

Characterization of the Phenolic Compound, Gallic Acid from *Sansevieria roxburghiana* Schult and Schult. f. Rhizomes and Antioxidant and Cytotoxic Activities Evaluation

Rajalekshmi Maheshwari, Chandrashekara Shastry Shreedhara, Picheswara Rao Polu, Renuka Suresh Managuli¹, Seena Kanniparambil Xavier, Richard Lobo, Manjunath Setty, Srinivas Mutalik¹

Departments of Pharmacognosy and ¹Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India

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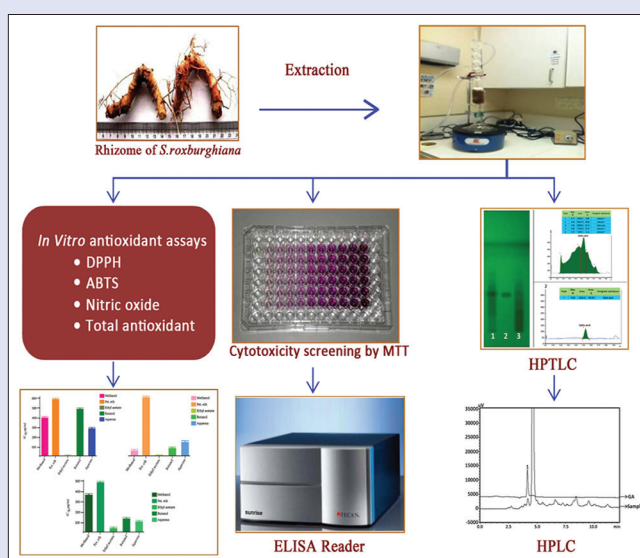
ABSTRACT

Background: *Sansevieria roxburghiana* Schult. and Schult. f. (Asparagaceae) grows in India, Indonesia, Sri Lanka, and tropical Africa. Even though the plant has been traditionally used for the treatment of many ailments, the antioxidant and antiproliferative activities of *S. roxburghiana* methanol extract and its fractions have not yet been explored. **Materials and Methods:** Quantitative estimation of phenols and different antioxidant assays were performed using standard methods. Anti-proliferative effect of the extract and fractions were evaluated in HCT-116, HeLa, MCF-7, HepG2, and A-549 cancer cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sulforhodamine B (SRB) assay methods. High-performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC) fingerprint profiling were carried out for extract and different fractions. **Results:** Significant antioxidant and anti-proliferative activity were detected in ethyl acetate fraction. Ethyl acetate fraction showed prominent scavenging activity in 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and nitric oxide antioxidant assays with an concentration yielding 50% inhibition (IC₅₀) 15.33 ± 1.45, 45.3 ± 1.93 and 48.43 ± 0.46 µg/ml, respectively. Cytotoxicity of ethyl acetate fraction was the highest among other fractions against HCT-116, HeLa, and MCF-7 cancer cell lines with IC₅₀ values 16.55 ± 1.28, 12.38 ± 1.36, and 8.03 ± 1.9 µg/ml, respectively, by MTT assay and 15.57 ± 0.70, 13.19 ± 0.49, and 10.34 ± 0.9 µg/ml, respectively, by SRB assay. The presence of gallic acid in the ethyl acetate fraction of *S. roxburghiana* rhizomes was confirmed by HPLC and HPTLC analysis. **Conclusion:** Results suggested that ethyl acetate fraction exhibited effective antioxidant and antiproliferative activities. The phenolic compounds identified in ethyl acetate fraction could be responsible for the activities.

Key words: Antioxidant, antiproliferative, high-performance liquid chromatography, high-performance thin layer chromatography, *Sansevieria roxburghiana*

SUMMARY

- Sansevieria roxburghiana* has been selected for *in vitro* antioxidant and cytotoxicity screening
- Ethyl acetate fraction of methanol extract of *S. roxburghiana* exhibited effective antioxidant and antiproliferative activities
- The activity of ethyl acetate fraction may be due to the presence of phenolic compound which is identified by high-performance liquid chromatography and high-performance thin layer chromatography techniques.



Abbreviations used: %: Percent, °C: Celsius, µg: Microgram, µl-Microlitre, ANOVA: Analysis of variance, DMSO: Dimethyl sulfoxide, g: Grams, IC₅₀: Concentration yielding 50% inhibition, Kg: Kilogram, mg: Milligram, min: Minutes, ml: Milliliter, HPLC: High-performance liquid chromatography, HPTLC: High-performance thin layer chromatography, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, GAE: Gallic acid equivalents, SRME: Methanol extract of *S. roxburghiana*, ROS: Reactive oxygen species, SRPE: Petroleum ether fraction of *S. roxburghiana*, SREA: Ethyl acetate fraction of *S. roxburghiana*, SRAQ: Aqueous fraction of *S. roxburghiana*, DMEM: Dulbecco's Minimum Essential Medium, FBS: Fetal bovine serum, OD: Optical density, TPC: Total phenolic content, SRBU: Butanol fraction of *S. roxburghiana*.

Correspondence:

Dr. Chandrashekara Shastry Shreedhara,
Department of Pharmacognosy, Manipal College
of Pharmaceutical Sciences, Manipal University,
Manipal - 576 104, Karnataka, India.
E-mail: css.shim@manipal.edu
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INTRODUCTION

Many plants and plant-derived products are used in cancer therapy. Taxol is used to treat breast cancer, whereas the vinca alkaloids are used for leukemia. These compounds are effective as anticancer agents due to the presence of chemopreventive compounds.^[1] Many herbs are curative; chemotherapy and cytotoxic drugs are inherently destructive.^[2] In this era, some herbal medicines such as paclitaxel, etoposide, and vincristine are used for cancer treatment but are highly expensive due to less abundance of plants and much less amount of active constituent (example, 500 mg vincristine from 5 kg vinca). With this background, *Sansevieria roxburghiana* was selected for anticancer activity screening as it is reported to have cytotoxicity in brine shrimp lethality assay^[3] and anticancer activity against Ehrlich ascites carcinoma (EAC) in mice.^[4] *Sansevieria* species were traditionally used for cancer therapy specifically for abdominal tumors.^[5]

