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Sansevieria: An Evaluation Of Potential Cytotoxic Activity In Reference To Metabolomic And Molecular Docking Studies

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Abstract

Sansevieria trifasciata Prain. and *Sansevieria suffruticosa* N.E.Br. were selected to evaluate their cytotoxic activity against colon (CACO2), lung (A-549) and liver (HepG-2) carcinoma cell lines. Results indicated that *S. suffruticosa* N.E.Br. showed a significant cytotoxic effect on CACO2 with IC50= 30.9 ± 0.72 µg/ml. Evaluating the phenolic and flavonoid contents, *S. suffruticosa* N.E.Br. has higher total phenolic and flavonoid contents than *S. trifasciata* Prain. The phenolic content was estimated using HPLC detecting the presence of apigenin-7-glucoside and cinnamic acid only in *S. suffruticosa* N.E.Br. with p-hydroxybenzoic acid and p-coumaric acid being the major compounds. While, catechin and kaempferol are the major compounds detected in *S. trifasciata* Prain. Phytochemical investigation of ethyl acetate fractions of both species results in isolation of chlorogenic acid, kaempferol, quercetin, and catechin from *S. trifasciata* Prain. and apigenin-7-glucoside and rutin from *S. suffruticosa* N.E.Br. Isolated compounds are identified using recent spectroscopic methods. All compounds are isolated for the first time from these species.

Metabolomic profiling indicates the presence of phenolic acids, flavonoids and saponins in the alcoholic extracts of the two species. In addition, molecular docking study was done for further investigation of the possible targets involved in the cytotoxicity of the alcoholic extracts of the two *Sansevieria* species. The ability of some phenolic compounds to interact with EGFR cancer target site rationalizes the cytotoxic activity of *S. suffruticosa* N.E.Br. alcoholic extract as proven by their docking pattern and docking score.

Keywords: Sansevieria trifasciata; *Sansevieria suffruticosa*; cytotoxic activity; molecular docking; HPLC.

1. Introduction

Cancer is a health problem around the world that seriously threaten human life. A lot of strategies have been developed for cancer treatment including surgery, radiotherapy, chemotherapy and targeted therapy [1, 2]. It is difficult to prevent occurrences of adverse effects resulting from chemotherapeutic drugs during therapy. For instance, doxorubicin, a drug used widely in chemotherapy, induces cardiomyopathy and chronic heart failure with prevalence between 4-36 % (cardiomyopathy) and 0.2-8.7% (chronic heart failure). Cancer cells may develop drug resistance during treatment with chemotherapy which results in applying higher doses to achieve a similar tumoricidal effect as the initial dosage. Consequently, higher dosages have a higher possibility of severe side effects [3]. This highlights the need for a new herbal remedy which affects the cancer cells and safe at the same time. *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. are species in the genus *Sansevieria* in the family Asparagaceae. Both species are only used as ornamental plants in Egypt. *S. trifasciata* Prain. was used in folk medicine as a whole plant for treatment of alopecia, malaria, tonic and snake bite in Bangladesh [4], while, the leaves and rhizomes were used for treating bronchitis, asthma, cough, snake and insect bites [5]. It has been

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commonly used for treatment of ear pain, swellings, boils and fever. Many biological activities were reported for *S. trifasciata* Prain. such as analgesic, antipyretic [6], antiallergic, anti-anaphylactic [5], antidiabetic [7] and thrombolytic activities [8]. Cytotoxic activity of leaves and rhizome against colon, breast and hepatic cell lines was weak [9]. There is very little literature review concerning *S. suffruticosa* N.E.Br. including mild antibacterial activity against *Escherichia Coli* [10]. This study was performed to evaluate the cytotoxic activity of the alcoholic extracts of the aerial non-flowering parts of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. against three tumor cell lines; CACO2, A-549 and HepG-2 in correlation to phenolic content using metabolomic and molecular docking study for further investigation of the possible targets involved in the cytotoxicity of the alcoholic extracts of the two *Sansevieria* species.

2. Experimental

Plant material

The aerial non-flowering parts of the two *Sansevieria* species, *Sansevieria trifasciata* Prain. and *Sansevieria suffruticosa* N.E.Br. were collected from El Orman Botanical Garden, Giza, Egypt in September 2016. They were kindly authenticated by Dr. Reem Samir Hamdy, Assistant Professor, Taxonomy Department, Faculty of Science, Cairo University and vouched specimens were deposited within the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt with numbers: 8-9-2016 I and 8-9-2016 II, respectively.

Standard material

Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, vanillic acid, ferulic acid, rutin, *p*-coumaric acid, Apigenin-7-glucoside, rosmarinic acid, cinnamic acid, quercetin and kaempferol were purchased from Sigma-Aldrich.

Drugs and Chemicals

Ethanol, acetonitrile and methanol were purchased from SD fine chemical limited, Mumbai, India and was used without further purification. All chemicals were of analytical grade. Folin-Ciocalteu reagent, sodium carbonate, sodium nitrite, aluminium chloride and sodium hydroxide were purchased from Sigma-Aldrich, Mumbai, India.

Biological assay: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA); Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Apparatus

Unicam UV spectrophotometer was used for determining the UV spectra. NMR spectra were recorded in DMSO and CD_3OD, using Mercury plus (400 MHz) and BRUKER (400 MHz) using TMS as internal standard and chemical shifts were given in δ value. The mass spectra were accomplished by using Agilent Triple Quadrupole mass spectrometer (UK-EI) and electron energy 150 eve. Electrothermal 9100 apparatus (Electrothermal Engineering Ltd, Essex, England) was utilized for determination of the melting point of the isolated compounds. Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector with Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) analytical column and C18 (Phenomenex, Torrance, CA) guard column for HPLC analysis. XEVO TQD triple quadruple instrument, Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer was used for LC/MS and separation was carried out on C18 column (ACQUITY UPLC - BEH C18 1.7 µm particle size 2.1 × 50 mm Column).

