In vitro antiproliferative effect of aqueous extract of Solanum macranthum fruits on MDA-MB-231 triple negative breast cancer cell line

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ARTICLE INFO

Article history:
Received on: June 25, 2019
Accepted on: August 19, 2019
Available online: January 10, 2020

Key words:
S. macranthum, aqueous extract, breast cancer, MTT assay

ABSTRACT

The current investigation was focused on the assessment of antiproliferative effects of the Solanum macranthum aqueous fruits extract against the human breast cancer cell line MDA-MB-231 in vitro. Phytochemical analysis of the S. macranthum aqueous fruits extract was performed and the phytoconstituents found positive for alkaloids, phenols, and saponins. Quantitative analysis of the total alkaloid and phenol was determined using gravimetric analysis and folins-phenols reagent method, respectively. The total alkaloid present was estimated to be 7.58% and total phenol of 158.774 mg/Gallic Acid Equivalent. The cytotoxicity of S. macranthum aqueous fruits extract was screened using 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay and camptothecin was used as standard reference drug. At the concentration of 500 µg/ml, the aqueous extract potentially inhibited the growth of the MDA-MB-231 cell line in vitro reaching 19%, and the IC50 value was calculated to be 525.59 µg/ml. Thus, indicating that aqueous extract of the S. macranthum fruits possess potential anticancer property.

INTRODUCTION

Breast cancer ranks in second amongst all the prevalent cancer deaths reported globally [1]. Among Indian population, breast cancer is reported to be the highest with death ratio of 2:1 (out of two newly diagnosed breast cancers in women one dies) [2]. Despite the significant therapies available, such as hormone therapy, radiotherapy, chemotherapy, and surgery for the treatment of the breast cancer, their side effects and the chances of reoccurrence of the malignancy or metastatic cancer still projects a huge risk [3]. Therefore, there has been a hunt for the novel anticancer agent from the natural products which could endure as an alternative medicine for the treatment of cancer [4,5]. Some of the potent anticancer drugs, such as vinca alkaloids, vincristine, and vinblastine, have set up the foundation for search of several plant-based drugs [6]. The development of paclitaxel analogs was found to be potentially effective against breast cancer and Kaposi sarcoma, but in some duration of time, it was reported to be non productive in several cases [7]. Hence, there is an immediate need for the development of an effective drug which should be significantly effective in treatment of breast cancer but nontoxic to healthy cells of individuals. The identification of phytobioactive compounds from the medicinal plants against cancers with lower side effects has been the need of the hour in the recent decades [8]. Solanum macranthum (Dunal) is an ornamental plant belonging to Solanaceae family a native of Brazil and also found in different regions of Indian Territory displaying spectacular purple and white flowers has been undertaken for the study to unmask its potential against breast cancer [9]. The phyto-compounds isolated from Solanaceae family have been reported to possess several medicinal values including anticancer property [10]. Some species of Solanaceae have been traditionally used as folk medicines in treatment of various diseases. The current scientific research undertaken focused on aqueous extract of S. macranthum fruit to identify its cytotoxic properties against human breast cancer cell line MDA-MB-231.
2. MATERIALS AND METHOD

2.1. Chemical procured
Analitical grade chemicals were procured and used for the analysis. Acetic acid, Ammonium Hydroxide, Gallic acid monohydrate, and Folin–Ciocalteu reagent (FCR) were obtained from SDFCL Laboratories Pvt. Ltd (Mumbai, India). Camptothecin was obtained from Sigma-Aldrich. Dulbecco modified eagles medium (DMEM), MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide], Dimethylsulfoxide (DMSO), and Fetal Bovine Serum (FBS, Brazil, EU Approved) were obtained from Hi media Laboratories Pvt. Ltd.

2.2. Extraction of phytoconstituents
The S. macranthum fruit was collected from the medicinal garden of K.L.E’s P.C Jabin Science College Hubballi, Karnataka, India and shade dried. Dr. Harsha Hegde, ICMR—National Institute of Traditional Medicine taxonomically authenticated the plant with herbarium accession number RMRC-1402. The fruit appears oval in shape with a hard calyx on it. 200 g of dried fruit and 10 g of the fruit calyx were subjected for sequential extraction separately using four different solvents based on their increasing polarity for 144 hours, i.e., Petroleum Ether, Chloroform, methanol, and aqueous for 144 hours [10]. The extracts from each solvent were filtered and dried at room temperature. For the current study, aqueous extract has been used.

2.3. Analysis of the phytoconstituents
The dried aqueous extract of fruit and calyx was analyzed for the presence of various phytoconstituents, such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, and sterols separately using standard methods [11]. Since the aqueous fruit extract of S. macranthum was found to reveal more positive phytoconstituents in the preliminary qualitative analysis than the calyx of the fruit; therefore, further experimental study was executed using the aqueous fruit extract.