S. roxburghiana Schult. and Schult. f., the bowstring hemp, is a botanical source for *Murva* – an Ayurvedic drug is a rhizomatous herb belongs to the family Asparagaceae. Leaves are 8–9 in a tuft, up to 90 cm long and 2.5 cm wide, broad toward the middle, rigid, and cross-striped, with a rigid spine-like tip. Flowers are greenish to white tinged with violet in fascicles of 3–6 on long racemes. Perianth tube up to 1 cm long, lobes narrow. Fruit is a globose berry.^[6] *S. roxburghiana* is an ornamental plant, flowering during January to June and is distributed in India, tropical Africa, Indonesia, and Sri Lanka. It has been reported that whole plant is traditionally used for ailments mainly heart diseases, fever, itching, cough, indigestion, and rheumatism.^[4,7] Rhizomes are mucilaginous and used for cold, ear pain, and persistent coughs.^[4,7] Previous investigation of the plant reported the presence of alkaloids, carbohydrates, flavonoids, phenols, glycosides, proteins, anthocyanin and betacyanin, steroids, and saponins.^[8] Phytochemical investigation of the ethanolic extract of *S. roxburghiana* rhizomes led to the identification of nine compounds; palmitic acid, isorhamnetin-3-O- β -D-glucopyranoside, gallic acid, 6,4-dihydroxy-3-propenyl chalcones, bis (2-ethylhexyl) phthalate, bupranidine, caftaric acid, diisobutyl phthalate, and 4-propenoxy-7-hydroxy anthocyanins.^[9] The rhizomes of *S. roxburghiana* are reported to possess antidiabetic,^[7] analgesic,^[3] antimicrobial,^[8] and antioxidant^[3,4,10] activities. It also showed anticancer activity against EAC in mice^[4] and cytotoxicity in brine shrimp lethality assay.^[3]

There are no investigations, so far, on the anticancer activity of *S. roxburghiana* extract to the active molecule level. The present study is aimed to fill this gap and validate the active fraction/s responsible for antioxidant and anticancer activities by established methodology.

MATERIALS AND METHODS

Chemicals

Quercetin, gallic acid, curcumin, ascorbic acid, reagent for total phenolic content-folin-Ciocalteu reagent (FCR), chemicals for antioxidant assays-2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and for cytotoxicity-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sulforhodamine B were procured from Sigma-Aldrich Chemicals Co., USA and high-performance thin layer chromatography (HPTLC) plates from E. Merck, Germany. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were procured from Finar, India. Other reagents and solvents used were of analytical grade.

Cell lines

HCT-116 (colon), HeLa (cervical), MCF-7 (breast), HepG2 (hepatic), A549 (lung) cancer cell lines, and Vero normal cell lines were obtained

from the National Centre for Cell Science, Pune, India and cultured in media-Dulbecco's Minimum Essential Medium with 10% fetal bovine serum and 50 μ g/mL gentamicin; maintained in CO₂ incubator (5% CO₂ and 95% air) at 37°C.

Plant material

Fresh plant material collected from Udyavara, Karnataka during December 2013 was identified and authenticated by Dr. KN Sunil Kumar, Pharmacognosist, SDM Centre for Research in Ayurveda and Allied Sciences, Udyavara, Udipi, Karnataka, India. Sample herbarium was prepared (PP 612) and placed in the raw drug and herbarium museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India.

Extraction and fractionation

Fresh plant material was washed with tap water, cut into small pieces, and shade dried. Dried plant material was powdered and extracted with methanol (methanol extract *S. roxburghiana* [SRME]) by Soxhlet extractor. The extract was concentrated and fractionated with petroleum ether (petroleum ether fraction of *S. roxburghiana* [SRPE]), ethyl acetate (ethyl acetate fraction of *S. roxburghiana* [SREA]), butanol (butanol fraction of *S. roxburghiana* [SRBU]), and water (aqueous fraction of *S. roxburghiana* [SRAQ]). Individual fractions were concentrated and used for the current study.

Phytochemical analysis

Preliminary phytochemical analysis for the identification of major classes of secondary metabolites was carried out as per standard procedures.^[11,12]

Total phenolic content

Quantification of total phenol in extracts and fractions of *S. roxburghiana* was performed using FCR^[13] by spectrophotometric detection method. FCR was prepared by diluting 5 mL of FCR reagent 10 times with water. One milliliter of test solution was added to 5 mL of prepared FCR and 4 mL of sodium carbonate (0.7 M). The reaction mixture was allowed to incubate for 2 h and the absorbance was measured at 765 nm. Calibration curve for gallic acid was plotted and concentration of total phenols in extracts and fractions was determined.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical scavenging activity

Reaction of solution of ABTS (7 mM) and potassium persulfate (2.45 mM) was performed to produce ABTS free radical. This reaction mixture was incubated at room temperature for 16 h in dark. To acquire the absorbance of 0.705 \pm 0.02 at 730 nm, 1 mL of ABTS solution was diluted with methanol. Suitable concentrations of samples and standard (Ascorbic acid) were prepared. The reagent solution without sample was taken as blank. The extracts and standards of 20 μ L were mixed with 180 μ L of prepared ABTS solution and the absorbance was read after 20 min at 750 nm.^[14]

%scavenging = $\left(\frac{A_c - A_s}{A_c}\right) \times 100$; where A_c = Absorbance of control; A_s = Absorbance of sample.

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

Stable DPPH free radical was used to evaluate the scavenging activity of methanol extract and its fractions. Suitable concentrations of samples and standard (Ascorbic acid) were prepared. Samples were added to the prepared DPPH solution (1 mL of 0.1 mM) and allowed to rest in dark for 20 min, and the optical density (OD) was recorded at 517 nm.^[15] The reagent solution without sample was taken as blank. The percentage

scavenging activity of samples was determined using the formula mentioned under ABTS assay.

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging assay was carried according to Griess reagent method. Sodium nitroprusside (10 mM) reagent was prepared and mixed with phosphate-buffered saline (PBS). A volume of 0.5 ml of extracts in different concentrations were added subsequently and incubated at 25°C for 150 min. After incubation time, 0.5 ml of reaction mixture was mixed with 1 ml of sulfanilic acid (0.33% sulfanilic acid in 20% glacial acetic acid). This aliquot was allowed to rest for 5 min and incubated for another 30 min with the addition of 1 ml of naphthyl ethylenediamine and the absorbance was read at 540 nm.^[16] The reagent solution without sample was taken as blank. Curcumin was used as standard. the percentage scavenging activity of samples was determined using the formula mentioned under ABTS assay.