Preparation of Plant Extracts and fractions

The fresh aerial non-flowering parts (5Kg) of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. were macerated, separately, in 95% ethanol till exhaustion. The ethanolic extracts were completely dried under reduced pressure at 45 °C using rotary evaporator (Buchi®R- 300, USA) and stored at 20 °C until use. The alcoholic extracts were 350g and 400g for *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. Part of the alcoholic extract (200g) of both species was suspended in distilled water, separately, and successively extracted with n-hexane, dichloromethane, ethyl acetate and n-butanol, till exhaustion. The fractions were evaporated under reduced pressure till dryness to yield (18.5g), (15g), (12g) and (30g) of the extractives of *S. trifasciata* Prain., respectively, and (22.5g), (8g), (17g) and (27g) of the extractives of *S. suffruticosa* N.E.Br., respectively. The residue was kept in sealed glass vials in a refrigerator until use for biological and phytochemical studies.

Biological Study

Cytotoxic screening

Crystal violet stain (1%): composed of 0.5% (w/v) crystal violet and 50% methanol then complete to volume with distilled H_2O and filtered through Whatmann No.1 filter paper.

Mammalian cell lines: CACO2 (colon carcinoma), **A-549** cells (human lung cancer cell line) and **HepG-2** cells (human Hepatocellular carcinoma) were obtained from VACSERA Tissue Culture Unit, Egypt.

Cell line propagation

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) which is supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times per week.

Assessment of cytotoxic activity

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×104 cells per well in 100µl of growth medium. Fresh medium which contain different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the sample were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) with the aid of multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for 48 h. Three wells were used for each concentration of the sample. Control cells were incubated without sample and with or without DMSO. The small percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells at 37°C, various concentrations of sample were added and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

Media were aspirated, at the end of the incubation period, and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed with tap water until all excess stain is removed. Glacial acetic acid (30%) was added to all wells, mixed thoroughly then the absorbance of the plates was measured at 490 nm, after gentle shaking on Microplate reader (TECAN, Inc.). All results were corrected for absorbance detected in wells without added stain. Treated samples were compared with the cell control. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested sample was calculated. The optical density was measured by microplate reader (SunRise, TECAN, Inc, USA) for determination of the quantity of viable cells. Viability percentage was calculated as [1-(ODt/ODc)]x100%; ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. Relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with each extract [11, 12]. IC50; the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for every conc. using Graphpad Prism software (San Diego, CA. USA).

Quantitative determination of total phenolic and flavonoid contents

a) Spectrophotometric method

1- Total Phenolic Content

Total soluble phenolics of the extracts were determined with Folin-Ciocalteau reagent with the help of UV-VIS spectrophotometer according to Attard and Mruthunjaya *et al.* [13, 14] with some modifications. A stock solution of 0.05% of authentic gallic acid was prepared. Different aliquots equivalent to 390-25000 µg were separately introduced into 50 ml volumetric flask, 2 ml of Folin-Ciocalteau reagent and 2 ml of 20% sodium carbonate were added and the volume was completed with distilled water. The contents were mixed and allowed to stand for 30 minutes at room temperature, then the absorbance of the solutions was measured at 630 nm using UV spectrophotometer. Six replicates determinations, for each concentration, were carried out and the mean absorbance were plotted versus concentrations. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh parts. The coefficient of determination was r = 0.9989.

2- Total Flavonoid Content

Total flavonoid content was determined according to Atanassova *et al.* and Herald *et al.* [15, 16] with some modifications. A stock solution of 0.05% of rutin was prepared, different aliquots equivalent to 50-1000 µg were taken in separate test tubes, 4 ml distilled water and 0.3 ml 5% $NaNO_2$ solution were added. After 5 min, 0.3 ml 10% $AlCl_3$ solution was added and left to stand for 2 min. Finally, 2 ml of 1 N NaOH was added then the volume was completed to 10 ml with distilled water and the contents were mixed. The absorbance of the solutions was measured at 510 nm using UV spectrophotometer. Triplicate determinations, for each concentration were carried out and the mean absorbance were plotted versus concentrations. The total flavonoid content was expressed as milligrams rutin equivalent (RE) per gram of fresh parts. The coefficient of determination was r = 0.9946.

b) HPLC method

HPLC analysis was performed according to method presented by Kim *et al.* [17] in methanol extracts (38.7 mg extract/ml dissolved in the mobile phase) with some modifications. Mobile phase was acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B) in a gradient elution: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min, with flow rate 0.8 ml/min using UV detector at wave length 280 nm with injection volume 20 µl for 70 min. Before injection, all samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI). Peaks were identified by retention

times and UV spectra and compared with those of the available standards.

Phytochemical study

Material for chromatographic analysis

Column chromatography was performed over silica gel 60 (Merck), Sephadex LH20 (Fluka) and Diaion® HP-20 (Sigma-Aldrich Chemie, Geermany). TLC was carried out using silica gel plates 60 F254 (Reide-de Haen, Germany). The following solvent systems were prepared from analytical grade chemicals purchased from El–Gomhoreya Co., Egypt; S1 (ethyl acetate: methanol: formic acid: water; 100: 16: 13: drops v/v/v/v), S2 (butanol: glacial acetic acid: water; 4:1:5 v/v/v, upper phase), S3 (chloroform: acetone: formic acid; 75: 16.5: 8.5 v/v/v), S4 (dichloromethane: methanol; 8:2), S5 (ethyl acetate: formic acid: glacial acetic acid: water; 100: 11: 11: 27 v/v/v/v), S6 (chloroform: acetone: formic acid; 50: 33: 17 v/v/v) and S7 (ethyl acetate: formic acid: water; 12: 2: 3 v/v/v). The spraying reagents used were p-anisaldehyde-sulphuric acid, ferric chloride, aluminium chloride and ammonia vapour.

