22±0.1316</td>
<td>53.52±0.2607</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Total carbohydrate</td>
<td>31.4±3.1961</td>
<td>22.72±1.5204</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Fatty acid profile of flaxseed oil and *S. platensis* oil**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fatty acids</th>
<th>% composition</th>
<th>Flaxseed oil</th>
<th><em>S. platensis</em> oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic acid</td>
<td>6.7</td>
<td>58.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Palmitoleic acid</td>
<td>0.1</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Stearic acid</td>
<td>7.6</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Oleic acid</td>
<td>22.8</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Linoleic acid</td>
<td>12.5</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>α-Linolenic acid</td>
<td>49.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>γ-Linolenic acid</td>
<td>-</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Arachidic acid</td>
<td>0.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Behenic acid</td>
<td>0.2</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Cytotoxic studies of oil samples (S1-S6) on CM cells showing IC50 values**

<table>
<thead>
<tr>
<th>Samples</th>
<th>MTT assay (IC50 values in mg/ml)</th>
<th>Trypan blue assay (IC50 values in mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>44.44±0.33</td>
<td>45.97±0.35</td>
</tr>
<tr>
<td>S2</td>
<td>42.83±0.29</td>
<td>46.4±0.32</td>
</tr>
<tr>
<td>S3</td>
<td>50.29±0.41</td>
<td>44.7±0.29</td>
</tr>
<tr>
<td>S4</td>
<td>130.1±0.77</td>
<td>115.0±0.71</td>
</tr>
<tr>
<td>S5</td>
<td>92.96±0.55</td>
<td>99.9%±0.63</td>
</tr>
<tr>
<td>S6</td>
<td>98.24±0.59</td>
<td>123.5±0.68</td>
</tr>
</tbody>
</table>

Mean±SD of triplicates. SD: Standard deviation

**Fig. 1: Antiproliferative effects of oil samples (S1-S6) at 20, 40 and 80 mg/ml concentrations against Human CM leukemic cells in MTT assay and trypan blue assay after 48 h treatment. C stands for DMSO treated vehicle control.**

"Drugs from Nature: Plants as an important source of pharmaceutically important metabolites"

Guest Editor: Dr. Dhananjaya Bhadrapura Lakkappa
From the results, it was observed that the percentage of dietary fibre, total lipid and total carbohydrate content was high in flaxseeds compared to S. platensis. In similar manner higher concentrations of total ash, moisture content and crude protein were observed in S. platensis sample.

The values obtained in our investigations were comparable to the previous studies [37, 38]. In the flaxseed oil the percentage of α-linolenic acid was high (49.6). In contrast, the percentage of palmitic acid was higher in S. platensis oil (51.1), γ-linoleic acid was exclusively present in S. platensis oil. Whereas, arachidic and behenic acid was found only in flaxseed oil. The high polyunsaturated fatty acids (PUFAs) content makes flaxseed oil and S. platensis oil as important food additives.

The data on cytotoxic studies of these oil samples (S1-S6) in terms of IC50 values are presented in the table 3. All the oil samples (S1-S6) were evaluated for in-vitro cytotoxicity against CM cancer cell lines by MTT assay. Each oil sample was tested in triplicates. The data obtained after 48 h of treatment by MTT assay showed that oil sample S2 has maximum growth inhibitory activity and found to be more effective, followed by S1 and S3 respectively. The growth suppressing activities of S4, S5 and S6 noticeably decreased when compared to S1, S2 and S3 oil samples (fig 1).

The efficacy of the drugs for an anticancer treatment can be measured by their ability to suppress the proliferation of cancer cells. MTT is a reliable method to assess the proliferative rate [39]. Cytotoxic properties of flaxseed oil and S. platensis oil was revealed by MTT and trypan blue assay. The growth suppressing activity was decreased when the cell lines are treated with combinations of oils in different proportions when compared to the cytotoxic activities of oil samples alone. Thus the study indicates that the antiproliferative potency of S. platensis oil or flaxseed oil alone is high and decreases considerably in combination treatments.

