

ORIGINAL CONTRIBUTION

Open Access



In-vitro cytotoxic effects of *Solanum macranthum* fruit. Dunal extract with antioxidant potential

Vishal U. Kalebar^{1,2}, Joy H. Hoskeri³, Shivaprakash V. Hiremath² and Murigendra B. Hiremath^{1*}

Abstract

Background: The current study undertaken to evaluate antioxidant property of *Solanum macranthum* fruit methanol extract and its in-vitro cytotoxic effects on breast cancer MDA-MB-231 and mice embryo fibroblast (MEF-L929) cell line.

Methods: The total alkaloids, phenols and flavonoids content in the methanol extract of *S. macranthum* fruit were estimated. The antioxidant activity was evaluated by FRAP assay, H₂O₂ scavenging assay and DPPH assay. Anti-inflammatory protein denaturation inhibition assay was carried out using bovine serum albumin. Anticancer property of the extract was evaluated against breast cancer MDA-MB-231 by MTT assay and comparative study was carried out on normal cells using MEF-L929 cell line.

Results: Phytochemical estimation revealed that total alkaloid content was measurable high (13.6 g/100 g of extract), where as the total phenolics and flavonoids content was 115.5 mg/GAE and 142.6 mg/QE. Further, the antioxidant effect of methanol extract by H₂O₂ assay showed considerably higher scavenging activity with 99.6% at 400 µg/ml, where as by FRAP assay the relative reducing power was estimated to be 39.1% at 400 µg/ml and in case of DPPH assay it was found to be 47.9% at 400 µg/ml. EC₅₀ value of anti-inflammatory assay was found to be 112.96 µg/ml. Anticancer studies showed that the IC₅₀ value was found to be 373.77 µg /ml against MDA-MB-231 breast cancer cell line, where as the methanol extract showed negligible cytotoxic effect against normal cells (MEF -L929 cell line), indicating its cancer specific effect.

Conclusion: Based on the results obtained, methanol extract of *Solanum macranthum* fruit was found to possess significant antioxidant properties and also showed potential anticancer property against MDA-MB-231 breast cancer cell line with negligible cytotoxic effect on normal cells revealing its potential application as an anticancer agent.

Keywords: Antioxidant activity, Anti-inflammatory, cytotoxic effect, Breast Cancer; MDA-MB-231; MEF-L929 cell lines

Introduction

Globally Breast Cancer ranks second cause of death (i.e., overall 522,000 deaths annually) with the mortality rate of 6 per 100,000 in Eastern Asia and 20 per 100,000 in Western Africa (Globocan 2018). Among the Indian population breast cancer is Highest compared to other cancer with ASR incidences of 25.76 per 100,000 women with the death ratio of 2:1 (out of two newly diagnosed

breast cancer in women one dies) and the estimated incidence rate of 200,000 new diagnosed cases per year by 2030 [1–3]. Development of proper treatment against cancer has been a continuous global effort, with regard to this many of the natural products are being tested against various cell lines to identify and develop a potential anticancer agent. The currently available cancer treatment includes chemotherapy, radiation therapy, hormonal therapy and surgical removal of breast [4]. But these treatments generally pose life threatening side effects on morbid and economic weak population of developing countries like India and also such population

* Correspondence: murigendra@gmail.com

¹Department of Biotechnology and Microbiology, Karnatak University, Dharwad, Karnataka, India

Full list of author information is available at the end of the article

cannot afford the expensive synthetic drugs. According to the reports of National Cancer Institute (NCI) 35,000 plant species possess anticancer properties and as per the reports of World Health Organization (WHO), 252 molecules are FDA approved anticancer drugs, out of which 11% are derived from plants. India is considered to be possessing rich source of medicinal plants and hence poses major producer of plant based medicine owing upto 25,000 effective plant-based formulations used in ethanobotanical communities in India, with many reports available on anticancer property of plant extracts, but their respective bioactive phytochemicals have remained unexplored [5]. The present investigation is focused on unmasking the anticancer property of an important plant *Solanum manranthum* Dunal (syn. *S. wrightii* Benth) or 'Giant Potato tree', which is a shrub and an ornamental plant [6]. *Solanum* "Sunberry" (Solanaceae) consisting of approx 2700 species, covering nearly 60% species of the solanaceas family [7]. The solanaceae family exhibits a vivid variety of medicinal properties including antimicrobial, anti-inflammatory, antioxidant as well as anticancer application [8]. With this background the present investigation was undertaken to assess the anticancer potential of *S. macranthum* fruit extract. This investigation has also focused on validation of cancer specific effect of *S. macranthum* fruit extract through evaluation of its non-toxic property against normal cells in order to set a platform for its use as an anticancer agent.

Materials and methods

The *Solanum macranthum* plant Fruit was collected from the medicinal garden of K.L.E's P. C Jabin Science Hubballi. The fruits were dried and subjected to sequential extractions using Petroleum ether, Chloroform, Methanol and water solvents. The qualitative analysis of all the solvents extracts revealed that methanol extract of *S. macranthum* fruit possessed more phytochemical profile than the other solvent extract which has been discussed in detail in our pervious studies [8]. Therefore, the present investigation was carried out using methanol extract of *S. macranthum* fruit.

Determination of Total alkaloids

S. macranthum fruit methanol extract (5gm) was transferred to 150 ml of acetic acid (20%). The mixture was incubated for 4 h at room temperature and filtered. The filtrate was heated on boiling water bath until the

mixture was reduced to one fourth of its volume. The filtrate was cooled and ammonium hydroxide solution was added drop wise until the occurrence of the precipitation in the filtrate was ceased. The precipitate formed was allowed to settle down and filtered. The percentage of the alkaloid was determined applying the formula to dried filtrate of alkaloid obtained from the extract [9]. The results were analyzed in triplicate.

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

Determination of Total flavonoids

The estimation of total flavonoids was carried out by following method described by Zhishen et.al. [10]. 20 µg of the methanol extract and aliquots of standard quercetin ranging from 10 to 50 µg was diluted up to 1 ml using absolute methanol. 200 µl of distill water and 150 µl of (5%) sodium nitrate was added to the dilution. After incubating the mixture for 5 min, 150 µl of (10%) aluminium chloride was added and incubated for further 6 min. 2 ml of (4%) sodium hydroxide was added and kept for 15 min at room temperature. The development of pink colour indicates the presence flavonoids, which was measured at 510 nm (Labman UV Visible Spectrophotometer: LMSP-UV1200PC). The total flavonoid was tabulated using calibration curve and the results were analyzed in triplicates.

Determination of Total phenols

Total phenol content was determined by Folin-phenols reagent method described by Siddhuraju et.al. 2003 [11]. 20 µg of the methanol extract of the fruit was diluted up to 1 ml using distilled water. To this 500 µl of diluted Folin-phenols reagent (1:1 with water) and 2.5 ml of sodium carbonate were added. The mixture was incubated for 40 min in dark until the colour develops. The absorbance was measured at 725 nm (Labman UV Visible Spectrophotometer: LMSP-UV1200PC). Gallic acid (10-50 µg/ml) was used to construct the calibration curve. The results were analyzed in triplicate. The total phenol of methanol extract was expressed (mg of gallic acid) using standard graph.