Isolation and identification of the compounds

The ethyl acetate fraction obtained from partitioning of the ethanolic extract of the aerial non-flowering parts of *S. trifasciata* Prain. (10g) was applied on a chromatographic column packed with Diaion HP-20 and eluted with gradient solvent system water-methanol (100:0 → 0:100). Similar fractions were pooled together then concentrated under reduced pressure yielding 120 fractions (Fr.). Fr. (40-58) 150 mg eluted with 30% methanol was further purified on column chromatography of Sephadex LH20 using methanol for elution. Subfraction (S. Fr. III) was introduced to preparative TLC using S1 solvent on precoated TLC plates to give compound E1 (17mg).

Fr. (66-78) 210 mg eluted with 50% methanol was rechromatographed on Silica gel column using dichloromethane-ethyl acetate (100:0 → 0:100). Subfraction IV (S. Fr. IV) eluted with 20% ethyl acetate was introduced to preparative TLC using S3 solvent on precoated TLC plates to give compound E2 (14mg). Subfraction VII (S. Fr. VII) eluted with 40% ethyl acetate was purified on Sephadex column using dichloromethane: methanol (50: 50) yielded two precipitated subfractions. The first precipitated subfraction afford compound E3 (10mg).

Fr. (79-91) 50 mg eluted with 70% methanol was applied on TLC affording only one major spot on silica gel TLC which is isolated by preparative TLC using S1 giving compound E4 (15mg).

The ethyl acetate fraction obtained from partitioning of the ethanolic extract of the aerial non-flowering parts of *S. suffruticosa* N.E.Br. (10g) was concentrated under reduced pressure at 40°C to yield a yellow precipitate (Fr. A; 1.20g) and mother liquor (Fr. B). Fr. A was purified on a column of Sephadex LH20 using methanol for elution and afforded compound E5 (25mg). Fr. B was evaporated to dryness to yield a residue (4.5g) which was then subjected to column chromatography on silica gel 60 using eluted with a solvent gradient of CHCl3-MeOH (100:0 →0:100). Similar fractions were pooled together then concentrated under reduced pressure yielding eight subfractions (S. Fr). S. Fr. (1-4) (100mg) was further purified on column chromatography of Sephadex LH20 using methanol for elution to give compound E6 (30 mg).

Metabolomic analysis by LC/MS

Metabolomics profiling was performed on alcoholic extracts of both *Sansevieria* species according to Raheem *et al.*, Abdelhafez *et al.* and Abdelmohsen *et al.* [18, 19, 20] with some modifications. ESI-MS positive and negative ion acquisition mode was performed. Processing of peaks and spectra was performed using the Maslynx 4.1 software and tentatively identified by comparing its retention time and mass spectrum with reported data.

The sample (100 µg/ml) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent of methanol, filtered using a membrane disc filter (0.2 µm) then subjected to LC-ESI-MS analysis. Samples injection volumes (10 µl) were injected. Before injection, sample mobile phase was prepared by filtering using 0.2 µm filter membrane disc and degassed by sonication.

Solvent A was water acidified with 0.1 % formic acid, solvent B was methanol acidified with 0.1 % formic acid. Solvents were set at flow rate 0.2 ml/min. Elution was gradient: 90% A to 70% A in 5 min, 70% A to 30% A in 10 min, 30% A to 10% A in 10 min, 10% A to 0% A in 4 min and 0% A to 90% A in 3 min.

Mass conditions: parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, de-solvation temperature 440 °C, cone gas flow 50 L/h and de-solvation gas flow 900 L/h. Mass spectra were detected in the ESI within m/z 100–1000.

Molecular docking

The crystal structure of protein targets were downloaded from the protein data bank [21]. The targets included the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase domain (PDB ID: 1M17), Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) Juxta membrane and Kinase Domains (PDB ID: 4ASD) and the Receptor for Advanced Glycation End Products (RAGE, also known as

Advanced Glycosylation End product-specific Receptor or AGER) (PDB ID: 2MOV). Proteins were prepared using Autodock tools and hydrogens and charges were added.

Ligands were downloaded from PubChem when possible or drawn and the 3D structures were generated. Co-crystalized ligands were extracted from the pdb file. Minimization was done using 500 steps of steepest descent followed by 500 steps of conjugate gradient algorithm and MMFF94 force field on Avogadro software.

Docking was done using Autodock Vina [22] in a grid box of 25 Å3 centred on the given co-crystallized ligand with exhaustiveness of 32. The generated docking poses were visualized and figures were generated with PyMOL software [23].

Statistical analysis

Statistical analysis was performed using SPSS (statistical package of social sciences, version 22), SPSS Inc., Chicago, IL, USA. Statistical significance was acceptable to a level of $p < 0.05$.

3. Results

Cytotoxic activity

From figure 1 and 2: The cytotoxic activity of the alcoholic extracts of the two *Sansevieria* species were studied *in vitro* against HepG-2, A-549 and CACO2 cell lines at different concentrations (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/ml). The results obtained showed that *S. suffruticosa* N.E.Br. has significant cytotoxic effect on CACO2 with IC50 = 30.9 ± 0.72 µg/ml and certain activity against HepG-2 with IC50 = 45.8 ± 2.59 µg/ml. On the other hand, low cytotoxic activities were revealed by *S. suffruticosa* N.E.Br. against A-549 with IC50 = 77.8 ± 7.89 µg/ml and by *S. trifasciata* Prain. against HepG-2 with IC50 = 81 ± 18.8 µg/ml. While, *S. trifasciata* Prain. has no significant activity with IC50 > 100 µg/ml against A-549 and CACO2 cell lines.