Table 4: Glucose uptake studies of oil samples (S1-S6) using porcine diaphragm

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Glucose uptake at 20 mg sample (S1-S6) (mg/g/30 min)</th>
<th>Glucose uptake at 40 mg sample (S1-S6) (mg/g/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without insulin With insulin</td>
<td>Without insulin With insulin</td>
</tr>
<tr>
<td>S1</td>
<td>27.68±0.22</td>
<td>29.56±0.23</td>
</tr>
<tr>
<td>S2</td>
<td>24.92±0.19</td>
<td>29.51±0.19</td>
</tr>
<tr>
<td>S3</td>
<td>24.83±0.21</td>
<td>29.90±0.24</td>
</tr>
<tr>
<td>S4</td>
<td>22.60±0.19</td>
<td>26.28±0.22</td>
</tr>
<tr>
<td>S5</td>
<td>22.31±0.18</td>
<td>27.24±0.23</td>
</tr>
<tr>
<td>S6</td>
<td>24.10±0.21</td>
<td>26.62±0.25</td>
</tr>
<tr>
<td>Control</td>
<td>22.12±0.17</td>
<td>25.94±0.24</td>
</tr>
</tbody>
</table>

mean±SD of triplicates. SD: Standard deviation

The in-vitro hypoglycemic activities of the oil samples were measured by the glucose uptake assay using porcine diaphragm. In the presence of insulin (0.4 units), porcine diaphragm showed increased uptake from 22.12±0.17 mg to 25.94±0.24 mg of glucose/g/30 min in the control well. The glucose uptake by the diaphragm at 20 mg/ml concentrations of the oil sample was found to be 27.68±0.22 mg. In the presence of insulin, the uptake was further increased to 29.56±0.23 mg of glucose/g/30 min. Similarly, the diaphragm incubated with different oil samples (S1-S6) at 20 & 40 mg/ml concentrations showed an increase in the uptake of glucose, which was further increased in the presence of insulin in the medium (table 4).

The study was to examine the synergistic effects of different oil samples (S1-S6) against inflammation and other associated diseases. From the experimental data obtained in HRBC membrane stabilization assay, flaxseed oil and S. platensis oil at 40 mg/ml concentrations exhibited an inhibition of 72.11% and 75.89%, respectively. The inhibitory effects were decreased in the...
combination studies and the samples S4, S5 and S6 at 40 mg/ml concentrations showed 58.37, 64.35 and 37.12% of inhibition respectively (fig 3).

![Fig 3: The anti-inflammatory effects of different oil samples (S1-S6) by HRBC membrane stabilization assay showing percentage inhibition](image)

In the protein denaturation assay, the flaxseed oil and S. platensis oil at 40 mg/ml concentration showed 74.11% and 82.35% of inhibitory activity, respectively. Whereas, in the samples S1, S3 and S6 at 40 mg/ml concentration, the inhibitory activity decreased and exhibited an inhibition of 55.29, 62.35 and 43.52% respectively (fig 4).

In protein anti-denaturation assay, the heat induced protein (albumin) denaturation has been studied. The heat induced protein anti-denaturation assay, the heat induced protein denaturation involves delayed type III hypersensitivity and arthritis like diseases. The heat induced hemolytic study (HRBC membrane stabilization assay) has been carried out by using HRBC. The heat causes damage to the membrane and leads to the release of serum proteins and fluids into the tissues which increase the membrane permeability that leads to inflammation. The anti-inflammatory activity of flaxseed oil was attributed to the presence of alpha-linolenic acid. Whereas, S. platensis oil contains gamma linolenic acid, which may be involved in the possible anti-inflammatory activity. The oils studied in different combinations (S1, S3 and S6) does not show any synergistic effects against inflammation in both the assays. This may be due to antagonistic behavior of oils in combination that causes a decline in the biological potentiality.

**CONCLUSION**

Flaxseed oil and S. platensis oil was assessed individually and in different proportions for the in-vitro antiproliferative, hypoglycemic and anti-inflammatory properties. Compositional analysis of oils showed that they are rich in omega-3 and omega-6 fatty acids. The present findings suggest that oils alone have predominant biological effects and the efficiency decreases considerably in combination studies. From these observations, it can be concluded that, the blending of flaxseed oil and S. platensis oil will antagonize the overall biological properties and thus provide an experimental evidence that, the combination of oils does not show any synergistic effects. Hence, the combination of certain type of oils is not preferable for the dietary purposes which forms the basis of the principles of Ayurveda, the Indian system of medicine.

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**CONFLICT OF INTERESTS**

The authors have declared no conflict of interest.

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