2.4. Total alkaloid content
The dried aqueous fruit extract of 1.5 g was added into 20% of acetic acid and the solution was incubated for 4 hours at 37°C. Furthermore, the solution was filtered and was reduced to its one fourth of its volume on water bath. Once the solution was cooled, concentrated ammonium hydroxide was added to it, in drop wise until the precipitation ceased. Using pre-weighed filter paper, the solution was filtered and dried. The percentage of crude dry weight of the alkaloid was determined by the formula [12]. The results were analyzed in triplicate.

Percentage of total alkaloids (%) = \( \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken}} \)

2.5. Total phenol content
The total phenol present in aqueous fruit extract was estimated using the Folinis-phenols reagent method described by Siddhuraju et al. [13] with slight modification. Aqueous extract of 0.5 ml (700 μg) was diluted to 1 ml using distilled water. 0.5 ml of Folinis-phenols reagent and 2.5 ml of 20% sodium carbonate were added to the solution and incubated at 37°C in dark until colour develops. The absorbance was measured at 725 nm (Labman UV Visible Spectrophotometer: LMSP-UV1200PC) and total phenol content was determined and expressed as mg/g Gallic Acid Equivalent (GAE) using calibration curve where Gallic acid (50–200 μg/ml) is used as a standard and the results were analyzed in triplicate [13].

2.6. Cytotoxic effect of S. macranthum aqueous extract on MDA-MB-231 cell line
Breast cancer MDA-MB-231 cell lines were procured from National Centre for Cell Science (NCCS), Pune, India. The trypsinized monolayer cells were subcultured in DMEM in which 10% FBS was added, approximately 20,000 cells (200 μl) were seeded in 96-microtitre plate and the cells were incubated in 5% CO₂ atmosphere at 37°C for 24 hours. 200 μl of different concentration of aqueous extract of the fruit and standard drug camptothecin was added to the partial monolayer (aspirated) of MDA-MB-231 cell suspension. Further the incubation was carried out in 5% CO₂ atmosphere at 37°C for 24 hours. Media containing the drug was aspirated and MTT reagent (10%) was added to each well of the microtitre plate followed by 3 hours of incubation in 5% CO₂ atmosphere at 37°C. After incubation 100 μl DMSO was added where solubilisation of formazan was observed. The percentage of inhibition was calculated and the results were analyzed in triplicate (n = 3), by measuring absorbance of microtitre plate at 570 and 630 nm using enzyme-linked immunosorbent assay reader (Biobase-EL10B) [14].

2.7. Statistical analysis
All the tests were carried out in triplicates (n = 3) and statistically analyzed and is presented as mean ± SE. Further Tukey t-test was carried out and statistical significance of the result was estimated were in Single * refers to p ≤ 0.05 and ** refers to p ≤ 0.01.

3. RESULTS AND DISCUSSION
The total yield of aqueous crude extract of fruit and calyx under the controlled parameters was found to be 15.04 g for 200 g of dried fruit and 1.303 g for 10 g of calyx (Table 1). The qualitative analysis of the aqueous extract was found to be positive for different phytoconstituents which is depicted in Table 2. Secondary metabolites present in most of the plant species naturally, comprise vivid pharmacological properties in them, because of which these metabolites have gained a wide importance in the field of medicine [15]. As per the earlier reports, alkaloids and phenols are the secondary metabolites present in many in the species of the plant kingdom. Some of the pharmacological therapeutic properties of these metabolites in the Solanum species are reported to display potentially antiinflammatory, antitumor, anticholinergic antiviral, antiplasmodial, and antimicrobial activities [16].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aqueous fruit extract</th>
<th>Aqueous calyx extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of sample</td>
<td>200</td>
<td>10 g</td>
</tr>
<tr>
<td>Total hours of extraction</td>
<td>144 hours (6 days)</td>
<td>144 hours (6 days)</td>
</tr>
<tr>
<td>Yield</td>
<td>15.04 g</td>
<td>1.303 g</td>
</tr>
<tr>
<td>Percentage of extract yield</td>
<td>7.52%</td>
<td>13.03%</td>
</tr>
<tr>
<td>Colour of extract</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
</tbody>
</table>

Table 1: The extracted yield of the S. macranthum aqueous fruit and calyx extract.
antihypertensive, antiulcer, analgesic, antioxidant, regulating carcinogen metabolism, oncogenesis expression, inhibiting DNA binding and cell adhesion [16,17]. The total alkaloid content present in aqueous extract of the *S. macranthum* was determined using the gravimetric analysis method described by Senguttuvan et al. [12] with slight modification. The total alkaloid content present in the aqueous extract of the fruit was calculated to be 7.58%. Using Gallic acid as a standard, the total phenol content present in the aqueous extract of the *S. macranthum* fruit was estimated to be 158.774 mg/GAE; this was calculated using the calibration curve (Fig. 1 and Table 3).

Evaluation of anticancer property of the plant will enable to identify the intrinsic toxicity of the plant and the effects of acute overdose [18,19]. *Solanum* plant species have been well documented for their various therapeutic properties, including anti-inflammatory, antidiabetic, antioxidant, and also anti-cancer activity [16,17]. The bioactive compounds, such as glycoalkaloids, solamargine, solasodine, and solasonine, present in these species have been reported to possess anticancer activity and correlate to the toxic effect exhibited by them.