Determination of antioxidant property by in-vitro assays

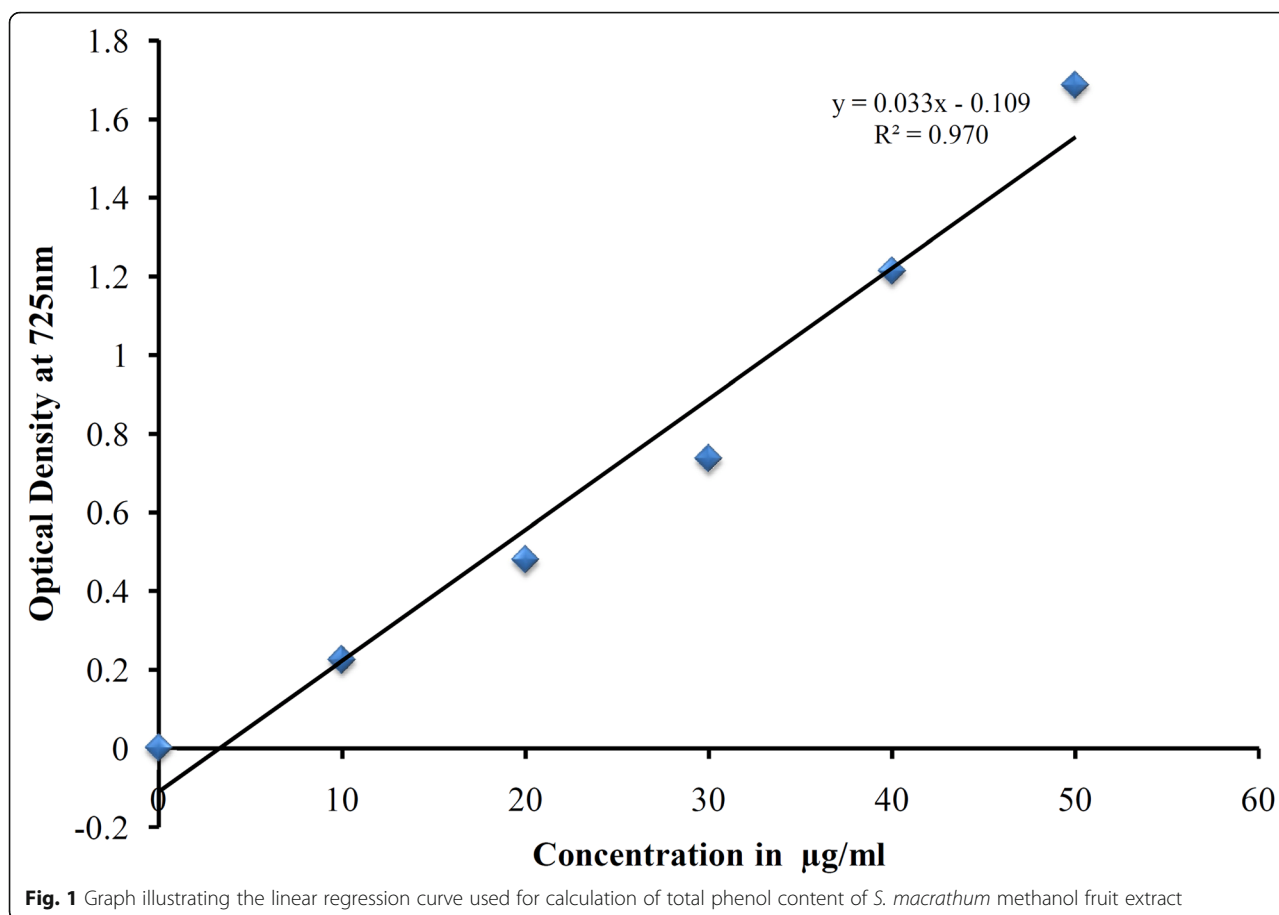
Ferric ion reducing antioxidant power (FRAP) assay

The total antioxidant capacity of the methanol extract was evaluated by the FRAP assay method with slight modifications according to the procedure described by Benzie and

Table 1 Phytoconstituents present in the *S. macranthum* Fruit methanol extract (SMFE)

Parameters	Alkaloids	Flavonoids	Glycosides	Phenols	Saponins	Tannins	Terpenoids	Steroids	Carbohydrates
SMFE	++	++	++	++	++	+	+	+	+

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



Strain 1996 [12] and the results were analyzed in triplicate. 0.5 mL of various concentrations (25, 50, 100, 200, 400 µg/mL) of *S. macranthum* fruit methanol extract and standard were mixed with 2 mL of freshly prepared FRAP reagent. The control used here contained the reagent but devoid of the extract or the drug. The reaction mixture was mixed thoroughly and incubated for 30 min under dark conditions. Absorbance was read at 593 nm (Labman UV Visible Spectrophotometer: LMSP-UV1200PC). Ascorbic acid is been used as reference standard.

$$\text{Relative\% of reducing power} = \frac{[(As - Ac) / (A_{max} - Ac)] \times 100}{}$$

Where, 'As' is absorbance of Sample, 'Ac' is absorbance of control and A_{max} is highest absorbance of Standard.

H₂O₂ scavenging assay

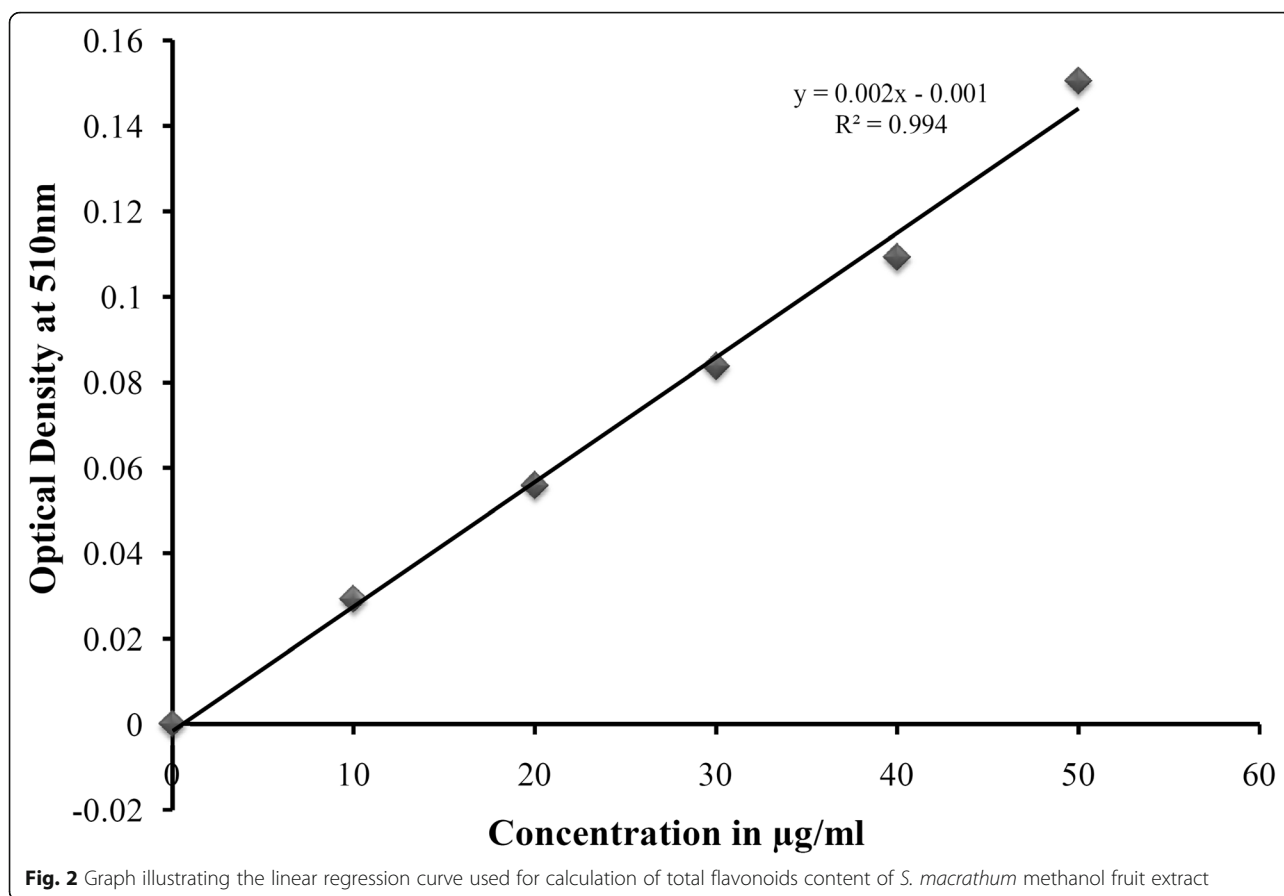
Solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). *S. macranthum* fruit methanol extract (25, 50, 100, 200, 400 µg/mL) in distilled water were added to a 0.6 mL of hydrogen peroxide solution (40 mM) and final volume was adjusted to 1 mL with distilled water. The control used here contained the reagent but

devoid of the extract or the drug. Absorbance of hydrogen peroxide at 230 nm (Labman UV Visible Spectrophotometer: LMSP-UV1200PC) was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both methanol extract and standard compounds were calculated using the formula:

Table 2 Total Phenol content of *S. macranthum* Fruit methanol extract (SFME)

Concentration µg/ml	Absorbance at 725 nm
Control	0.000 ± 0.01
10	0.224 ± 0.01**
20	0.478 ± 0.01**
30	0.734 ± 0.01**
40	1.212 ± 0.01**
50	1.685 ± 0.01**
SFME (20 µg/ml)	0.185 ± 0.01**

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. Significantly different in comparison with control



$$\% \text{Scavenged } [\text{H}_2\text{O}_2] = [(A_C - A_S)/A_C] \times 100.$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample of extracts or standards. Ascorbic acid was used as reference standard [13]. The results were analyzed in triplicate.