Total Phenolic Content

Higher phenolic content was found in the *S. suffruticosa* N.E.Br. (3.73 ± 0.78 mg GAE/g fresh parts) than *S. trifasciata* Prain. (1.96 ± 0.26 GAE/g fresh parts). These results clearly demonstrate that the content of phenolic compounds is higher in *S. suffruticosa* N.E.Br. than *S. trifasciata* Prain.

Total Flavonoid Content

The flavonoid content in alcoholic extracts is 1.57 ± 0.34 and 2.81 ± 0.26 mg RE/g fresh parts of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. These results demonstrate that the flavonoidal content is higher in *S. suffruticosa* N.E.Br. than *S. trifasciata* Prain.

HPLC analysis

From table 1 and figure (3-5), it was found that p-hydroxybenzoic acid and p-coumaric acid are major phenolics in *S. suffruticosa* N.E.Br., while catechin and kaempferol are major in *S. trifasciata* Prain. Apigenin-7-glucoside and cinnamic acid are detected only in *S. suffruticosa* N.E.Br.

Identification of the compounds

Compound E1: is a white amorphous powder, soluble in organic solvents with melting point 208°C and R_f =0.26 in S1. EI/MS spectrum showed molecular ion peaks $[M]^+$ at m/z 354 and a prominent fragment ion at m/z 190.7. From the previous physical, spectral data, co-chromatography with chlorogenic acid standard and by comparing the obtained data with those reported for chlorogenic acid [24], compound E1 was identified as chlorogenic acid.

Compound E2: yellow powder soluble in methanol with R_f = 0.92 in S2, 0.52 in S3 and 0.85 in S4. UV λ max nm (MeOH): 269,369; NaOMe: 278, 320,415; NaOAc: 274, 435; NaOAc/H_3BO_3: 270, 384; $AlCl_3$:271, 361 (sh), 420; $AlCl_3$/HCl: 273, 359 (sh), 420. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.091 (2H, m, H-2', H-6'), 6.883 (2H, d, J= 8.4, H-3', H-5'), 6.386 (1H, d, J= 3.6, H-8), 6.182 (1H, d, J= 1.8, H-6). From the previous physical, spectral data, co-chromatography with kaempferol standard and by comparing the obtained data with those reported for kaempferol [25], compound E2 was identified as kaempferol.

Compound E3: yellow needle crystals, soluble in methanol with with melting point 314°C, R_f = 0.96 in S2, 0.4 in S3 and 0.93 in S5. UV λ max nm (MeOH): 256, 371; NaOMe: 234, 327, 428; NaOAc: 275, 447; NaOAc/H_3BO_3: 263, 439; $AlCl_3$: 271, 449; $AlCl_3$/HCl: 256, 421. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.737 (1H, d, J=2.1, H-2'), 7.651 (1H, dd, J=2.4, J=9, H-6'), 6.902 (1H, d, J=10.2, H-5'), 6.407 (1H, d, J=2.1, H-8), 6.197 (1H, d, J=2.1, H-6). From the previous physical, spectral data, co-chromatography with quercetin standard and by comparing the obtained data with those reported for quercetin [25], compound E3 was identified as quercetin.

Compound E4: white amorphous powder, soluble in organic solvents with melting point 175°C, R_f = 0.85 in S1. UV λ max nm (MeOH): 277, 220. EI/MS spectrum showed molecular ion peaks m/z=290 $[M]^+$. From the previous physical, spectral data, co-chromatography with catechin standard and by comparing the obtained data with those reported for catechin [26], compound E4 was identified as catechin.

Compound E5: pale yellow powder, soluble in methanol with melting point 242°C, R_f = 0.32 in S2, 0.26 in S6 and 0.33 in S7. UV λ max nm (MeOH): 257, 359; NaOMe: 268, 327, 403; NaOAc: 273, 323, 365; NaOAc/H_3BO_3: 262, 379; $AlCl_3$: 273, 430; $AlCl_3$/HCl: 268, 398. ^{1}H NMR (400 MHz, CD_3OD) δ ppm aglycone: 7.48 (1H, dd, J= 2 and 9, H-6'), 7.395 (1H, d, J= 3, H-2'), 6.685 (1H, d, J= 9, H-5'), 6.195 (1H, d, J=3, H-8), 5.995 (1H, d, J=3, H-6); glycoside: 5.23 (1H, m, H-1''), 4.0-3.2 (sugar protons, m), 0.937 (3H, protons of rhamnosyl CH_3). From the previous

physical, spectral data, co-chromatography with rutin standard and by comparing the obtained data with those reported for rutin [25], compound E5 was identified as rutin.

Compound E6: white amorphous powder, soluble in methanol, R_f = 0.23 in S1. ESI-MS spectrum showed molecular ion peaks m/z 432 $[M]^+$. ^1H NMR (400 MHz, DMSO) δ ppm aglycone: 12.97 (s, OH-5), 6.43 (1H, d, J= 2.2, H-6), 6.82 (1H, d, J= 2.2, H-8), 7.95 (d, J=8.9, H-2', H-6'), 6.93 (d, J=8.9, H-3', H-5'), 10.51 (s, OH-4'), 6.87 (s, H-3); glycoside: 5.44 (d, J=7.4, H-1''), 3.27-3.47 (5H, m, H-3''-H-6''), 3.71 (m, H-2''), 5.12 (s, OH-2''), 5.07 (s, OH-3''), 5.05 (s, OH-4''), 4.65 (s, OH-6''). From the previous physical, spectral data, co-chromatography with apigenin-7-glucoside standard and by comparing the obtained data with those reported for apigenin-7-glucoside [27], compound E6 was identified as apigenin-7-glucoside.