Total antioxidant assay

Assay was carried out according to phosphomolybdenum method. In brief, 300 µl of extract (1 mg/ml) were added to 900 µl of reagent solution. The reagent solution was prepared by adding sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). After adding the reagent the aliquot was incubated at 95°C for 90 min and the absorbance was read at 695 nm.^[17,18] The reagent solution without sample was taken as blank. Standard graph of ascorbic acid was plotted. The results of total antioxidant capacity were expressed as ascorbic acid equivalents.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Assay was performed to ensure the ability of extract and fractions of *S. roxburghiana* to inhibit proliferation of cancer cells. Ninety-six well plates were seeded with 100 µl of cell suspension of all the five cancer cell lines and normal cell line of density 1×10^4 cells/well. Appropriate concentrations (10–400 µg/ml) of extract and fractions were made by serial dilution with media and added to 96 well plates. The plate was incubated for next 48 h. control group was treated with only media containing dimethyl sulfoxide (DMSO) (0.1% v/v). The media was removed and the 96 well plates were washed with 200 µL of PBS. A volume of 100 µL of MTT (1 mg/ml in PBS) were added to each well of plate and allowed to rest for 4 h at 37°C. DMSO (100 µL) was added to dissolve the formazan crystals and the OD was measured at 540 nm.^[19] Doxorubicin was used as standard.

Sulforhodamine B assay

The 96 well plates were seeded with 100 µl of cell suspension of all the five cell lines and normal cell line of density 1×10^4 cells/well and incubated for 24 h. Appropriate concentrations (10–400 µg/ml) of extract and fractions were made by serial dilution with media and added to 96 well plates and incubated for 48 h. The cells were fixed with ice-cold trichloroacetic acid (100 µl per well, 10%w/v) at 4°C for 1 h. The plate was washed, air dried, and stained by sulforhodamine B (SRB) dye solution (0.057%w/v in 1% aqueous acetic acid) and kept for 30 min. To remove unbound dye-stained well plate was washed with 1% acetic acid. The bound SRB dye was solubilized by the addition of Tris base (100 µL of 10 mM) to the 96 well plates. The OD was measured at 540 nm, and the percentage cell viability was calculated.^[20]

High-performance liquid chromatography analysis

The ethyl acetate fraction of methanol extract of rhizomes of *S. roxburghiana* was subjected to reversed phase HPLC (RP-HPLC)

separation technique so as to identify the presence of gallic acid. Shimadzu HPLC instrument (LC-2010 CHT, Shimadzu Corporation, Kyoto, Japan) was used which was equipped with quaternary low pressure gradient pumps, dual wavelength ultraviolet detector, SPD-M20A prominence photodiode array detector, degasser unit, column oven, and high throughput autosampler. LC Solution 5.57 software was used to monitor/control the chromatography system and process the obtained chromatograms. The separation of compound was achieved with a mobile phase consisting of orthophosphoric acid (1%v/v) and methanol in the ratio of 60:40%v/v. The mobile phase was flowed at a rate of 0.8 mL/min through Phenomenex Luna C18 (250 mm × 4.6 mm, 5 µm) column. Sample stock solution of 1 mg/ml was prepared, and injection volume was 20 µL and the wavelength was set to 275 nm. detection wavelength was 280 nm, and identification of compounds was carried out by comparing with the retention time of compounds from active fraction and standard compounds.

High-performance thin layer chromatography analysis

HPTLC fingerprint profile was carried out to detect the compounds presence in extract and fractions. HPTLC was performed using CAMAG (Muttentz, Germany) HPTLC instrument. Ten microliters of sample (5 mg/ml) was applied in a precoated silica gel G F₂₅₄ plates (Merck) using CAMAG Linomat 5 applicator. Chloroform:methanol (9:1) was used as mobile phase for a good separation of constituents. Toluene: ethyl acetate:formic acid:methanol (3:3:0.8:0.2) solvent system was used for the detection of gallic acid. CAMAG twin trough chamber which was previously saturated with mobile phase was used for the development of plate. Developed plate was dried and visualized and photographed at 254 nm, 366 nm, and 620 nm in a visualizing chamber Reprostar 3 and scanned at 254 nm and 366 nm using CAMAG scanner 3. The plate was derivatized with anisaldehyde-sulfuric acid, heated at 120°C for 5 min, and scanned at 620 nm. The R_f values were calculated.^[21]

Statistical analysis

The results were represented as mean ± standard error of mean values for three reproducible data and then evaluated using GraphPad Prism 5.02 Software. Analysis of variance was used for statistical comparison and level of significance was determined by *post hoc* Tukey's test. Correlation analysis was performed using Pearson's correlation method. The value of $P < 0.05$ was considered statistically significant.

RESULTS

The current study was intended to evaluate the antioxidant and anticancer activities of *S. roxburghiana* methanol extract and its active fraction/s. The ability these extracts to scavenge free radicals was assessed by different *in vitro* antioxidant assays. Rhizomes of *S. roxburghiana* were previously reported for cytotoxicity in brine shrimp lethality assay^[3] and anticancer activity against EAC model in mice.^[4] Whereas, *in vitro* anticancer evaluation of *S. roxburghiana* rhizomes (methanol extract and its fractions) has not yet been reported in HCT-116 (Colon), HeLa (Cervical), MCF-7 (Breast), HepG2 (Hepatic), and A-549 (Lung) cancer cell lines till date. Therefore, the present study was performed with the attempt to identify the active fractions responsible for the anticancer activity in the above five cancer cell lines by established *in vitro* methodology. HPLC and HPTLC analysis were also performed to identify the compounds responsible for antioxidant and anticancer activities.

Total phenolic content

Polyphenols, an important class of secondary metabolite of plants are naturally occurring antioxidants^[22] having numerous biological activities such as antifungal, antiviral, antibacterial, antiulcer, anticancer, and anti-cholesterol.^[23] *Terminalia chebula*,^[24,25] *Datura metel*,^[26] *Cynodon dactylon*,^[26] *Alocasia indica*,^[27] *Crataegus oxyacantha*,^[28] etc., have antioxidant activity mainly due to the presences of polyphenols.