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability assay

DPPH of 2ml working solution (0.2 mM) was mixed with 0.5 ml of different concentrations (25, 50, 100, 200, 400 µg/ml) of *S. macranthum* fruit methanol extract and standard solution followed by incubation for 30 min in dark at room temperature. The control used here contained the reagent but devoid of the extract or the drug. The absorbance was measured at 517 nm (Labman UV Visible Spectrophotometer: LMSP-UV1200PC). The percent antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{Antioxidant activity} = [(A_C - A_S)/A_C] \times 100$$

Where, 'Ac' and 'As' are the absorbance of control and sample, respectively. Ascorbic acid is been used as reference standard [14]. The results were analyzed in triplicate.

In vitro anti-inflammatory activity

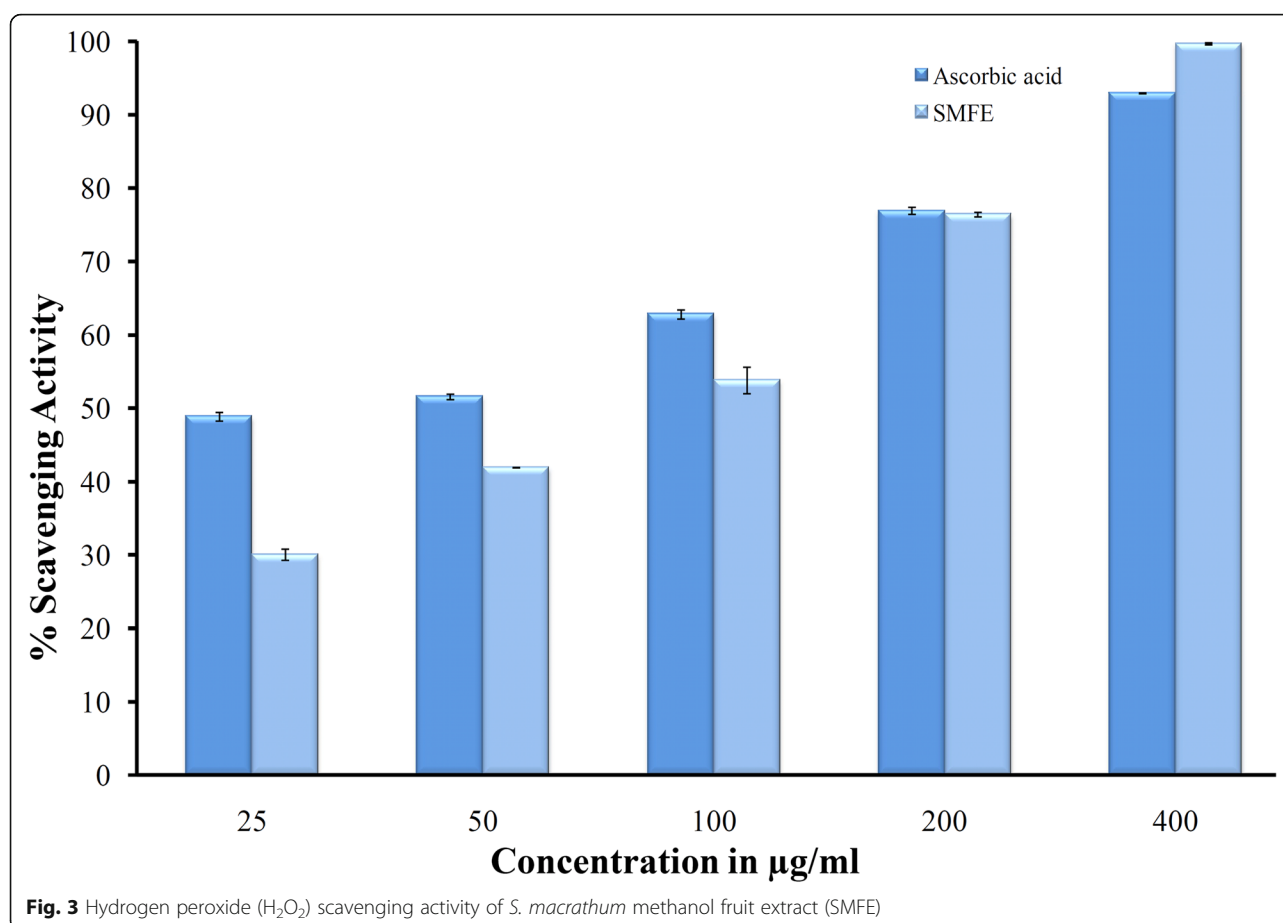
The anti inflammatory activity of methanol extract of *S. macranthum* fruits was assessed according to Chandra et al. 2012 [15] and Sangeetha et al. 2011 [16] using the inhibition of albumin denaturation method with slight modification and the results were analyzed in triplicate.

Test solution: 0.2% w/v aqueous solution of BSA (100 µl) and test solution of different concentrations (50, 100, 150, 200, and 250 µg/ml) of methanol extract of *S.*

Table 3 Total Flavonoid content of *S. macranthum* Fruit methanol extract (SFME)

Concentration µg/ml	Absorbance at 510 nm
Control	0.01 ± 0.01
10	0.03 ± 0.01**
20	0.06 ± 0.01**
30	0.08 ± 0.01**
40	0.11 ± 0.01**
50	0.15 ± 0.01**
SFME (20 mg/ml)	2.85 ± 0.01**

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



macranthum fruit along with phosphate buffer saline (PBS) pH 6.4 (2 ml) were used.

Test control solution: 0.2% w/v aqueous solution of BSA (100 µl) and PBS (2 ml) were used.

Standard solution: 0.2% w/v aqueous solution of BSA (100 µl) and Diclofenac sodium 100 µg/ml was used.

The assay mixture was incubated at 37 °C for 20 min followed by incubation at 70 °C for 5 min. The assay mixture was cooled and the absorbance was measured

using Labman UV Visible Spectrophotometer: LMSP-UV1200PC at 660 nm. The absorbance values were determined by calculating the difference in the OD values of all the samples prior incubation and after incubation time. The control represents 100% protein denaturation. The percentage inhibition of protein denaturation was calculated by the following formula:

$$\% \text{ of Inhibition} = \{(\text{Abs Control} - \text{Abs sample}) / \text{Abs Control}\} \times 100$$

Table 4 Antioxidant assays of *S. macranthum* Fruit methanol extract (SFME)

Concentration µg/ml	DPPH Assay Absorbance at 517 nm		FRAP Assay Absorbance at 593 nm		H ₂ O ₂ Assay Absorbance at 230 nm	
	Ascorbic acid	SFME	Ascorbic acid	SFME	Ascorbic acid	SFME
Control	1.4 ± 0.01	1.4 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.74 ± 0.01	0.74 ± 0.01
25	1.01 ± 0.01**	1.15 ± 0.01**	1.36 ± 0.01**	0.54 ± 0.01**	0.38 ± 0.01**	0.52 ± 0.01**
50	0.95 ± 0.02**	1.13 ± 0.01**	1.90 ± 0.01**	0.58 ± 0.01**	0.36 ± 0.01**	0.43 ± 0.01**
100	0.69 ± 0.03**	1.06 ± 0.01**	2.39 ± 0.01**	0.74 ± 0.01**	0.28 ± 0.01**	0.34 ± 0.01**
200	0.42 ± 0.01**	0.96 ± 0.01**	2.56 ± 0.03**	0.97 ± 0.01**	0.17 ± 0.01**	0.18 ± 0.01**
400	0.11 ± 0.01**	0.73 ± 0.01**	2.69 ± 0.01**	1.27 ± 0.01**	0.05 ± 0.01**	0.01 ± 0.01**

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

Comparative cytotoxic evaluation of *S.macranthum* methanol fruit extract on MDA-MB-231 and MEF-L929 cell line

Breast cancer MDA-MB-231 and noncancerous MEF-L929 cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. The monolayer cell culture was trypsinized cells, were subcultured using Dulbecco modified eagles medium supplemented with 10% FBS, such that 200 μ l of suspension contains approximately 20,000 cells which was seeded to the 96 microtitre late and incubated at 37 °C with 5% CO₂ atmosphere for 24 h.

When partial monolayer was formed after 24 h of incubation the culture was aspirated, 200 μ l of different test concentrations (100,200,300,400,500 μ g/ml) of methanol extract of *S.macranthum* fruit along with reference standard drug camptothecin (15 μ M) were added to the partial monolayer of the cell suspension of both the cell lines separately and incubated at 37 °C with 5% CO₂ atmosphere for 24 h. The drug containing media was aspirated and 10% MTT reagent was then added to each well and incubated at 37 °C with 5% CO₂ atmosphere for 3 h. After incubation, solubilisation of formazan occurred by the

addition of 100 μ L di-methylsulfoxide. The percentage of inhibition was calculated in triplicate ($n = 3$) by measuring observance of microtitre plate at 570 nm and 630 nm using microtiter plate reader [17].

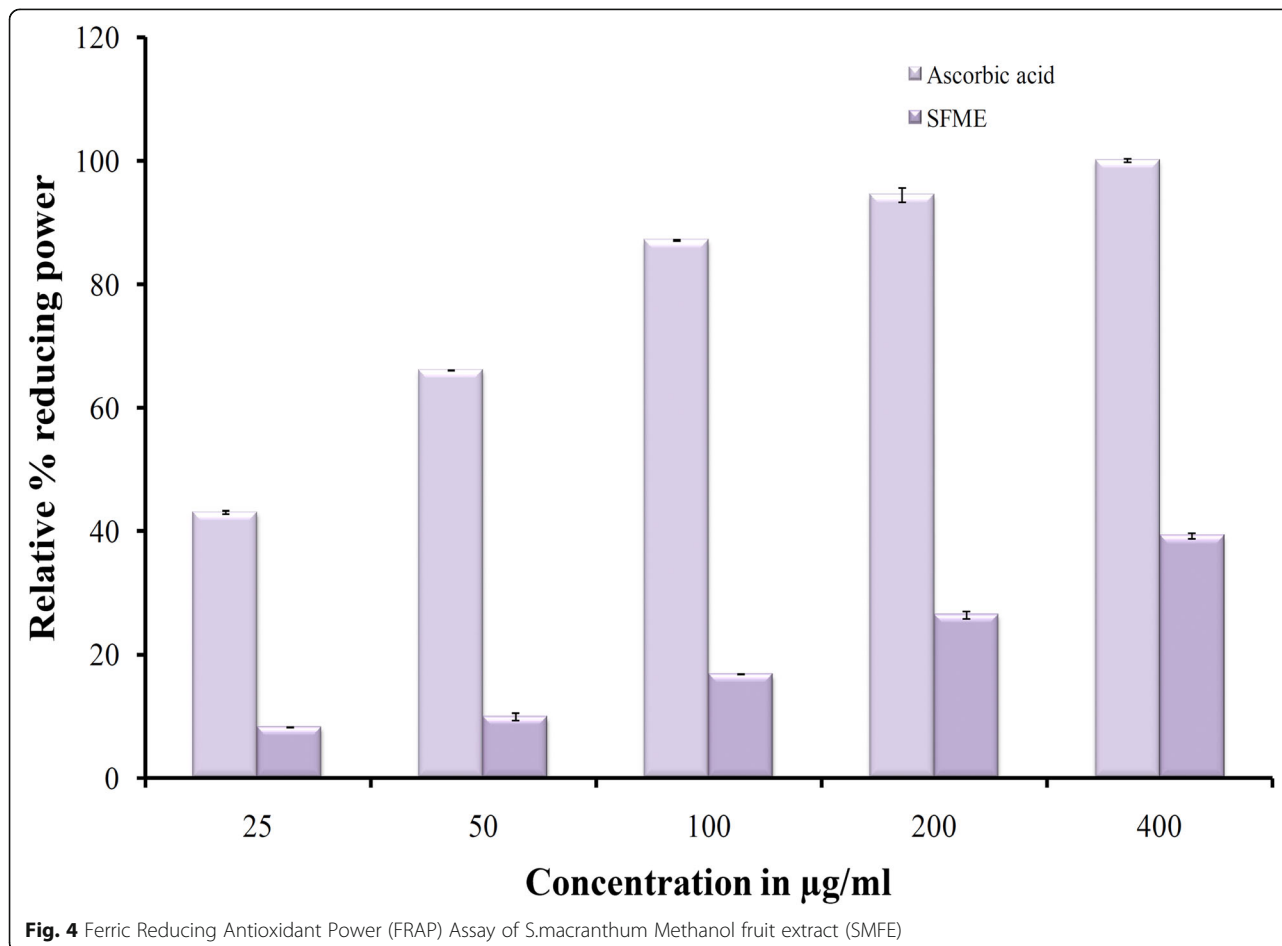
Statistical analysis

All the tests were carried out in triplets ($n = 3$) and statistically analyzed and is presented as mean \pm S.E. using ezANOVA statistical programme. Further Tukey's t-test was carried out and statistical significance of the result was determined where e * refers to $p \leq 0.05$ and ** refers to $p \leq 0.01$.

Results

Phytochemical analysis

The qualitative analysis of the methanol extract were found to posses more phytoconstituents which was revealed by qualitative analysis indicating the presence of alkaloids, falvonoids, glycosides, phenols, saponins, tannins, terpenoids, steroids and carbohydrates which is briefed in the Table 1.



Total alkaloid content

The alkaloids of the methanol extract of *S. macranthum* fruit was determined by the method followed by Sengutuvan et.al. 2014 with slight modification. Gravimetric analysis for alkaloids content in the extract sample was found to be 13,600 mg/100 g.

Total phenols content

Total phenols content of the methanol extract of *S. macranthum* fruit was estimated by using Gallic acid as standard. The total phenol present in the extract sample was found to be 115.5 mg/GAE which was calculated using the standard graph Fig. 1 and Table 2.

Total flavonoids

The total flavonoids content present in the methanol extract of *S. macranthum* fruit was found to be 142.6 mg/quercetin equivalent, which was calculated using the standard graph Fig. 2 and Table 3.

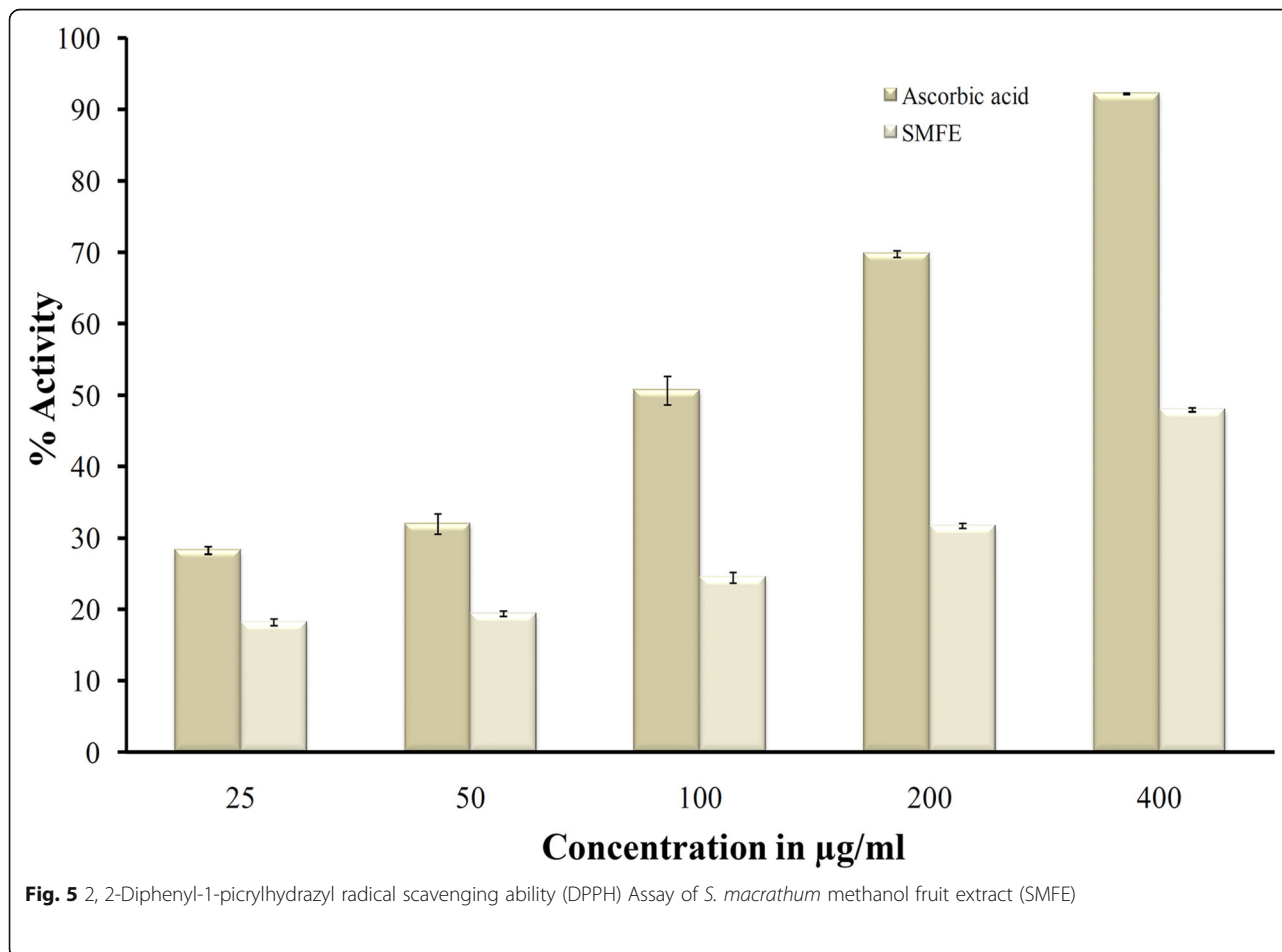
Antioxidant activity

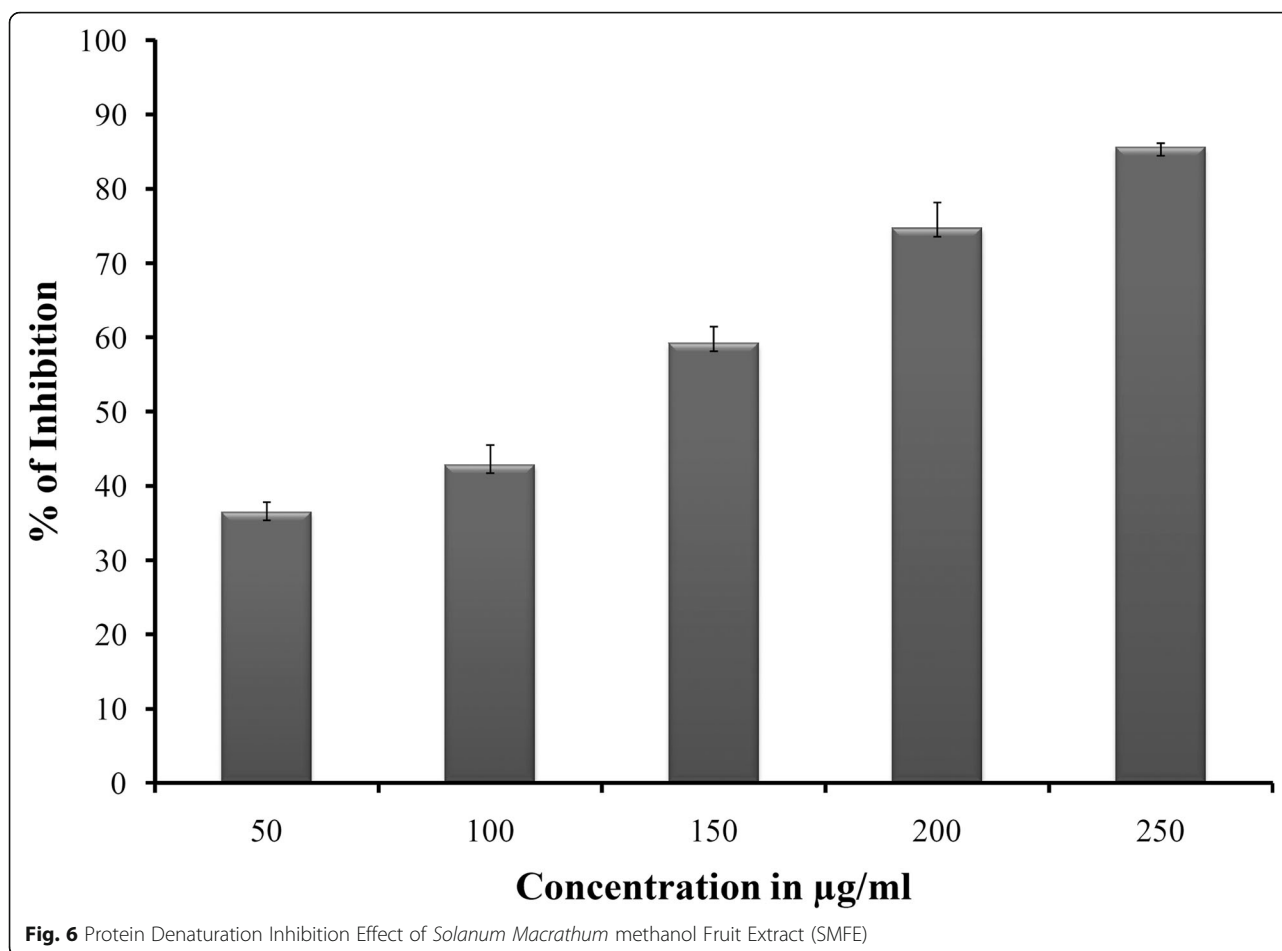
Hydrogen peroxide (H_2O_2) scavenging activity

Hydrogen peroxide (H_2O_2) scavenging activity of *S. macranthum* fruit methanol extract was carried out along with reference standard ascorbic acid. The results obtained revealed that effective concentration (EC_{50}) of methanol extract and standard was 97.24 μ g/ml and 14.83 μ g/ml respectively. At 200 μ g/ml concentration the percentage of scavenging activity of methanol extract was significantly similar at par with reference standard ascorbic acid i.e., 76.38% and 76.89% respectively. Further with the increase in the concentration of 400 μ g/ml, methanolic extract antioxidant activity significantly increased and had higher percentage of scavenging activity when compared with standard reference ascorbic acid i.e., 99.6% and 92.8% respectively. Hydrogen peroxide (H_2O_2) scavenging activity of *S. macranthum* fruit methanol extract along with standard reference ascorbic acid is depicted in Fig. 3 and Table 4.

Ferric reducing antioxidant power (FRAP) assay

The assay was based on reducing power of the antioxidant, where the reduction of ferric ion (Fe^{3+}) to the





ferrous ion (Fe^{2+}) take place by donating electron. The methanolic extract of *S. macranthum* fruit was subjected to FRAP assay and ascorbic acid was used as standard. The methanol extract showed significant antioxidant activity with increase in concentration and EC_{50} value was calculated to be 515.144 $\mu\text{g/ml}$. Ferric Reducing Antioxidant Power Assay of *S. macranthum* fruit methanol extract along with standard reference ascorbic acid is depicted in Fig. 4 and Table 4.

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability (DPPH) assay

The assay is based on accepting of hydrogen donor by DPPH compound from antioxidant molecule. The methanol extract of *S. macranthum* fruit obtained was subjected to DPPH assay with ascorbic acid as reference. The extract showed a significant antioxidant activity with increase in the concentration and was maximum with 47.9% at 400 $\mu\text{g/ml}$ and EC_{50} was calculated to be 426.112 $\mu\text{g/ml}$. DPPH Assay of *S. macranthum* fruit methanol extract along with standard reference ascorbic acid is depicted in Fig. 5 and Table 4.

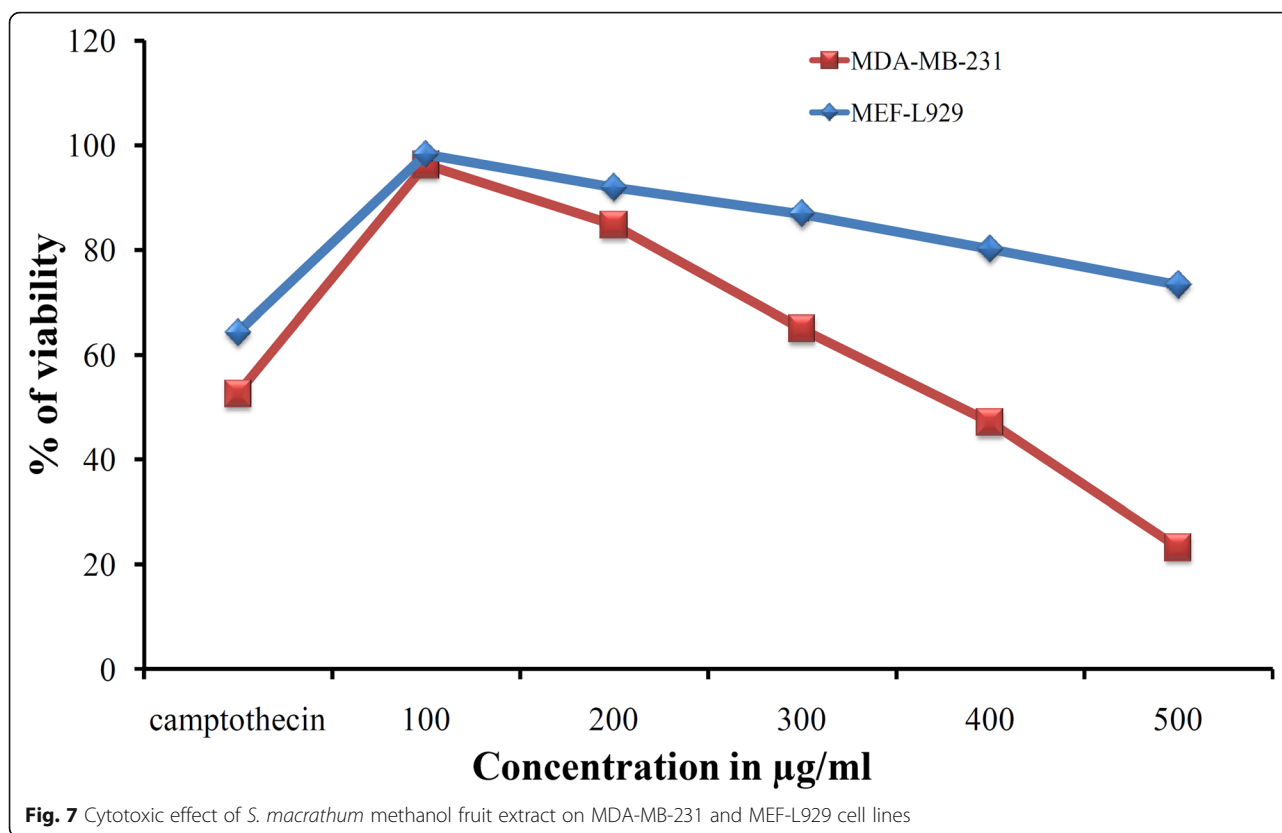
Anti-inflammatory assay

The SMFE showed a significant protein denaturation inhibitory effect in concentration dependant manner which has been depicted in the Fig. 6 and Table 5. The SMFE was found to have a maximum inhibition of protein denaturation of 85% at 250 μg concentration which is at par with standard Diclofenac sodium whose percentage of inhibition was to found 73% at 100 $\mu\text{g/ml}$. The EC_{50} of the SMFE was calculated to be 112.96 $\mu\text{g/ml}$.

Table 5 Anti-inflammatory effect of *S. macranthum* Fruit methanol extract (SFME)

Concentration $\mu\text{g/ml}$	Absorbance at 660 nm	% of Inhibition
Control	0.037 \pm 0.01	–
50	0.023 \pm 0.01*	36.36364
100	0.021 \pm 0.01**	42.72727
150	0.014 \pm 0.01*	60.90909
200	0.009 \pm 0.01**	74.54545
250	0.005 \pm 0.01**	86.27273

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



Comparative cytotoxic evaluation of *S. macrathum* methanol fruit extract on MDA-MB-231 and MEF-L929 cell line

MTT assay measures the cell proliferation rate and conversely when metabolic activity decreased eventually leads to apoptosis or necrosis, representing reduction in cell viability. The methanolic extract of *S. macrathum* fruit through MTT assay revealed significant cytotoxic effect on MDA-MB-231 cell line, however no growth inhibition was induced on MEF-L929 cells. The cytotoxic effect of the methanolic extract had similar effects as that of standard camptothecin against MDA-MB-231 cell lines. Morphologically the MDA-MB-231 cells appeared to be shrink, followed by the death of the cells induced by the methanolic extract of *S. macrathum* fruit. The IC_{50} value of *S. macrathum* fruit methanolic extract was 373.77 µg/ml against MDA-MB-231 breast cancer cell lines. However, *S. macrathum* fruit methanolic extract showed negligible toxicity against normal cell line MEF-L929, where in the highest tested concentration (500 µg/ml) itself showed 73.29% cell viability, whereas the IC_{50} was found out to be in negative concentration hence revealing its non toxic property against normal cells. This study clearly indicated the cancer specific cytotoxic effect of *S. macrathum* fruit methanolic extract (Figs. 7 and 8 and Table 6).

Discussion

Plant derived compounds are drawing a great interest due to their versatile applications, they have been richest bio-source of drugs among the traditional medicine, modern medicine, food supplements and as well as chemical entities of synthetic drugs [18]. The qualitative phytochemical analysis of the *S. macrathum* fruit had been carried for four different solvent extracts i.e., petroleum ether, chloroform, methanol and water extract. Among them methanol extract was found to be positive for various phytoconstituents such as alkaloids, tannins, terpenoids, flavonoids, saponins and phenols [8]. The prominent phytoconstituents which are reported for their importance in combating with various diseases and disorders are likely to be alkaloids, flavonoids and phenols. These bioactive compounds have predominantly available in the vast variety of plant species. In this study the methanol extract of *S. macrathum* fruit expressed a greater amount of these bioactive compounds, among these alkaloid concentration (13,600 mg/100 g) tend to be higher followed by flavonoids (142.6 mg/QE) and phenols (115.5 mg/GAE). These bioactive compounds also display a potentially antioxidant property.

Reactive oxygen species (ROS) majorly constitute as pathological factor involved in serious diseases, neurodegenerative disorders, damaging the cells and one of the

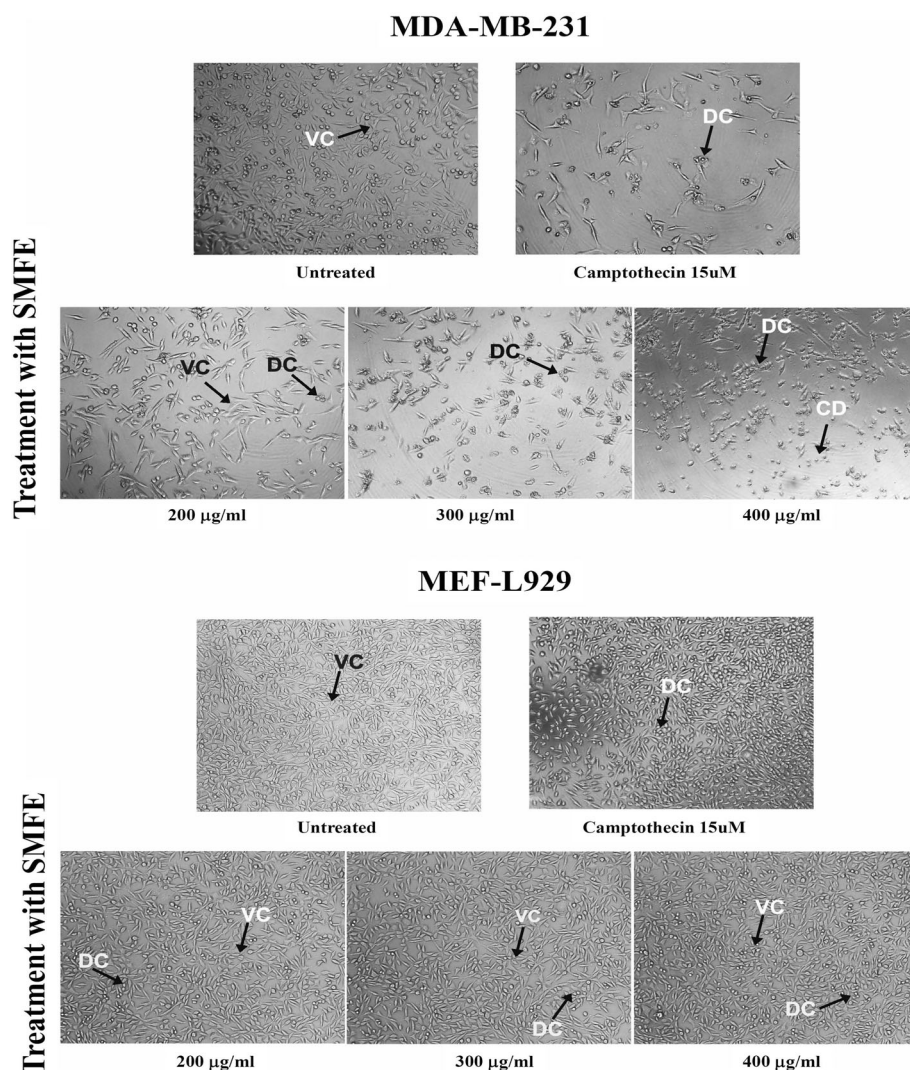


Fig. 8.

causative factors to induce cancer. There are reports which signifies and implies that oxidative stress has significant correlation between the occurrences of breast cancer especially in premenopausal women [19]. Many of the phytoconstituents have the ability to suppress these free radicals by donating the electrons and balancing their valences [20]. Solanum species are known to possess a great variety of medicinal properties including antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic, microbicidal, cytotoxic, anti-convulsant, antiulcerogenic and also against sexually transmitted diseases [21]. The study focused on evaluating the antioxidant property of *Solanum macranthum* extract, since there have been no reports of antioxidant activity of the plant till date. The methanol extract of the *S. macranthum* fruit was tested for their antioxidant properties by subjecting to the H₂O Assay, DPPH assay

and FRAP assay. Hydrogen peroxide (H₂O₂) assay is one of the most widely accepted assay to determine the antioxidant activity of any compound, the activity is based on the ability of antioxidant to inhibit the formation of hydroxyl radicals and malondialdehyde (MDA) and thereby reduce or prevent the degradation of 2-deoxyribose [20]. When methanol extract of *S. macranthum* fruit was tested for its antioxidant activity by H₂O₂ assay, it had similar antioxidant activity at par with reference standard ascorbic acid at 200 µg/ml concentration, but at 400 µg/ml concentration the antioxidant activity of methanol extract increased appreciably than reference ascorbic acid, with EC₅₀ value of 51.872 µg/ml, which reveals that methanol extract of *S. macranthum* fruit had major radical scavenging activity.

FRAP assay, based on the reduction of ferric tripyridyl-triazine complex to its ferrous colored form. This

Table 6 Cytotoxic effect of *S.macranthum* Fruit methanol extract (SFME) on MDA-MB-231 and MEF-L929 cell line

Concentration µg/ml	MDA-MB-231		MEF-L929	
	Absorbance at 630 nm	% of Viability	Absorbance at 630 nm	% of Viability
Blank	0.04 ± 0.01	–	0.04 ± 0.01	–
Control	1.13 ± 0.15**	100	0.61 ± 0.01**	100
100	1.08 ± 0.16*	96.27	0.90 ± 0.01**	98.29
200	0.96 ± 0.14*	84.74	0.85 ± 0.01**	91.93
300	0.75 ± 0.06**	64.89	0.80 ± 0.01**	86.87
400	0.55 ± 0.02**	47.08	0.75 ± 0.01**	80.17
500	0.29 ± 0.01**	23.14	0.68 ± 0.01**	73.29
Camptothecin (15µM)	0.61 ± 0.09*	52.51	0.92 ± 0.01**	64.26

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

reduction of 2,4,6-Tripyridyl-S-Triazine (TPTZ)-Fe (III) complex to TPTZ-Fe(II) by the methanolic extract of *S.macranthum* fruit was used to assess the total reducing power of antioxidants. Under the acidic condition when an TPTZ-Fe³⁺ complex is reduced by electron donating antioxidants, the change from colourless Fe³⁺ to blue colored Fe²⁺ was measured at 593 nm [22]. The results revealed that methanolic extract had prominent antioxidant activity with increase in the concentration and with EC₅₀ value of 515.144 µg/ml, establishing that the bioactive compounds present in the methanol extract of *S.macranthum* fruit has an notable antioxidant property.

DPPH Assay is based on hydrogen donating ability of the compound. The DPPH free organic nitrogen radical is very stable which contains an odd electron when reacts with antioxidant compounds that can donate hydrogen atoms, after accepting the electron it reduces and the purple color is changed to yellow. This degree of reduction in absorbance measurement at 512 nm is indicative of scavenging potential of compounds [23]. From the experimental data methanolic extract showed properties of scavenging the free radicals (EC₅₀ = 426.112 µg/ml) and posses antioxidant activity with increase in the concentration.

Denaturation of the proteins has been well documented as cause inflammation in the individual. When external stress or compound, such as strong acid or base, a concentrated inorganic salt, organic solvent or heat is exerted on these proteins loses its primary and secondary structure configuration. Under such condition most of the biological proteins get denatured [24].

Precisely chronic inflammation has also been implicated to complications such as arthritis, stroke, and cancer [25]. There has been an substantial epidemiologic and experimental data which configures significant role of inflammation has impacted in establishment, progression, and/or aggressiveness of various malignancies, for most of the biological entities involved in signaling pathways in apoptosis, cell proliferation, and angiogenesis

were found to be common in both inflammation and carcinogenesis [26]. As part of the current investigation the SMFE exhibited its anti-inflammatory property ranging from 36.36% to 85.45% with EC₅₀ value of 112.96 µg/ml, signifying its ability to inhibit the protein denaturation at par with standard drug.

The bioactivity assessment of plant extracts its ability to inhibit the proliferation of cancerous cells will help in its utilization as a therapeutic agent [27]. For evaluating the preliminary cytotoxic effect of the synthetic derivatives, natural products and natural products extracts MTT assay is most widely preferred, where the reduction of tetrazolium salts by mitochondrial dehydrogenase by pur-ple formazan product [28].

Various reports have been documented of Solanaceae family for their medicinal applications [29]. Among them few of the species have proven to have anticancer property but at very higher concentration such as, methanol extract of *S.nigrum* ripe berries had effective cytotoxic activity with IC₅₀ value of 4.8 mg/ml on HL-60 promyelocytic leukemia cell line [23] and on *HeLa* cell line having IC₅₀ value of 265.0 mg/ml [30]. Ethyl acetate leaves extract of *Solanum anguivi* was found to be toxic for HepG2 and MCF-7 cell lines whose IC₅₀ value was reported to be 0.625 mg/ml and 1.25 mg/ml respectively [31]. On the Ehrlich Ascites Carcinoma cells (EAC) the ethanolic extract of *Solanum torvum* fruit berries showed its toxicity with IC₅₀ value of 600 µg/ml [32]. This cytotoxic effect is majorly due to certain bioactive active compounds such as glycoalkaloids, solamargine, solasodine, and solasonine, found in these species specially solamargine are responsible to induce apoptosis in cancer cell lines [33–35].

In this study, the in vitro cytotoxicity was assessed using non cancerous L929-Mouse fibroblast Cell Line and MDA-MB- 231 breast cancer Cell line. The results obtained revealed that the methanol extract significantly inhibited the growth of MDA-MB- 231 cell line with IC₅₀ value of 373.77 µg/ml which is considerably higher

cytotoxic effect than the solanum plant species stated above, while the methanol extract of *S. macranthum* fruit did not exhibit any toxicity against proliferation of normal MEF-L929 cells even at higher concentration. The results also confirmed that the differential effect induced by the extracts and standard drug in cancerous and normal cells. As a result the inhibition of the growth of the cancerous cells by *S. macranthum* methanol fruit extract can be conceivably due to the several bioactive components present in them.

Conclusion

In conclusion methanol extract of the *S. macranthum* exhibits strong antioxidant potential, anti-inflammatory response and possess specific cytotoxic effect towards breast cancer cells while posing no toxic effect on the normal cell line. Thus further investigations should be made in order to find the potential bioactive phytoconstituent present in the fruit of *S. macranthum* and assess its anticancer effects in in-vivo models.

Acknowledgements

The authors would like to acknowledge 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 Cytixon Biosolutions Pvt. Ltd. Hubballi.

Authors' contributions

VUK: Accomplishment of the experimental works as well as analysis and acquisition of data. JHH: - Study design of the experiment and final draft of the manuscript. SVH:- reviewed and gave critical analysis of the manuscript MBH: Supervised and designed the study as well as drafted and corrected all versions of the manuscript. All authors read and approved the final manuscript.

Funding

This research study was not granted any specific fund.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Biotechnology and Microbiology, Karnatak University, Dharwad, Karnataka, India. ²Department of Biotechnology, P.C. Jabin Science College, Vidyannagar, Hubballi, Karnataka 580031, India. ³Department of Bioinformatics and Biotechnology, Karnataka State Akkamahadevi Women's University, Vijayapura, Karnataka 568108, India.

Received: 8 June 2019 Accepted: 12 March 2020

Published online: 23 April 2020

References

- Singh D, Singh P, Gupta A, Solanki S, Sharma E, Nema R. Qualitative estimation of the presence of bioactive compound in *Centella asiatica*: an important medicinal plant. *Int J Life Sci Med Sci*. 2012;24–7.
- Kulkarni BB, Kulkarni SS, Hallikeri UR, Patil BR, Gai PB. Decade of breast cancer-trends in patients profiles attending tertiary cancer care center in south India. *Asian J Epidemiol*. 2012;5:103–3.
- Ali R, Mathew A, Rajan B. Effects of socio-economic and demographic factors in delayed reporting and late-stage presentation among patients with breast cancer in a major cancer hospital in South India. *Asian Pac J Cancer Prev*. 2008;4:703–7.
- Hortobagyi GN. Multidisciplinary management of advanced primary and metastatic breast cancer. *Cancer*. 1994;74:416–23.
- Sateesh MK, Mohsin B. Screening of the ethnobotanicals against MDA-MB-231 and MCF-7 breast cancer cell lines. *Int J Phytopharmacy*. 2014;4:140–7.
- Essien EE, Walker TM, Newby JM, Ogunwande IA, Setzer WN, Ekundayo O. The floral essential oil composition and biological activity of *Solanum macranthum* Dunal. *Am J Essent Oils Nat Prod*. 2016;4:36–9.
- Yadav R, Rathi M, Pednekar A, Rewachandani Y. A detailed review on Solanaceae family. *Euro J Pharm Med Res*. 2016;3:369–78.
- Kalebar VU, Hoskeri JH, Hiremath SV, RV ABK, Sonappanavar KL, Agadi BS, et al. Pharmacognostical and phytochemical analysis of *Solanum macranthum* (Dunal) Fruits. *J Pharmacogn Phytochem*. 2019;8:284–90.
- Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. *Asian Pac J Trop Biomed*. 2014;4:59–67.
- Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. 1999;64:555–9.
- Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agr Food Chem*. 2003;51:2144–55.
- Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239:70–6.
- Keser S, Celik S, Turkoglu S, Yilmaz O, Turkoglu I. Hydrogen peroxide radical scavenging and total antioxidant activity of *hawthorn*. *Chem J*. 2012;2:9–12.
- Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol*. 1995;28:25–30.
- Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effect of ashwagandha: a preliminary study in vitro. *Pharmacognosy J*. 2012;4:47–9.
- Sangeetha M, Kousalya K, Lavanya R, Sowmya C, Chamundeeswari D, Reddy CU. In-vitro anti-inflammatory and anti-arthritis activity of leaves of *Cleodendron inerme*. *Res J Pharm Biol Chem Sci*. 2011;2:822–7.
- Alley MC, Scudiero DA, Monks A, Czerwinski MJ, Shoemaker RH, Boyd MR. Variation of an automated microculture tetrazolium assay (MTA) to assess cell growth and drug sensitivity of human tumor cell lines. *InProc Am Assoc Cancer Res* 1986;2:389.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*. 2011;1:98–106.
- Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, et al. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res*. 1999;59:602–6.
- Afsar T, Razak S, Shabbir M, Khan MR. Antioxidant activity of polyphenolic compounds isolated from ethyl-acetate fraction of *Acacia hydasipica* R. Parker. *Chem Cent J*. 2018;12:5.
- Jayakumar K, Meenu Krishnan VG, Murugan K. Evaluation of antioxidant and antihemolytic activities of purified caulophyllumine-A from *Solanum mauritianum* Scop. *J Pharmacogn Phytother*. 2016;5:195–9.
- Muselik J, Garcia-Alonso M, Martín-López MP, Žemlička M, Rivas-Gonzalo JC. Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. *Int J Mol Sci*. 2007;8:797–809.
- Bokhari J, Khan MR, Shabbir M, Rashid U, Jan S, Zai JA. Evaluation of diverse antioxidant activities of *Galium aparine*. *Spectrochim Acta A Mol Biomol Spectrosc*. 2013;102:24–9.
- Jayashree V. n in-vitro anti-inflammatory activity of 4-benzylpiperidine. *Asian J Pharm Clin Res*. 2016;9:108–10.
- Osman NI, Sidik NJ, Awal A, Adam NA, Rezali NI. In vitro xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. *J Intercult Ethnopharmacol*. 2016;5:343.
- Wong RS. Role of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in cancer prevention and cancer promotion. *Adv Pharmacol Sci*. 2019;2019:1–10.

27. Gabrani R, Jain R, Sharma A, Sarethy IP, Dang S, Gupta S. Antiproliferative effect of *Solanum nigrum* on human leukemic cell lines. *Indian J Pharm Sci.* 2012;74:451–3.
28. McCauley J, Zivanovic A, Skropeta D. Bioassays for anticancer activities. In *Metabolomics Tools for Natural Product Discovery*. 2013;1055:191–205.
29. Yu S, Sheu HM, Lee CH. *Solanum incanum* extract (SR-T100) induces melanoma cell apoptosis and inhibits established lung metastasis. *Oncotarget.* 2017;8:103509–17.
30. Patel S, Gheewala N, Suthar A, Shah A. In-vitro cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and Vero cell line. *Int J Pharmacy Pharm Sci.* 2009;1:38–46.
31. Gandhiappan J, Rengasamy R. Antiproliferative activity of *Solanum anguivi* against cancer cell lines. *Der Pharm Lett.* 2012;4:875–80.
32. Panigrahi SW, Sundaram Muthuraman M, Natesan RA, Pemiah BR. Anticancer activity of ethanolic extract of *Solanum torvum* sw. *Int J Pharmacy Pharm Sci.* 2014;6:93–8.
33. Kupchan SM, Barboutis SJ, Knox JR, Cam CA. Beta-solamarine: tumor inhibitor isolated from *Solanum dulcamara*. *Science.* 1965;150:1827–8.
34. Cham BE, Meares HM. Glycoalkaloids from *Solanum sodomaeum* are effective in the treatment of skin cancers in man. *Cancer Lett.* 1987;36:111–8.
35. Liu LF, Liang CH, Shiu LY, Lin WL, Lin CC, Kuo KW. Action of solamargine on human lung cancer cells—enhancement of the susceptibility of cancer cells to TNFs. *FEBS Lett.* 2004;577:67–74.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)