Metabolomic analysis by LC/MS

Analysis of the secondary metabolites of the alcoholic extracts of the two *Sansevieria* species resulted in identification of different classes of compounds as shown in table 2 and fig.(6-9). The identification of compounds was based on comparing the molecular ion peak in the negative and positive modes and the product ion with the literature and previously identified compounds. Identified compounds were mostly phenolic acids, flavonoids and saponins.

Molecular docking

Docking study was done for further investigation of possible targets involved in the cytotoxicity of the extracts from *Sansevieria sp*. against HepG-2, A-549 and CACO2 cell lines. This was done through docking studies of the identified compounds in the extracts in the active sites of known cancer targets. Three targets were chosen which are Epidermal Growth Factor Receptor (EGFR), Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) and receptor for advanced glycation end-products (RAGEs). Scores of docked compounds in the active sites of the three targets and scores for redocking of the internal ligands are also shown in table 3.

Identified compounds in general showed better docking scores with EGFR (PDB ID: 1M17). EGFR is co-crystalized with the erlotinib which a known EGFR inhibitor. Erlotinib is known to form two key hydrogen bonds with EGFR. These includes two hydrogen bonds between quinazoline ring of erlotinib with Met769 and with Thr766 through a water bridge [39]. Redocking of Erlotinib was able to predict the crystallization pose with RMSD of 1.491 and a docking energy of -7.1 kcal/mol (Figure 10A). Among the identified compounds, ruscogenin, rutin and apigenin-7-glucoside showed best binding with EGFR with docking energies less than -9.0 kcal/mol which is better than erlotinib. Out of the three compounds only apigenin-7-glucoside was able to satisfy the 2 key hydrogen bonds (Figure 10B and C) while the 2 other compounds were docked far from these key amino acids (Figure 11) and they were rejected for that. The sugar part of apigenin glycoside was involved in one of the key interactions between Thr766 with glucose 6-OH. The sugar also forms several hydrogen bonds with Leu764, Ala719, Glu738, Asp831 and Thr830. The other key hydrogen bond with Met769 is formed with the 5 OH of the flavonoid. In addition to the hydrogen bonds, several hydrophobic interactions are contributing to the binding energy. For example, rings A and B of apigenin is sandwiched between Val702 and Leu820. Also, ring C is in close proximity to Phe699 (Figure 10B). These findings suggest the potential inhibitory effect of apigenin-7-glucoside for EGFR which need further study.

All the identified compounds showed weaker binding in the active site of VEGFR-2 when compared to the co-crystalized ligand, sorafenib (**Table 3**). This suggest that these compounds might not show comparable binding and inhibition of VEGFR-2 relative to sorafenib. Tested compounds also showed weaker binding to RAGE compared to their binding with EGFR as can be deduced from docking energy values of each compound in the active sites of RAGE and EGFR (Table 3).

4. Discussion

Cancer comes after heart disease in leading causes of death. There are not any extremely effective drugs to treat most cancers. Nowadays, there is a general call for new drugs that are highly effective, possess low toxicity and have a minor environment impact. Natural products offer many opportunities for innovation in drug discovery. Actually, natural products play a major role in cancer prevention and treatment. A large number of antitumor agents currently used in the clinic are of natural origin. In fact, majority of anticancer prescription drugs approved internationally were natural products or their derivatives. Plants have been the chief source of natural compounds used for medicine [40].

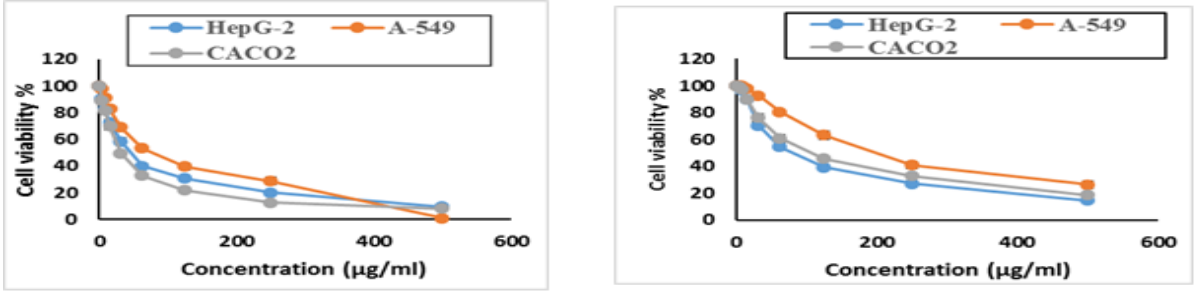
Phenolic compounds are secondary metabolites in plants with aromatic ring bearing one or more hydroxyl groups. Isolated phenolic compounds from plants include simple phenols, phenolic acids, flavonoids, lignins and lignans, tannins, xanthones and coumarins. These compounds exhibit potent anti-cancer activities as well as combat many diseases associated with oxidative stress. Previous studies have demonstrated that the health beneficial effects of dietary phenols are due to their ability to exhibit antioxidant and anti-inflammatory activities [41].

In the current study, an evaluation of the cytotoxic activities of the alcoholic extracts of *S. trifasciata*

Prain. and *S. suffruticosa* N.E.Br. was performed. *S. trifasciata* Prain. showed low cytotoxic activity against HepG-2 with IC50 = 81 ± 18.8 µg/ml and no activity against CACO2 and A-549. This agrees with the Kamalova *et al.* study which proved that *S. trifasciata* Prain. does not possess antitumor activity against A-549 [42] and with Abdul-Hafeez *et al.*who proved the low cytotoxic activity of *S. trifasciata* Prain. against colon, breast and hepatic cell lines [9]. On the other hand, *S. suffruticosa* N.E.Br. exhibited significant cytotoxic effect on CACO2 (IC50 = 30.9 ± 0.72 µg/ml) and certain activity against HepG-2 (IC50 = 45.8 ± 2.59 µg/ml). These results corresponds to the higher phenolic and flavonoid contents in *S. suffruticosa* N.E.Br. than *S. trifasciata* Prain. HPLC analysis of both *Sansevieria* extracts using available authentics results in identification of 14 phenolic compounds in *S. trifasciata* Prain. and 16 phenolic compounds in *S. suffruticosa* N.E.Br. Catechin and kaempferol are the major compounds identified 22.13 and 17.17 mg/ 100 g, respectively in the fresh aerial parts of *S. trifasciata* Prain., while p-hydroxybenzoic acid and p-coumaric acid are the major compounds identified 17.29 and 15.86 mg/ 100 g, respectively in the fresh aerial parts of *S. suffruticosa* N.E.Br. Apigenin-7-glucoside and cinnamic acid are detected only in *S. suffruticosa* N.E.Br. Trials to isolate compounds from ethyl acetate extracts of both *Sansevieria* species was done. Seven compounds have been isolated and reported for the first time from these species. These compounds are chlorogenic acid, kaempferol, quercetin and catechin from *S. trifasciata* Prain. and apigenin-7-glucoside and rutin from *S. suffruticosa* N.E.Br.

Metabolomic study using LCMS of the two *Sansevieria* extracts under study results in identification of many compounds of different classes; nine phenolic acids, five flavonoids and seven saponins. TIC of both species (figure 6-9) showed higher percentage of saponins when compared to phenolics. Ruscogenin was identified in both species and this agrees with Gonzalez *et al.* [43] who isolate ruscogenin from *S. trifasciata* Prain. Teponno *et al.* [38] proved the presence of trifasciatosides A-J in *S. trifasciata* Prain. planted in Cameroon, but in Egypt only trifasciatosides A and F were identified by LCMS. All identified compounds by LCMS were used in the molecular docking study to relate the cytotoxic activity of the extracts with the identified compounds. The first target chosen was EGFR because several types of cancers are linked to change or activation of EGFR. For example, mutation (L858R) and the deletion (E746-A750) of EGFR are very common in non-small cell lung cancer (NSCLC) patients [44]. Also, EGFR abnormalities may have a relevant role in both carcinogenesis and clinical progression of colon cancer [45]. VEGFR-2; second target chosen; has an important role in the angiogenesis. Angiogenesis, or formation of new blood vessels, is very critical for tumour growth and progression [46]. Another target that was studied is RAGEs which binds to the advanced glycation end-products leading to the activation of transcription factors which is responsible for carcinogenesis [47].

As mentioned in the result section, all identified compounds showed weak binding in the active sites of both VEGFR-2 and RAGEs. While, they showed better docking results with EGFR, especially apigenin-7-glucoside which satisfied the two key hydrogen bonds with EGFR. Apigenin-7-glucoside was proved to have potential inhibitory effect for EGFR which is a cancer target, this agrees with Smiljkovic *et al.* study [48] which proved that apigenin-7-glucoside was more prominent in cytotoxic activity on colon cancer cells *in vitro* compared to apigenin. Since, apigenin-7-glucoside is only detected in *S. suffruticosa* N.E.Br., this declares the better cytotoxic activity of *S. suffruticosa* N.E.Br. than that of *S. trifasciata* Prain. against colon cancer cells.



(A) **(B)**

Figure 1: Cytotoxic activity of ethanolic extracts of (A) S. suffruticosa N.E.Br. and (B) S. trifasciata Prain. on Liver cell line (HepG-2), Lung cell line (A-549) and Colon cell line (CACO2)

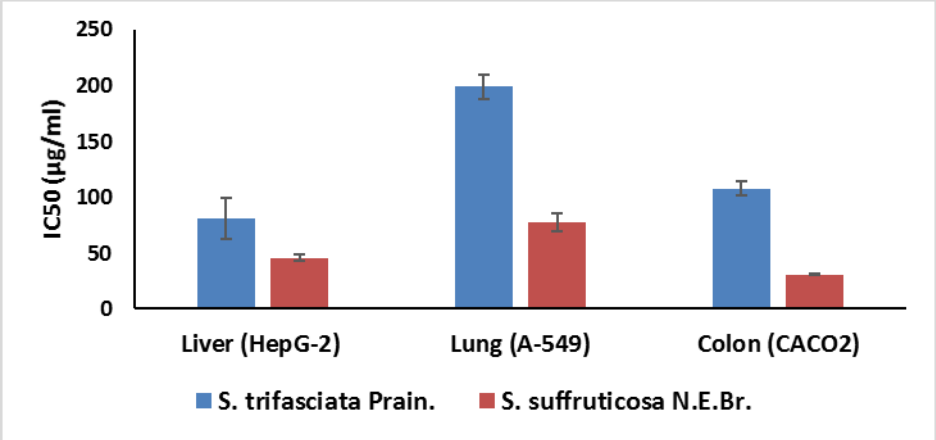


Figure 2: **IC50 of alcoholic extracts of the studied *Sansevieria* species on HepG-2, A-549 and CACO2 cell lines**

Table 1. Results of HPLC analysis of flavonoids and phenolic acids in alcoholic extracts of fresh aerial non-flowering parts of Sansevieria species

Compound \ Species	RT (min.)	*S. trifasciata* Prain.	*S. suffruticosa* N.E.Br.
		mg/ 100 g fresh aerial parts	mg/ 100 g fresh aerial parts
Gallic acid	4	0.34	0.31
Protocatechuic acid	8.1	2.40	2.62
***p*-hydroxybenzoic acid**	12.6	5.11	17.29
Catechin	15.6	22.13	7.32
Chlorogenic acid	16.7	0.09	0.10
Caffeic acid	17.7	0.34	0.02
Syringic acid	19.4	1.42	0.12
Vanillic acid	21.4	1.09	0.08
Ferulic acid	29.2	0.34	1.70
Rutin	32	0.07	3.07
***p*-coumaric acid**	33.9	0.22	15.86
Apigenin-7-glucoside	36	ND	1.60
Rosmarinic acid	39.7	3.05	0.34
Cinnamic acid	45.2	ND	0.82
Quercetin	52	1.42	1.60
Kaempferol	54.3	17.17	6.06

ND: not detected; RT: retention time

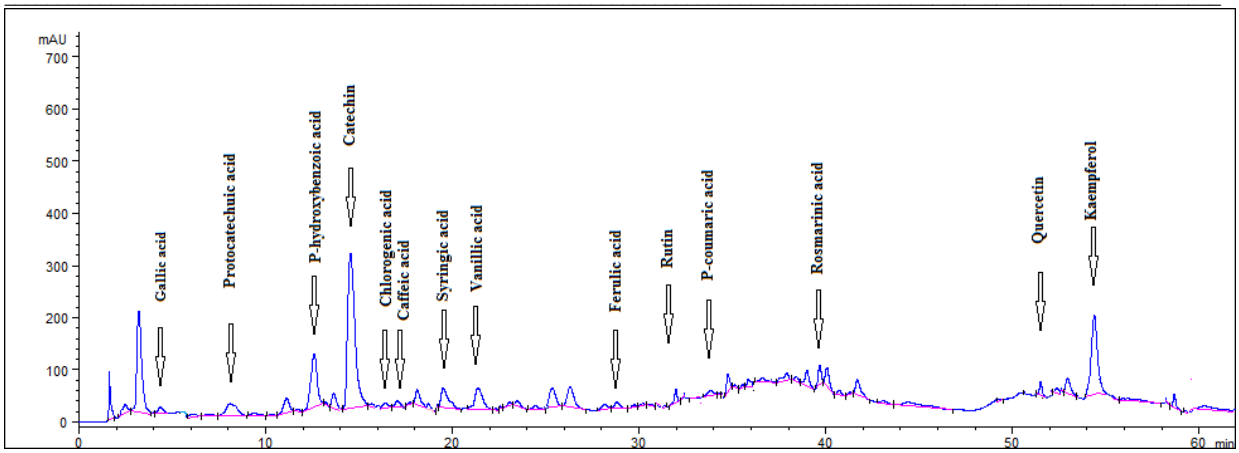


Figure 3. HPLC chromatogram of flavonoids and phenolic acids in alcoholic extract of S. trifasciata Prain. fresh parts

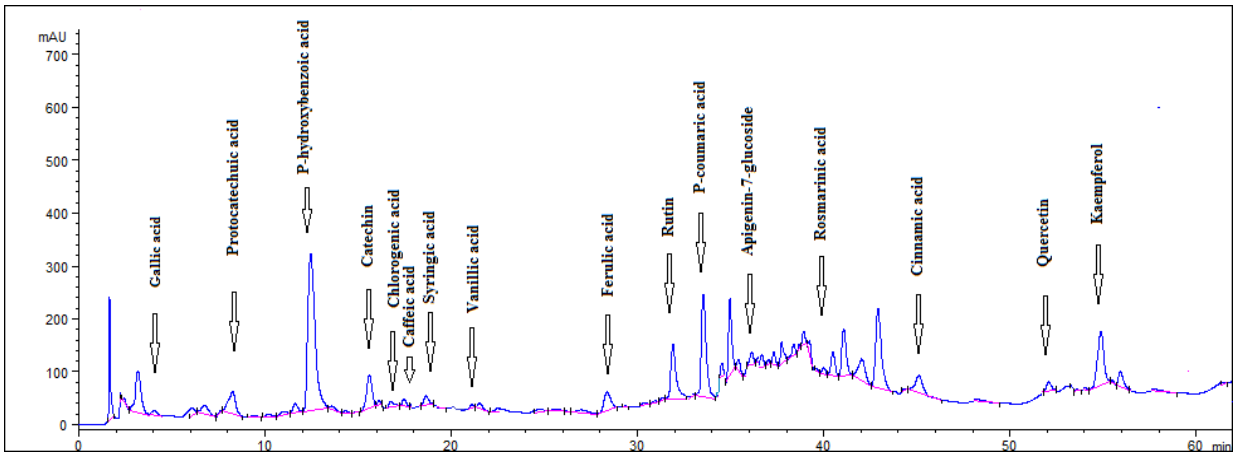


Figure 4. HPLC chromatogram of flavonoids and phenolic acids in alcoholic extract of S. suffruticosa N.E.Br. fresh parts