The MTT assay is being frequently used as an *in vitro* method to determine the cytotoxicity of the crude extract as well as isolated compounds. The MTT assay is mainly based on the reduction of tetrazolium salts by the metabolically active cells to form a purple colored formazan product by the action of dehydrogenase enzymes [20,21]. The earlier reports on the essential oils extracted from the *S. macranthum* fruit, had 79.39% cytotoxic effect at 250 μg/ml on Hs 578T cell line and 47.43% on PC-3 cell line at 100 μg/ml [22]. In the present study MDA-MB-231 breast cancer cell line was used to evaluate *in vitro* cytotoxic potential of aqueous extract of *S. macranthum* fruit. The extract was found to be very potent to inhibit the growth of MDA-MB-231 breast cancer cell line at low concentration, i.e., at 50 μg/ml concentration and toxic effect was elevated with increased concentration and the IC$_{50}$ value was calculated to be 525.59 μg/ml (Table 4, Figs. 2 and 3). The cytotoxic effect of the aqueous extract was similar to that of standard camptothecin at 40μM.

### Table 2: The table depicts the phytoconstituents present in the *S. macranthum* aqueous fruit and calyx extract.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Phenols</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous fruit extract</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aqueous calyx extract</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

++: Moderately Present, +: poorly present, -: Absent.

### Table 3: Total phenol content of aqueous extract of *S. macranthum* fruit (AESF).

<table>
<thead>
<tr>
<th>Concentration (µg)</th>
<th>Absorbance at 725 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>00</td>
</tr>
<tr>
<td>50</td>
<td>0.359 ± 0.01**</td>
</tr>
<tr>
<td>100</td>
<td>0.816 ± 0.02**</td>
</tr>
<tr>
<td>150</td>
<td>1.073 ± 0.01**</td>
</tr>
<tr>
<td>200</td>
<td>1.456 ± 0.03**</td>
</tr>
<tr>
<td>250</td>
<td>1.844 ± 0.01**</td>
</tr>
<tr>
<td>AESF</td>
<td>0.793 ± 0.01**</td>
</tr>
</tbody>
</table>

Data is presented as mean standard error of the mean (n = 3). Statistical significance was assessed using one-way ANOVA. *p < 0.05 **p < 0.01.

### Table 4: Cytotoxic effect of aqueous extract of *S. macranthum* fruit on MDA-MB-231 cell line.

<table>
<thead>
<tr>
<th>Concentration µg</th>
<th>Absorbance at 630 nm</th>
<th>Inhibitory activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.04 ± 0.01</td>
<td>–</td>
</tr>
<tr>
<td>100</td>
<td>1.12 ± 0.25*</td>
<td>99.08</td>
</tr>
<tr>
<td>200</td>
<td>1.07 ± 0.18**</td>
<td>94.49</td>
</tr>
<tr>
<td>300</td>
<td>0.93 ± 0.27*</td>
<td>81.65</td>
</tr>
<tr>
<td>400</td>
<td>0.77 ± 0.21*</td>
<td>66.97</td>
</tr>
<tr>
<td>500</td>
<td>0.63 ± 0.16*</td>
<td>54.12</td>
</tr>
<tr>
<td>Camptothecin (40 µM)</td>
<td>0.61 ± 0.18*</td>
<td>52.29</td>
</tr>
</tbody>
</table>

Data is presented as mean standard error of the mean (n = 3). Statistical significance was assessed using one-way ANOVA. *p < 0.05 **p < 0.01.

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**Figure 1:** Depicts the calibration curve of Gallic acid standard for the Determination of total phenol content of aqueous extract of *S. macranthum* fruits.

**Figure 2:** Depicts cytotoxic effects of *S. macranthum* fruits aqueous extract on MDA-MB-231 cell line, concentrations (100–500 µg/ml) in triplicate (standard deviations in parentheses); Human breast cancer cell line IC$_{50}$ = 525.59 µg/ml; negative controls, medium and DMSO had no effects in the assay; Reference compound camptothecin at 40μM concentration.
4. CONCLUSION
In conclusion, the aqueous extract of *S. macranthum* fruit tends to possess potential anticancer property on MDA-MB-231 Breast cancer cell line. The phyto constituents, such as alkaloids, phenols, and saponins present in the extract may contribute to its cytotoxic property. Thus, further investigation need to be performed to identify the pharmacological bioactive compound present in the aqueous extract of *S. macranthum* fruit to assess its anticancer effects in *in vivo* models.

ACKNOWLEDGMENT
The authors would like to acknowledge Department of Biotechnology Karnatak University Dharwad and P.C Jabin Science College Hubballi for providing the laboratory facility to carry out the research. The authors would also like to acknowledge the support rendered by Cytxon Biosolutions Pvt Ltd Hubballi.

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How to cite this article: