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RESEARCH ARTICLE

Phytochemicals of *Salacia oblonga* responsible for free radical scavenging and antiproliferative activity against breast cancer cell lines (MDA-MB-231)

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Abstract *Salacia oblonga*, an inhabitant of tropical regions has been used in traditional Indian medicinal systems. Phytochemicals were extracted in methanol from the plant and analyzed for various biological activities. The results of biochemical tests for total phenolics (297 ± 0.005 and 275 ± 0.006) and flavonoids (95 ± 0.004 and 61.6 ± 0.004) in the aerial and root parts were indicated as Gallic acid and quercetin equivalents respectively. The Aerial and root extracts showed strong reducing ability based on reducing power and FRAP assays. The extracts exhibited significant IC_{50} values in DPPH, super oxide and nitric oxide radical scavenging assays. The extracts displayed low IC_{50} values (<50 μg/ml) when assessed for antiproliferative activity against breast cancer cell lines using the MTT assay. GC-MS analysis of methanolic extracts have revealed the presence of compounds viz. n-Hexadecanoic acid, N-Methoxy-N-methylacetamide, Ursa-9(11), 12-dien-3-ol, Gamma-sitosterol etc., that might be potential candidates for the biological activity exhibited by the extract.

Keyword *Salacia oblonga* · Antioxidant activity · Free radical scavenging activity · Reactive oxygen species · Antiproliferative activity

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Introduction

Plant natural products contain wide variety of active components that can be exploited to treat many chronic and infectious diseases (Duraipandiyan et al. 2006). Natural antioxidants extracted from plant sources have renewed the interest of scientists, in this area as they are helpful in scavenging the free radicals produced in the body with less side effects. These free radicals generated in the body through various physiological processes have been implicated in many pathological conditions (Jomova and Valko, 2011).The disease progression and risk of chronic diseases can be reduced either by enhancing the antioxidant defense or by supplementation of dietary antioxidants (Stanner et al. 2000). The role of polyphenolics and flavonoids in scavenging the free radicals has been reported in several plant species (Djeridane et al. 2006) as they exhibit significant antioxidant activity (Abourashed, 2013). There are reports of natural antioxidants from fruits and vegetables, however, antioxidants from herbs and tree species have not been exploited much. The plants used in traditional medicinal system can act as sources of natural antioxidants due to the presence of flavonoids and phenolic compounds responsible for free radical scavenging abilities (Wojdylo et al. 2007). Production of reactive oxygen and nitrogen species is a regular phenomenon in human physiology, these reactive species when over-produced (due to oxidative stress) can react with bio-molecules causing cellular damage and injury, that leads to chronic diseases viz. cancer, cardio-vascular diseases etc. The antioxidant activity of plants can be attributed by various mechanisms like prevention of chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen extraction, reducing power and radical scavenging activities (Yuan et al. 2006). Cancer is a major public health problem in the world, since starting of human civilization plants have

been used for therapeutic purposes and they are also part of source of modern medicine. Many therapeutic medicines for cancer treatment are identified and isolated from the plant natural products, hence there is renewed interest for plant derived natural products to combat cancer. (Prakash et al. 2013; Solowey et al. 2014).

Salacia oblonga an inhabitant of tropical regions has been used in ayurveda and traditional Indian system of medicine for its potent medicinal properties. The bark and roots of *S.oblonga* have been used in the treatment of diabetes, polyuria, gonorrhea, rheumatism, asthma and fever (Yuhao et al. 2008). *S. oblonga* root extracts inhibit the inflammation by increased vascular permeability in male albino rats using carrageenan-induced rat paw oedema (acute inflammation) and cotton pellet granuloma (Ismail et al. 1997). Anti-inflammatory activity of *S. oblonga* exhibited by the phytochemicals of the root crude extracts include polyphenols, flavonoids, alkaloids, tannins, saponins and other secondary metabolites. These phytochemicals might inhibit the COX 1 (cyclooxygenase 1) and COX 2 gene expression during inflammation in rats (Murugesan and Deviponnuswamy, 2014). *S. oblonga* extracts have displayed hypoglycemic, antioxidant and cytoprotective activities (Palani et al. 2011; Chawla et al. 2013). Phytochemicals of *S. oblonga* bind to the intestinal enzymes responsible for breakdown of carbohydrates in the body. These enzymes called alpha-glucosidases, convert carbohydrates to glucose, the sugar that circulates throughout the body. These enzymes bind to the salcinol and kotanol present in the herbal extracts of *S. oblonga* rather than to carbohydrate so that less glucose gets into the blood stream resulting in low blood glucose level and insulin. Presence of various phytochemicals viz., salcinol, kotanol and mangiferin from *S. obloga* extracts have proven biological activities (Huang et al. 2006; Nakata et al. 2011). Salcinol and kotanol have shown anti-diabetic or hypoglycemic activities (Kumar et al 1999). Mangiferin is one of the major active components in tubers of *Salacia* species including *Salacia oblonga* (Chawla et al. 2013; Kaliappan et al. 2014). Antioxidant and cytoprotective activities of *Salacia oblonga* are due to the presence of mangiferin, phenolics, flavonoids and other phytochemicals present in the crude extracts. These secondary metabolites can scavenge the free radicals by donating the hydrogen molecules to oxidizing agents. *S. oblonga* extracts displayed antimicrobial activity against different human pathogens (Rao and Giri, 2010; Musini et al. 2013). Antimicrobial properties of *S. oblonga* and its mode of action may depend on several factors viz., disruption of the membrane structure and function, disruption of the DNA/RNA synthesis and function, interference with intermediate metabolism (proteins), induction of coagulation of cytoplasmic content and interference with intracellular communications (Quorum sensing). The present study was designed to evaluate the antioxidant, free radical scavenging and anticancer activities of methanolic aerial and root extracts of *S.oblonga.* The extracts were also subjected to phytochemical analysis to investigate the presence of compounds.

Materials and methods

Preparation of extracts

S.oblonga plants were collected from the Western Ghats, India, and authenticated by Dr. Sidda Mallaya, Research officer, Regional Research Institute, Bangalore, India. The shade dried plants were separated into aerial and root parts and ground into a fine powder using an electric blender. The phytochemicals were extracted in methanol with the help of a soxhlet apparatus and concentrated using a rotatory evaporator (IKA, Germany) under reduced pressure.

Total phenolic content

Total phenolic content of plant extracts was determined using Folin-Ciocalteu's reagent (Kim et al. 2003). Plant extract (250 μg) was dissolved in distilled water and the final volume was adjusted to 2 ml whereas control was maintained with distilled water without extracts. 1 ml of 10 % Folin-Ciocalteu's reagent was added to all the tubes including control (2 ml distilled water). After a brief vortex, 1 ml of 10 % sodium carbonate was added to it and the mixture was incubated at room temperature for 1 h, the absorbance was measured at 760 nm using a UV-Visible spectrophotometer (Shimadzu). The phenolic content was expressed as GAE (Gallic acid equivalence) in terms of μg/mg of dry weight of the extracts obtained from the standard graph of Gallic acid.

Total flavonoid content

Total flavonoid content of the extracts (aerial and roots) was measured by the aluminium chloride colorimeter assay (Kumar et al. 2008). 250 μg of extract was mixed with 2 ml of H_20. To the above mixture, initially 0.3 ml of 5 % $NaNO_2$ was added followed by addition of 0.3 ml of 10 % $AlCl_3$. After 6 min 1 ml NaOH was added and the total volume was made up to 5 ml with distilled water. The absorbance was measured at 510 nm and calculated from the standard graph of quercetin. The results were expressed as μg of quercetin equivalent/mg weight of the sample.

Reducing power assay

Reducing power of *S. oblonga* extracts was determined by the method of Yen and Chen (1995). Plant extracts (250 μg), were

incubated with 2.5 ml of phosphate buffer (0.2 M pH 6.6) and 2.5 ml of 1 % potassium ferricynade (K_3 Fe (CN) $_6$) at 50 °C for 20 min. The reaction was terminated by adding 2.5 ml of 10 % TCA (Trichloro acetic acid) solution and centrifuged at 3000 rpm for 10 min. The supernatant was incubated at room temperature for 10 min along with 2.5 ml of H_20 and 1 ml of 0.1 % $FeCl_3$ (Ferric chloride) solution and the absorbance was measured at 700 nm along with controls, butylated hydroxyl toluene (BHT) and quercetin (250 μg/ml). The reducing power of the extracts was directly proportional to the absorbance which in turn is associated with high colour intensity.

Ferric reducing antioxidant power assay (FRAP)

FRAP assay was carried out according to the method of Benzie and Strain (1999) which is based on the reduction of TPTZ-Fe^{3+} complex to TPTZ-Fe^{2+} in the presence of antioxidants. The FRAP solution was prepared by mixing 25 ml acetate buffer (300 Mm, pH 3.6), 2.5 ml TPTZ solution (10 Mm in 40 mM HCl) and 2.5 ml $FeCl_3$ solution (20 mM). Extracts (250 μg/ml) were made up to 2 ml with distilled water and incubated with 1 ml of FRAP reagent for 20 min. A standard graph of $FeSO_4$ was plotted, from which the reducing ability of extracts, BHT and quercetin (250 μg/ml) was calculated.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity

DPPH radical-scavenging activity of *S. oblonga* extracts was determined as described by Burits and Bucar (2000). The capacity of extracts to scavenge lipid-soluble 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, resulted in purple colour exhibited by the stable DPPH radical. 1.0 ml of extracts (100–500 μg/ml), quercetin and BHT (100–500 μg/ml) in ethanol were added separately to 4 ml of 0.004 % methanolic solution of DPPH. After incubation for 30 min at room temperature in the dark, the absorbance was measured at 517 nm. Tests were carried out in triplicate. The ability of extracts, quercetin and BHT to scavenge DPPH radical was calculated using following equation:

$$\text{Radical scavenging activity } (\%) = [A_0 - A_1 / A_0] \text{ x } 100.$$

Where A_0 was absorbance of negative control (containing all reagents except test compounds) and A_1 was absorbance of the extracts or quercetin or BHT. DPPH scavenging activity of extracts and standard was expressed as IC_{50}, which was interpolated from a graph constructed using percentage of inhibition (Y-axis) against concentration (X-axis) of extracts and standards.

Super oxide radical scavenging activity

The ability of *S.oblonga* extracts, quercetin and BHT to quench generation of superoxide radicals was determined according to the method of Nishikimi and Rao (1972) with a slight modification. Superoxide radicals were generated in PMS-NADH system by oxidation of NADH and analysed by NBT reduction. 1 ml of extracts (100–500 μg/ml) and quercetin/BHT (100–500 μg/ml) were mixed separately with 2.9 ml of phosphate buffer (40 Mm, pH 7.4) and 1 ml of Nitroblue tetrazolium solution (150 μM in 40 mM phosphate buffer, pH 7.4). The reaction was initiated by the addition of 1 ml phenazine methosulfate (60 μM in 40 mM phosphate buffer, pH 7.4) to the mixture. After incubation at 25 °C for 5 min, absorbance was measured at 560 nm. Negative control was subjected to same procedures as extracts, except that the extracts were replaced with solvent control. All the experiments were made in triplicate. The ability of extracts, quercetin and BHT to scavenge superoxide radical was calculated using following equation:

$$\text{Superoxide radical scavenging activity } (\%) = [A_0 - A_1 / A_0] \text{ x } 100$$

Where A_0 was absorbance of negative control at 560 nm and A_1 was absorbance of the extracts or quercetin/BHT at 560 nm. IC_{50} value, which represents concentration of extracts and standards that caused 50 % inhibition, was determined by a linear regression analysis.

Nitric oxide scavenging activity

The method devised by Rai et al. (2006) based on spontaneous generation of nitric oxide (NO·) from sodium nitroprusside (SNP) buffered solution was used to assess NO· scavenging ability of *S.oblonga* extracts. Briefly, 0.5 ml SNP (10 mM) in phosphate buffer –saline was mixed with 1 ml of extracts (100–500 μg/ml) and incubated in the dark at room temperature for two and half hour. A control was set up as above by replacing the extracts with the water. After incubation, 1 ml of sulfanilic acid reagent (0.33 % sulfanilic acid in 20 % glacial acetic acid) was added to 0.5 ml of reaction mixture. After 5 min, reaction mixture was incubated further with 1 ml 0.1 % naphthyethylenediamine dihydrochloride (NEED) for 30 min at 25 °C. Absorbance of chromophore formed was read at 540 nm. Results were expressed as a percentage of scavenged nitric oxide with respect to negative control. The percentage scavenging of NO· by extracts and standards was calculated using following equation:

$$\text{NO·scavenging activity } (\%) = [A_0 - A_1 / A_0] \text{ x } 100$$

Where A_0 was absorbance of negative control and A_1 was absorbance of extracts or standards. NO· scavenging activity of extracts and standard was expressed as IC_{50}, which was

interpolated from graph constructed using percent inhibition (Y axis) against concentration (X-axis) of the extracts and standards.

Cell lines and culture conditions

In the present study Human Breast cancer cell lines (MDA-MB-231) were used that were procured from National Centre for Cell Science (NCCS) Pune, India. The cell lines were cultured in DMEM (PAN, Germany) containing 10 % FBS (PAA, USA), 50 IU of Penicillin and 50 μg/ml of streptomycin (Sigma–Aldrich, USA) at 37 °C in a humidified atmosphere containing 5 % CO_2.

Cell proliferation assay

Cytotoxic effect of plant methanolic aerial and root extracts on MDA-MB-231 breast cancer cells was assessed using 3-(4, 5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay (Kavitha et al. 2009). Cells were seeded (1×10^5 cells/well) in duplicate in 96-well plate. After 24 h, plant extracts were added at a concentration range of 20–100 μg/ml and incubated for 24 h. MTT reagent (5 mg/ml, Sigma–Aldrich, USA) was added and incubated for 4 h. The blue MTT formazan precipitate was then solubilized in DMSO and incubated for 2 h. Absorbance was measured at 540 nm on a multi well ELISA plate reader scanning spectrophotometer (Multiscan spectrum, USA). Cells grown in culture media alone or with appropriate concentrations of methanol were used as controls. Percentage of cell proliferation was calculated as a ratio of optical density to that of control.

Phytochemical analysis of aerial and root extracts of *Salacia oblonga* by GC-MS (gas chromatography and mass spectrum)

GC - MS analysis, was done by injecting the samples into a HP - 5MS capillary column (30 m length × 250 μm dia. × 0.25 μm film thickness), Agilent Technologies, USA GC-MS model, consisting of 6890 N Gas Chromatograph coupled with 5973 insert MSD [Mass Selective Detector]. The injector was set at 250 °C and the detector at 280 °C. The stepped temperature program-was as follows: held at 50 °C for 2 min, then, from 50 to 280 °C at the rate of 10 °C/min, held for 5 min. The total running time was of 30 min. The GC-MS interface temperature was at 280 °C. The injection volume was 1 μl. The solvent delay was 3 min and was injected in a split less mode. The MS scan range was from 35 to 6000 Da. Their mass spectra (MS) and retention indices (RI) were compared with Wiley library literature data and spectra database (Vaughn and Berhow 2005).

Statistical analysis

The data obtained from the results was calculated as mean ± standard deviation in triplicate. The data was compared by least significant difference ($p \leq 0.05$) test using Statistical Analysis System ver.9.2.

Results and discussion

Total phenolic content

Organic solvents such as methanol, ethanol, and acetone were often used for extraction of bioactive compounds from the plants (Eloff 1998). In our study methanol (polarity index 5.1) was used as a solvent of choice for preparation of extracts. The quantitative determination of the total phenolic content of *S. oblonga* methanolic aerial and root extracts was carried out by Folin-Ciocalteu method and expressed as gallic acid equivalent. Total phenolic content of the aerial and root extracts were found to be 297 ± 0.005 and 275 ± 0.006 μg GAE/mg dry weight of the extracts respectively. Phenolic compounds are one of the most important and ubiquitously found groups in the plant kingdom synthesized during the developmental process (Naczk and Shahidi 2004). The extracts with significant antioxidant activities are rich in polyphenols, that has been established from the earlier studies (Mitsuru et al. 2011). The total phenolic content of *S. oblonga* aerial and root extracts was much higher in comparison to earlier reports from other medicinal plants (Shahriar et al. 2013; Kaur and Mondal, 2014).

Total flavonoid quantification

The role of flavonoids in antioxidant activities and their effect on human health and nutrition has been reported (Pourmorad et al. 2006). Total flavonoid content of the extracts was determined by aluminium chloride method and expressed as percentage of quercetin equivalent. The total flavonoid content of methanolic aerial and root extracts were found to be 95 ± 0.004 and 61.6 ± 0.004 μg quercetin/mg dry weight of the extracts respectively. In the present study aerial extracts of *S.oblonga* have shown more total phenolic and flavonoid content than the root extracts. A comparative study of total phenolic and flavonoid content of *Salacia* species has proven that *S. oblonga* has maximum polyphenol and flavonoid content (Chavan et al. 2013, Subhasree et al. 2013), that is also in corroboration with our findings with *S. oblonga*.

Reducing power assay

The present study demonstrates that, the reducing ability of *S. oblonga* aerial (1.542 ± 0.0085) and root (1.489 ± 0.078)

extracts was superior to standard BHT and lower than quercetin (Table 1). The reducing power of any substance is dependent on the presence of reductants which show anti-oxidative potential by breaking the free radical chain reaction (Ebrahimzadeh et al. 2008). Presence of reductants or antioxidants in methanolic aerial and root extracts caused the reduction of ferric (Fe^{3}+)/ferricyanide complex to the ferrous form (Fe^{2}+) which is determined by the intensity of perl's prussian blue colour. Reducing power of compounds specify that, they are electron donors and reduce the oxidized intermediates (Yen and Chen 1995). The reducing ability of aerial and root extracts of *S. oblonga* (Table 1), revealed that they are good electron and hydrogen donors and could stop the radical chain and remove the free radicals to produce more stable product.

Ferric reducing antioxidant power (FRAP) assay

The aerial and root extracts (250 µg/ml) along with the standards BHT and quercetin, were used to deduce the ferric reducing ability obtained from the $FeSO_4$ standard graph and expressed as mg Fe(II)/mg dry weight of plant material. The results indicated that, quercetin (410.8 ± 0.012) has the highest reducing ability followed by BHT, aerial extract and finally the roots extract (Table 1). The reducing property indicates the efficiency of antioxidant compounds as electron donors, thereby lowering the formation of oxidized intermediates during the lipid peroxidation process (Eloff 1998). FRAP measures the change in absorbance at 593 nm owing to the formation of blue coloured Fe^{2+}-tripyridyltriiazine (TPTZ) compound from the colourless oxidized Fe^{3+} form by the action of electron donating antioxidants (Chellaram et al. 2014). FRAP assay displayed (Table 1) that aerial and root extracts of *S. oblonga* were able to reduce ferric ions efficiently. Dua et al (2013) reported that extracts having higher amount of polyphenols and flavonoids show more reducing property, supporting the antioxidant property of this plant.

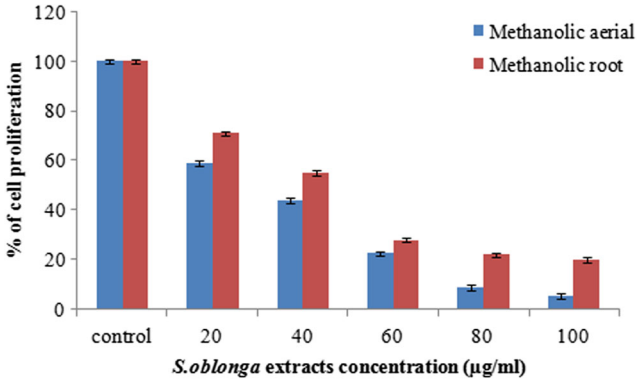

Fig. 1 % of cell proliferation after treatment with methanolic aerial and root extracts by MTT Assay. Values are mean ± SD (n = 3). *p < 0.05

DPPH radical scavenging activity

The aerial and root extracts of *S.oblonga* exhibited potent free radical scavenging activity which was evident from the DPPH radical quenching assay, based on the percentage of inhibition IC_{50} values of extracts and standards was calculated. Both aerial and root extracts have exhibited strong DPPH radical scavenging activity with the IC_{50} being 260 and 260 µg/ml respectively (Table 1). The scavenging activity of the extracts was comparable to control BHT, however, quercetin exhibited the low IC_{50} value 150 µg/ml. Radical scavenging activity is one of the important antioxidant mechanisms. Electron donating capability of natural product can be measured by DPPH purple colored solution bleaching (Krishnaiah et al. 2011). DPPH radical scavenging activity of the *S. oblonga* aerial and root extracts showed more and significant percentage of inhibition and low IC_{50} values indicating better radical scavenging activity, suggesting its antioxidant potency.

Superoxide radical scavenging activity

The aerial and root extracts of *S. oblonga* exhibited inhibition of superoxide radical generation with the IC_{50} values of 170

Table 1 Reducing power assays and comparative scavenging activities of plant extracts, BHT and Quercetin

S.no	Concentration (250 µg/ml)	FRAP[a]	Reducing Power[b]	DPPH IC_{50}(µg/ml)	Super oxide	Nitric oxide
1	Aerial	86 ± 0.051	1.542 ± 0.0085	260	170	185
2	Root	56 ± 0.092	1.489 ± 0.078	260	230	220
3	BHT	119 ± 0.069	1.402 ± 0.059	250	170	225
4	Quercetin	410.8 ± 0.012	1.984 ± 0.0593	150	135	160

Values are mean ± SD (n = 3)

[a] mg $FeSO_4$/mg dry extract

[b] expressed as OD at 700 nm

*p < 0.05

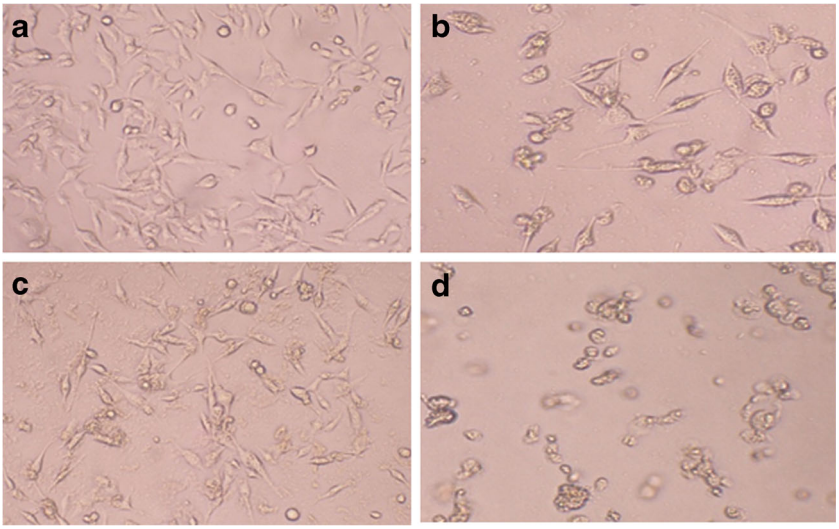

Fig. 2 Morphology of the breast cancer cell lines **a** Control cells. **b** Cells treated with plant aerial extracts 35 μg/ml **c** Treated with plant root extracts 44 μg/ml. **d** Treated with 40 μg/ml of cisplatin

and 230 μg/ml respectively (Table 1). Among the standards, quercetin has shown the highest percentage of inhibition, whereas the values obtained for BHT was similar to the aerial extracts. In the PMS-NADH-NBT system, superoxide anion, derived from dissolved oxygen from the coupling reaction of PMS-NADH, reduce NBT. The aerobic photochemical reduction of NBT leads to the formation of formazan that gives the characteristic purple colour. Whereas, in the presence of antioxidant that donates an electron to NBT, the typical purple colour of NBT starts disappearing that can be visualized spectrophotometrically at 560 nm. The decrease in absorbance was observed with all the extracts, indicating the consumption of super oxide anion in the reaction mixture.

Nitric oxide scavenging activity

Nitric oxide scavenging activity revealed the IC_{50} values of *S. oblonga* aerial and root extracts were found to be 185 and 220 μg respectively. Whereas the IC_{50} values of Quercetin and BHT were 160 and 225 μg respectively (Table 1). As the extracts were able to completely scavenge the $NO^{\cdot}$ released from SNP during the assay, the phytochemicals might have the necessary mechanism to prevent excessive nitric oxide generation thereby preventing the human body from ill effects. Nitric oxide often generated in the human body through various cellular mechanisms, that exhibit a range of beneficial functions, including regulation of vascular tone, neuro-transmission, inhibiting tumor cells and other homeostatic mechanisms. However, higher levels of nitric oxide have been implicated in an array of patho-physiological conditions including inflammation, cancer and cardiovascular diseases (Moncada et al. 1991). The aerial and root extracts of *S. oblonga* exhibited good nitric oxide scavenging activity, with aerial extracts showing higher efficiency in comparison to the root extracts and synthetic standard BHT.

Antiproliferative activity by MTT assay

The Breast cancer cell lines (MDA-MB-231) were treated with various concentrations of the extracts ranging from 20

Table 2 Identification of phytocomponents present in high quantity in the aerial and root extracts of *S. obloga* by GC-MS analysis

Compound name	*S. oblonga* extracts[a]	
	Aerial	Root
n-Hexadecanoic acid	11.94	13.72
6-Octadecanoic acid	2.24	13.34
Hexadecanoic acid, 3-hydroxy methyl ester	1.84	3.75
N-Methoxy-N-methylacetamide	–	17.38
Phytol	0.58	–
1,2 benzene dicarboxylic acid mono(2-ethylhexyl) ester	0.95	2.03
γ –sitosterol	6.73	–
Ursa-9(11), 12-dien-3-ol	8.45	–
2-Ethylacridine	–	6.09

[a] Relative area percentage (peak area relative to the total peak area percentage)

to 100 μg/ml for 24 h in order to evaluate the cytotoxic effect by MTT assay (Fig. 2). Since methanol was the choice of solvent for extract preparation, the cells with methanol were used as vehicle control.

The results indicate that after treatment with plant extract at a concentration of 30 μg/ml, the cell viability decreased dramatically (Fig. 1). The observed inhibition was concentration dependent and morphological changes were recorded in the cell lines and compared with the standard anticancer drug cisplatin (Fig. 2). Based on the above studies, the $IC_{50,}$ value for methanolic aerial and root extracts on breast cancer cells was determined as 35 and 44 μg/ml after 24 h of incubation. This is the first report of anticancer activities of *S. oblonga* aerial and root extracts against human breast cancer cell lines. Extracts have shown good inhibitory activity against this cell line.

GC/MS analysis of methanolic aerial and root extracts

Phytochemicals contribute to the medicinal value of the plant. Both the methanolic aerial and root extracts were subjected to GC-MS analysis for identification of varied phytoconstituents. Methanolic aerial extracts exhibited 32 compounds in which n-Hexadecanoic acid, Ursa-9(11),12-dien-3-ol and γ -sitosterol were present in higher quantities, whereas, methanolic root extracts have shown the presence of 30 compounds in which N-Methoxy-N-methylacetamide, nHexadecanoic acid, 6-Octadecenoic acid and 2-Ethylacridine were present in higher quantities (Table 2).

GC-MS analysis of *S. oblonga* extracts revealed the presence of N-Hexadecanoic acid, and N-Methoxy-N-methylacetamide in higher quantites. The therapeutic potential of which has been established from other plant species (Yizhou et al. 2010; Weil et al. 2011). γ-sitosteral, one of the major constituents found in the aerial extract could be responsible for its anticancer activity by promoting apoptosis based on the study of *Acacia nilotica* (Sundarraj et al. 2012). 1, 2 benzene dicarboxylic acid mono (2-ethylhexyl) ester isolated from the ethanol extract of *Polygonum chinense* was attributed with anti inflammatory and antioxidant activities (Ezhilan and Neelamegam, 2012). Whereas, Phytol isolated from *Peperomia pellucida* leaf extract exhibited anticancer and antibacterial activities (Weil et al. 2011). The mechanism of action of these compounds from *S. oblonga* can be termed polyvalent and additive resulting in the enhanced potency of the active constituents, hence causing synergistic effect. The implications of these findings have a great impact on human health to combat the diseases.

Conclusion

With our study it can be concluded that plyphenols may be the potent antioxidant and free radical scavenging compounds in *S. oblonga* aerial and root extracts. The GC-MS analysis has revealed the presence of phytochemicals which contribute to activities like antimicrobial, antioxidant and anticancer. However, further investigations are needed to identify the active compounds responsible for biological activity and to elucidate the mechanism at molecular level, which can pave way in the rational design of effective molecules for use as therapeutic agents.

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