The amount of TPC of SREA was found to be more compared to other fractions (23.40 ± 0.38 mg gallic acid equivalents (GAE)/g of plant extract) and less in SRPE (2.82 ± 0.16 mg GAE/g of plant extract) [Table 1].

Antioxidant assays

Reactive oxygen species (ROS) are highly reactive chemical molecules having a vital role in living organisms. In general, there is in equilibrium between the development and neutralization of ROS. The different ROS generated inside the cells include hypochlorous acid, hydrogen peroxide, and free radicals such as the hydroxyl radical and the superoxide anion.^[29] Moderate level of these species promotes cell development and proliferation, but high level causes damages in proteins, lipids, and DNA. DNA damage produces mutations and leads to cancer,^[30] whereas proteins damage causes enzyme inhibition and degradation of protein.^[31]

Table 1: Total phenolic content in *Sansevieria roxburghiana* rhizomes methanol extract and fractions

Extract/fractions	Percentage yield	Total phenols (mg GAE/g of plant extract), mean \pm SEM
SRM	26	8.59 \pm 0.12
SRPE	5	2.82 \pm 0.16
SREA	2.5	23.40 \pm 0.38
SRBU	10	11.34 \pm 0.63
SRAQ	80	9.80 \pm 0.66

SRM: Methanol extract of *S. roxburghiana*; SRPE: Petroleum ether extract of *S. roxburghiana*; SREA: Ethyl acetate fraction of *S. roxburghiana*; SRBU: Butanol of *S. roxburghiana*; SRAQ: Aqueous fraction of *S. roxburghiana*; *S. roxburghiana*: *Sansevieria roxburghiana*; GAE: Gallic acid equivalent; SEM: Standard error of mean, SRPE: Petroleum ether fraction of *S. roxburghiana*, SRBU: Butanol fraction of *S. roxburghiana*

ROS are found in the environment as pollutants and are produced inside the cells through different mechanisms mainly by mitochondrial respiration.

Anomalous over production of ROS due to inappropriate biological reaction resulting in oxidative stress is considered responsible for many disease conditions including diabetes,^[32] Parkinson's disease,^[33] Alzheimer's disease,^[34] Rheumatoid arthritis,^[35] and neurodegeneration in motor neuron diseases,^[36] inflammation, and gastrointestinal tract disorders.^[37] Moreover, also for the oxidation of low-density lipoprotein which directs to cardiovascular disease.^[38] Oxidative stress leads to cellular dysfunction and death. Therefore, ROS homeostasis should be maintained for normal cell growth through the scavenger of the elevated free radicals formed or control their production.^[39] The ability of *S. roxburghiana* extract and its fractions to scavenge free radicals were evaluated by *in vitro* antioxidant assays.

In vitro free radical scavenging activity

SREA was effectively scavenging DPPH free radical and ABTS free radicals with an IC₅₀ 15.33 ± 1.45 μ g/ml ($P < 0.005$) and 45.3 ± 1.93 μ g/ml ($P < 0.005$), respectively [Table 2]. In correlation analysis, total phenolic content (TPC) showed moderate correlation with DPPH scavenging assay ($R = -0.7412$) and ABTS scavenging assay ($R = -0.7046$) [Table 3].

Nitric oxide scavenging activity

The highest nitric oxide scavenging activity was observed in SREA with an IC₅₀ 48.43 ± 0.46 μ g/ml ($P < 0.005$) [Table 2]. A moderate correlation of TPC with nitric oxide radical scavenging activity ($R = -0.7525$) was observed in correlation analysis [Table 3].

Total antioxidant capacity

The highest antioxidant capacity was found in SREA extract with 218.62 ± 5.04 AAE/mg followed by SRME [Table 2]. Moderate correlation ($R = 0.7702$) between TPC and total antioxidant capacity [Table 3] was achieved in the correlation analysis.

Table 2: Free radical scavenging and antioxidant capacity of *Sansevieria roxburghiana* rhizomes methanol extract and fractions

Extract/fractions	IC ₅₀ (μ g/mL), mean \pm SEM (n=3)			
	DPPH scavenging	ABTS scavenging	Nitric oxide scavenging	Total antioxidant capacity (μ g ascorbic acid equivalents/mg extract)
SRM	435.4 \pm 2.24 ^a	158.3 \pm 1.45 ^a	361.06 \pm 0.26 ^a	149.27 \pm 2.96 ^a
SRPE	>1000	>1000	NA	71.2 \pm 1.66 ^b
SREA	15.33 \pm 1.45 ^b	45.3 \pm 1.93 ^b	48.43 \pm 0.46 ^b	218.62 \pm 5.04 ^c
SRBU	507.77 \pm 1.9 ^a	327.3 \pm 2.65 ^c	147.8 \pm 0.48 ^c	71.6 \pm 4.11 ^d
SRAQ	248.17 \pm 1.4 ^a	584.3 \pm 1.08 ^d	130 \pm 0.25 ^d	53.66 \pm 4.11 ^a
Ascorbic acid	5.836 \pm 0.08 ^c	2.05 \pm 0.28 ^c	-	-
Curcumin	-	-	21.59 \pm 0.98 ^e	-

^{a-c}The significant difference ($P < 0.05$). Values expressed as mean \pm SEM (n=3). NA: not active; SEM: Standard error of mean; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; SRM: Methanol extract of *S. roxburghiana*; SRPE: Petroleum ether extract of *S. roxburghiana*; SREA: Ethyl acetate fraction of *S. roxburghiana*; SRBU: Butanol of *S. roxburghiana*; SRAQ: Aqueous fraction of *S. roxburghiana*; *S. roxburghiana*: *Sansevieria roxburghiana*; IC₅₀: Concentration yielding 50% inhibition, SRPE: Petroleum ether fraction of *S. roxburghiana*, SRBU: Butanol fraction of *S. roxburghiana*

Table 3: Correlation (R) between total phenolic content of *Sansevieria roxburghiana* rhizomes methanol extract and fractions versus Concentration yielding 50% inhibition values of various antioxidant assays

	Correlation coefficient (R)			
	DPPH scavenging	ABTS scavenging	Nitric oxide scavenging	Total antioxidant capacity
Total phenolic content	-0.7412	-0.7046	-0.7525	0.7702

DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

Cytotoxicity studies

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and sulforhodamine B assays

Cytotoxicity of SRME and its fractions was determined in HCT-116, HeLa, MCF-7, HepG2, A-549, and Vero cell lines by MTT and SRB assays. SRME showed significant inhibitory effect on cell proliferation in HCT-116, HeLa, and MCF-7 cancer cell lines with an IC_{50} 46.69 ± 0.65 μ g/ml ($P < 0.005$), 34.50 ± 0.56 μ g/ml ($P < 0.005$), and 74.0 ± 0.45 μ g/ml ($P < 0.005$), respectively, by MTT assay. Among all fractions of SRME, SREA showed potent cytotoxicity in three cancer cell lines with an IC_{50} 16.55 ± 1.28 μ g/ml (HCT-116) ($P < 0.005$), 12.38 ± 1.36 μ g/ml (HeLa) ($P < 0.005$), and

8.03 ± 1.9 μ g/ml (MCF-7) ($P < 0.005$) by MTT assay [Table 4] and 15.57 ± 0.70 μ g/ml (HCT-116) ($P < 0.005$), 13.19 ± 0.49 μ g/ml (HeLa) ($P < 0.005$), and 10.34 ± 0.9 μ g/ml (MCF-7) ($P < 0.005$), respectively, by SRB assay [Table 5].

High-performance thin layer chromatography analysis

HPTLC fingerprint profile of SRME and its fractions revealed the presence of many spots corresponding to various phytoconstituents when visualized in TLC visualizing chamber [Figure 1]. Scanning under different wavelengths showed the presence of many peaks of individual compounds. Ethyl acetate fraction demonstrated the presence of gallic

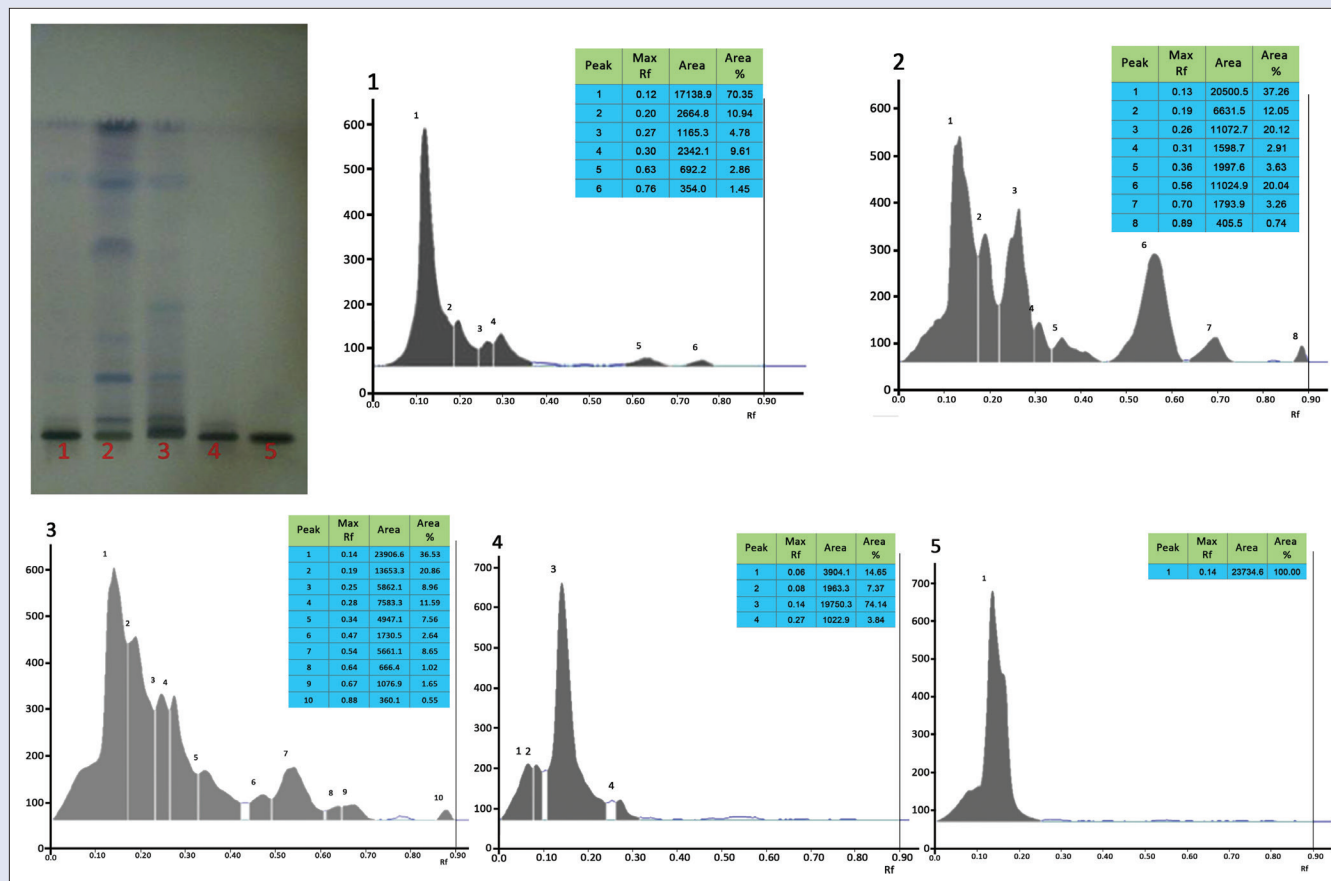


Figure 1: High-performance thin layer chromatography fingerprinting profile of *Sansevieria roxburghiana* rhizomes methanol extract and fractions. Track 1-methanol extract *S. roxburghiana* (10 μ l); Track 2-Petroleum ether fraction of *S. roxburghiana* (10 μ l); Track 3-Ethyl acetate fraction of *Sansevieria roxburghiana* (10 μ l); Track 4-Butanol fraction of *S. roxburghiana* (10 μ l); Track 5-Aqueous fraction of *S. roxburghiana* (10 μ l)

Table 4: Concentration yielding 50% inhibition (IC_{50}) values of *Sansevieria roxburghiana* rhizomes methanol extract and fractions and doxorubicin by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Extract/fraction	IC_{50} (μ g/mL)					
	HCT-116	HeLa	HepG2	MCF-7	A-549	Vero
SRM	46.69 ± 0.65^a	34.50 ± 0.56^a	181.0 ± 0.35^a	74.0 ± 0.45^a	323.7 ± 2.5^a	423.7 ± 0.4^a
SRPE	198.1 ± 0.98^b	232.01 ± 1.17^a	274.09 ± 0.02^b	228.41 ± 1.7^b	328.04 ± 1.6^c	442.05 ± 0.54^b
SREA	16.55 ± 1.28^c	12.38 ± 1.36^b	128.14 ± 1.02^c	8.03 ± 1.9^c	194.02 ± 1.06^b	387.02 ± 1.8^c
SRBU	112.4 ± 1.27^d	261.72 ± 3.22^a	375.34 ± 0.64^d	209.8 ± 0.90^d	298.24 ± 2.86^c	492.12 ± 0.94^d
SRAQ	616.2 ± 1.45^e	517.13 ± 2.29^a	463.3 ± 0.15^e	354.06 ± 0.64^e	437.09 ± 1.9^d	467.45 ± 0.45^e
Doxorubicin	1.6 ± 0.18^f	0.36 ± 0.08^c	1.34 ± 0.12^f	1.90 ± 0.30^f	2.6 ± 0.20^c	2.9 ± 0.16^f

^{a-f}The significant difference ($P < 0.05$). Values expressed as mean \pm SEM ($n=3$). SEM: Standard error of mean; SRM: Methanol extract of *S. roxburghiana*;

SRPE: Petroleum ether extract *S. roxburghiana*; SREA: Ethyl acetate fraction of *S. roxburghiana*; SRBU: Butanol of *S. roxburghiana*; SRAQ: Aqueous fraction of *S. roxburghiana*; *S. roxburghiana*: *Sansevieria roxburghiana*; IC_{50} : Concentration yielding 50% inhibition

acid at 270 nm with R_f value of 0.54 in toluene:ethyl acetate:formic acid: methanol (3:3:0.8:0.2) mobile phase [Figure 2].

High-performance liquid chromatography analysis

HPLC fingerprinting of ethyl acetate fraction was performed to analyze the presence of gallic acid. Absorption maxima and retention time of standard gallic acid were matched with peak 1. The eluting time of standard gallic acid and peak 1 was 4.15 min [Figure 3]. The amount of gallic acid in ethyl acetate fraction was found to be 0.42% w/w.

The compound, identified from SREA by HPLC and HPTLC, is 3, 4, 5 – trihydroxybenzoic acid (gallic acid); a low molecular weight polyphenolic compound found in large amounts in various plants and vegetables. Being a powerful antioxidant, it has ability to prevent oxidation at cellular and physiological level due to its electron donating ability and stable radical intermediate forming capacity.^[40]

Gallic acid and its derivatives are reported to have many pharmacological and biological activities including strong antioxidant,^[41,42] antibacterial,^[43] anti-inflammatory,^[44,45] antiviral^[46] antimitagenic,^[47] and anticancer properties.^[48,49] Biological activities of gallic acid are mainly due to its pro-oxidant and antioxidant properties. Gallic acid

acts specifically on the cancer cells as apoptosis inducer.^[50-52] The pro-oxidant property of gallic acid depends on its concentration and the presence of metal ions.^[53]

Gallic acid has been averting the formation of neoplastic cells in animal and *in vitro* models. Previous studies suggested that gallic acid inhibitory effect on malignant cells development is mediated through the regulation of genes responsible for apoptosis.^[53] Gallic acid also stimulates the activation of Ataxia-telangiectasia mutated (ATM) kinase and induce ATM-dependent apoptosis,^[54] inhibit ribonucleotide reductase, and causing inhibition of DNA synthesis,^[55] inhibits activation of Akt signaling pathway and cyclooxygenase,^[56] induce apoptosis by the up-regulation of Bax and down-regulation of Bcl2 proteins^[57] and anti-angiogenic effect.^[58]

The study revealed that the SREA rhizomes have significant free radical scavenging capacity in all DPPH, ABTS, and nitric oxide scavenging assays. This indicates the extract contains active principle(s) with good antioxidant potential. SREA showed the highest total antioxidant capacity (218.62 ± 5.04 AAE/mg equivalent to ascorbic acid). The methanol extract (IC_{50} HCT 116– 46.69 ± 0.65 and HeLa– 34.50 ± 0.56 μ g/ml) and its ethyl acetate fraction (IC_{50} HCT 116– 16.55 ± 1.28 ; HeLa– 12.38 ± 1.36 μ g/ml and MCF 7– 8.03 ± 1.9 μ g/ml) is having significant antiproliferative activity. Gallic acid identified by HPTLC and HPLC fingerprint profiling may be responsible for the antioxidant and antiproliferative activity of the rhizomes of *S. roxburghiana*. Studies exploring the mechanism of action of bioactive molecule of rhizomes of *S. roxburghiana* are in progress.

CONCLUSION

The present study analyzed the free radical scavenging activity, cytotoxicity, HPLC, and HPTLC analysis of methanol extract and its fractions of rhizomes of *S. roxburghiana*. Gallic acid, a potent antioxidant molecule present in ethyl acetate fraction of methanol extract might be responsible for significant antioxidant and anticancer activity. Detection of other principles and its mechanism of action are under progress.

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Conflicts of interest

There are no conflicts of interest.

Table 5: Concentration yielding 50% inhibition values of *Sansevieria roxburghiana* rhizomes methanol extract, ethyl acetate fraction, and doxorubicin by sulforhodamine B assay

Extract/fraction	IC_{50} (μ g/mL)		
	HCT-116	HeLa	MCF-7
SRM	46.84 ± 1.73^a	35.57 ± 0.70^a	77.27 ± 1.11^a
SREA	15.57 ± 0.70^b	13.19 ± 0.49^b	10.34 ± 0.9^b
Doxorubicin	2.30 ± 0.36^c	0.51 ± 0.48^c	2.20 ± 0.30^c

^{a-c}The significant difference ($P < 0.05$); Values expressed as mean \pm SEM ($n=3$); SEM: Standard error of mean; SRM: Methanol extract of *S. roxburghiana*; SREA: Ethyl acetate fraction of *S. roxburghiana*; *S. roxburghiana*: *Sansevieria roxburghiana*; IC_{50} : Concentration yielding 50% inhibition

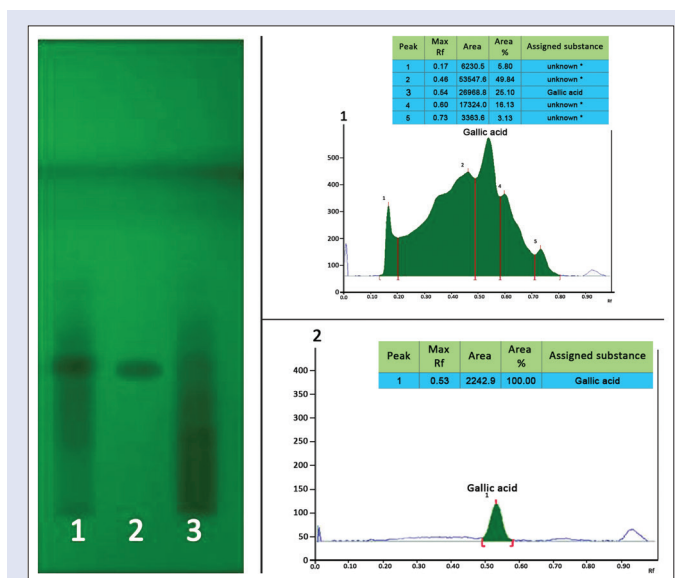


Figure 2: High-performance thin layer chromatography fingerprinting profile identifying the presence of gallic acid in *Sansevieria roxburghiana* ethyl acetate fraction. Track 1-ethyl acetate fraction of *Sansevieria roxburghiana* 5 μ l; Track 2-gallic acid; Track 3-SREA 10 μ l

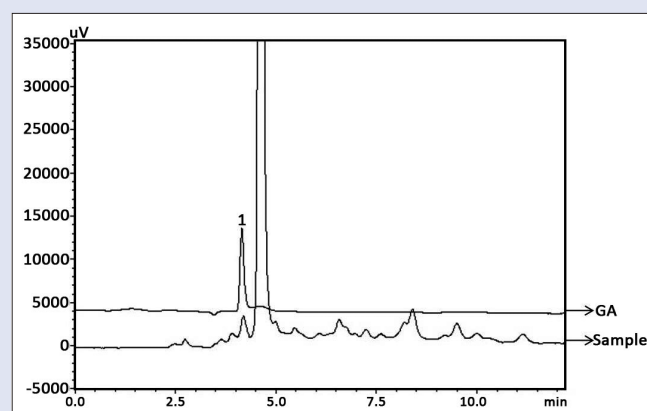


Figure 3: High-performance liquid chromatography fingerprinting profile identifying the presence of gallic acid in *Sansevieria roxburghiana* ethyl acetate fraction

REFERENCES

- Prakash O, Kumar A, Kumar P, Ajeet. Anticancer potential of plants and natural products: A Review. *Am J Pharmacol Sci* 2013;1:104-15.
- Alan NH. The Use of Herbs and Natural Plant Compounds in the Fight Against Cancer. Cancer support association, Information, Wellness and Healing. Available from: <http://www.canceractive.com/cancer-active-page-link.aspx?n=1415>. [Last accessed 2016 Jul 21].
- Jimuty R, Mohammed K, Bilkis B, Choudhury H. Evaluation of analgesic, cytotoxic and antioxidant activities of *Sansevieria roxburghiana* Schult. and Schult. f. *Asian Pac J Trop Biomed* 2012;2(2):S723-5.
- Haldar PK, Kar B, Bala A, Bhattacharya S, Mazumder UK. Antitumor activity of *Sansevieria roxburghiana* rhizome against Ehrlich ascites carcinoma in mice. *Pharm Biol* 2010;48:1337-43.
- Hartwell JL. Plants Used Against Cancer. Massachusetts: Quaterman Pub.; 1982. p. 340.
- Bhat KG. Flora of Udupi. Manipal, India: Manipal Press Limited; 2003. p. 118.
- Haldar PK, Biswakanth K, sanjib B, Asis B, Suresh K. Anti diabetic activity and modulation of antioxidant status by *Sansevieria roxburghiana* rhizome in streptozotocin- induced diabetic rats. *Diabetol Croat* 2010;39:115-23.
- Deepa P, Kaleena PK, Valivittan K, Girish Kumar CP. Phytochemical screening and antimicrobial activity of *Sansevieria roxburghiana* Schult. and Schult. f. *Middle East J Sci Res* 2011;10:512-8.
- Arun J, Maya B, Gauri PA. Phytochemical investigation of the rhizomes of *Sansevieria roxburghiana*. *Am J Pharm Health Res* 2013;1:16-26.
- Deepa P, Kaleena PK, Valivittan K. Antioxidant potential of *Sansevieria roxburghiana* Schult. and Schult. f. *Asian J Pharm Clin Res* 2012;5:166-9.
- Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall; 1998. p. 60-6.
- Kokate CK. Practical Pharmacognosy. New Delhi, India: Vallabh Prakashan; 1991. p. 107-11.
- Slinkard J, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. *Am J Enol Vitic* 1977;28:49-55.
- Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chem* 2007;103:381-8.
- Bansal P, Paul P, Nayak PG, Pannakal ST, Zou J, Laatsch H, *et al.* Phenolic compounds isolated from *Pilea microphylla* prevents radiation-induced cellular DNA damage. *Acta Pharm Sin B* 2011;1:226-35.
- Gouthamchandra K, Mahmood R, Manjunatha H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from *Clerodendrum infortunatum* L. *Environ Toxicol Pharmacol* 2010;30:11-8.
- Besada A. A facile and sensitive spectrophotometric determination of ascorbic acid. *Talanta* 1987;34:731-2.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of Vitamin E. *Anal Biochem* 1999;269:337-41.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
- Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc* 2006;1:1112-6.
- Sethi PD. High Performance Thin Layer Chromatography. 1st ed. New Delhi: CBS Publishers and Distributors; 1996. p. 1-56.
- Khadem S, Marles RJ. Monocyclic phenolic acids; hydroxy- and polyhydroxybenzoic acids: Occurrence and recent bioactivity studies. *Molecules* 2010;15:7985-8005.
- Bharti B, Neha S, Rita K. Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. *RSC Adv* 2015;35:27540-57.
- Eshwarappa RS, Ramachandra YL, Subaramaiha SR, Subbaiah SG, Austin RS, Dhananjaya BL. Anti-Lipoxygenase activity of leaf gall extracts of *Terminalia chebula* (Gaertn.) Retz. (Combretaceae). *Pharmacognosy Res* 2016;8:78-82.
- Alagesan V, Arumugam K, Shanmugam P, Venugopal S. Antioxidant, antibacterial activities and identification of bioactive compounds from *Terminalia chebula* bark extracts. *Free Radic Antioxid* 2017;7:43-9.
- Roy S, Pawar S, Chowdhary A. Evaluation of *in vitro* cytotoxic and antioxidant activity of *Datura metel* Linn. and *Cynodon dactylon* Linn. Extracts. *Pharmacognosy Res* 2016;8:123-7.
- Mulla W, Kuchekar S, Thorat V, Chopade A, Kuchekar B. Antioxidant, antinociceptive and anti-inflammatory activities of ethanolic extract of leaves of *Alocasia indica* (Schott.). *J Young Pharm* 2010;2:137-43.
- Amor L, Belhatab R. Antioxidant activity of aqueous extracts from *Crataegus oxyacantha* leaves. *Pharmacogn Commun* 2015;5:229-32.
- Valko M, Leibfriz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;39:44-84.
- Nakabeppu Y, Sakumi K, Sakamoto K, Tsuchimoto D, Tsuzuki T, Nakatsu Y. Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. *Biol Chem* 2006;387:373-9.
- Stadtman ER. Protein oxidation and aging. *Science* 1992;257:1220-4.
- Davi G, Falco A, Patrono C. Lipid peroxidation in diabetes mellitus. *Antioxid Redox Signal* 2005;7:256-68.
- Wood-Kaczmar A, Gandhi S, Wood NW. Understanding the molecular causes of Parkinson's disease. *Trends Mol Med* 2006;12:521-8.
- Christen Y. Oxidative stress and Alzheimer disease. *Am J Clin Nutr* 2000;71:621S-9S.
- Hitchon CA, El-Gabalawy HS. Oxidation in rheumatoid arthritis. *Arthritis Res Ther* 2004;6:265-78.
- Cookson MR, Shaw PJ. Oxidative stress and motor neurone disease. *Brain Pathol* 1999;9:165-86.
- Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz J Med Biol Res* 2002;35:523-34.
- Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006;444:875-80.
- Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? *Nat Rev Drug Discov* 2009;8:579-91.
- Cuvrelle ME, Richard H, Berset C. Comparison of the antioxidant activity of some acid phenols, structure activity relationship. *Biosci Biotechnol Biochem* 1992;56:324-5.
- Golumbic C, Mattill HA. The antioxidant properties of gallic acid and allied compounds. *J Am Oil Chem Soc* 1942;19:144-5.
- Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J Agric Food Chem* 2002;50:3713-7.
- Kang MS, Oh JS, Kang IC, Hong SJ, Choi CH. Inhibitory effect of methyl gallate and gallic acid on oral bacteria. *J Microbiol* 2008;46:744-50.
- Kroes BH, van den Berg AJ, Quarles van Ufford HC, van Dijk H, Labadie RP. Anti-inflammatory activity of gallic acid. *Planta Med* 1992;58:499-504.
- Kim SH, Jun CD, Suk K, Choi BJ, Lim H, Park S, *et al.* Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. *Toxicol Sci* 2006;91:123-31.
- Kratz JM, Andrighetti-Fröhner CR, Leal PC, Nunes RJ, Yunes RA, Trybala E, *et al.* Evaluation of anti-HSV-2 activity of gallic acid and pentyl gallate. *Biol Pharm Bull* 2008;31:903-7.
- Gichner T, Pospíšil F, Veleminský J, Volkeová V, Volke J. Two types of antimutagenic effects of gallic and tannic acids towards N-nitroso-compounds-induced mutagenicity in the Ames Salmonella assay. *Folia Microbiol (Praha)* 1987;32:55-62.
- Mirvish SS, Cardesa A, Wallcave L, Shubik P. Induction of mouse lung adenomas by amines or ureas plus nitrite and by N-nitroso compounds: Effect of ascorbate, gallic acid, thiocyanate, and caffeine. *J Natl Cancer Inst* 1975;55:633-6.
- Inoue M, Suzuki R, Sakaguchi N, Li Z, Takeda T, Ogihara Y, *et al.* Selective induction of cell death in cancer cells by gallic acid. *Biol Pharm Bull* 1995;18:1526-30.
- Yen GC, Duh PD, Tsai HL. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chem* 2002;79:307-13.
- Sergediene E, Jönsson K, Szymusiak H, Tyrakowska B, Rietjens IM, Cenas N. Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: Description of quantitative structure-activity relationships. *FEBS Lett* 1999;462:392-6.
- Aruoma OI, Murcia A, Butler J, Halliwell B. Evaluation of the antioxidant and pro-oxidant actions of gallic acid and its derivatives. *J Agric Food Chem* 1993;41:1880-5.
- Verma S, Singh A, Mishra A. Gallic acid: Molecular rival of cancer. *Environ Toxicol Pharmacol* 2013;35:473-85.
- Zhou BB, Elledge SJ. The DNA damage response: Putting checkpoints in perspective. *Nature* 2000;408:433-9.
- Madlener S, Illmer C, Horvath Z, Saiko P, Losert A, Herbacek I, *et al.* Gallic acid inhibits ribonucleotide reductase and cyclooxygenases in human HL-60 promyelocytic leukemia cells. *Cancer Lett* 2007;245:156-62.
- Reddy TC, Aparoy P, Babu NK, Kumar KA, Kalangi SK, Reddanna P. Kinetics and docking studies of a COX-2 inhibitor isolated from *Terminalia bellerica* fruits. *Protein Pept Lett* 2010;17:1251-7.
- Hsu WT, Pang CN, Sheetal J, Wilkins MR. Protein-protein interactions and disease: Use of *S. cerevisiae* as a model system. *Biochim Biophys Acta* 2007;1774:838-47.
- Sherr CJ. Cancer cell cycles. *Science* 1996;274:1672-7.