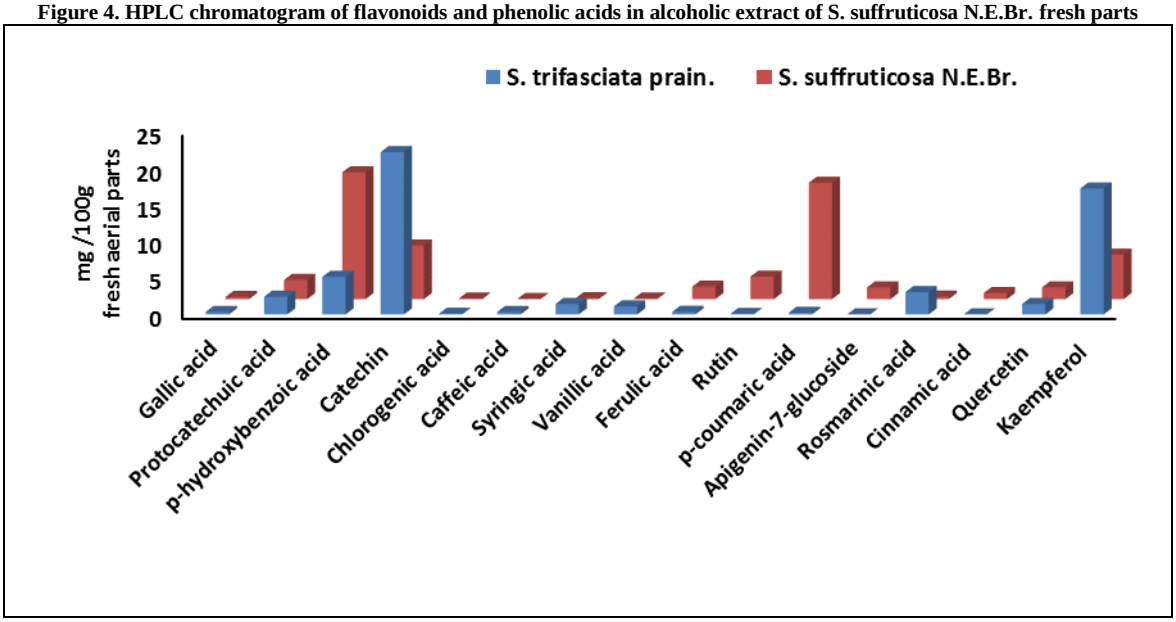


Figure 5. Flavonoids and phenolic acids in alcoholic extract of S. trifasciata Prain. and S. suffruticosa N.E.Br. fresh parts

Table 2: Compounds identified by LC/ MS of alcoholic extracts of non-flowering aerial parts of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br.

m/z	Rt (min)	*S. trifasciata* Prain.	*S. suffruticosa* N.E.Br.	Molecular weight	Identified compounds	Molecular forumla	Reference
169 [M-H]$^-$	**0.76**	+	+	170.12	Gallic acid	$C_7H_6O_5$	[28]
139 [M+H]$^+$	**1.2**	+	+	138.12	p-hydroxybenzoic acid	$C_7H_6O_3$	[29]
153 [M-H]$^-$	**1.3**	+	+	154.12	Protocatechuic acid	$C_7H_6O_4$	[30]
353 [M-H]$^-$	**1.5**	+	+	354.31	Chlorogenic acid	$C_{16}H_{18}O_9$	[30]
289 [M-H]$^-$	**1.91**	+	+	290.26	Catechin	$C_{15}H_{14}O_6$	[28]
431 [M-H]$^-$	**2.04**	-	+	432.38	Apigenin-7-glucoside	$C_{21}H_{20}O_{10}$	[31]
195 [M+H]$^+$	**2.22**	**+**	**+**	194.19	Caffeine	$C_8H_{10}N_4O_2$	[32]
181 [M+H]$^+$	**2.51**	+	+	180.16	Caffeic acid	$C_9H_8O_4$	[29]
287 [M+H]$^+$	**2.92**	+	+	286.23	Kaempferol	$C_{15}H_{10}O_6$	[32]
303 [M+H]$^+$	**4.41**	+	+	302.24	Quercetin	$C_{15}H_{10}O_7$	[32]
609 [M-H]$^-$	**4.95**	+	+	610.52	Rutin	$C_{27}H_{30}O_{16}$	[30]
383 [M+Na]$^+$	**7.05**	+	+	360.31	Rosmarinic acid	$C_{18}H_{16}O_8$	[33]
163 [M-H]$^-$	**7.30**	+	+	164.05	p-coumaric acid	$C_9H_8O_3$	[34]
195 [M+H]$^+$	**7.37**	+	+	194.18	Ferulic acid	$C_{10}H_{10}O_4$	[35]
453 [M+Na]$^+$	**7.65**	+	+	430.62	Ruscogenin	$C_{27}H_{42}O_4$	[36]
167 [M-H]$^-$	**10.18**	+	+	168.14	Vanillic acid	$C_8H_8O_4$	[28]
838 [M]$^-$ 706 [M-xylosyl]$^-$ 692 [M-rhamnosyl]$^-$ 559 [M- xylosyl-rhamnosyl]$^-$	**12.93**	-	+	838.01	Neoruscogenin-1-*O*-{*O*-α-L-rhamnopyranosyl -(1→2)-*O*-[β-D-xylopyranoxyl-(1→3)]-α-L-arabinoside}	$C_{43}H_{66}O_{16}$	[37]
1071 [M+Na]$^+$	**14.15**	-	+	1048.50	Trifasciatoside A	$C_{50}H_{80}O_{23}$	[38]
891 [M+Na]$^+$	**14.28**	+	+	868.45	Trifasciatoside B	$C_{44}H_{68}O_{17}$	[38]
1087 [M+Na]$^+$	**23.7**	+	-	1064.53	Trifasciatoside F	$C_{51}H_{84}O_{23}$	[38]
1071 [M+Na]$^+$ 1031 [M+H- H2O]$^+$ 869 [M+H- H2O -glu]$^+$ 723 [M+H- H2O-glu-rha]$^+$ 707 [[M+H- H2O-2glu]$^+$	**30.53**	+	+	1048.16	Ruscoside	$C_{50}H_{80}O_{23}$	[36]
891 [M+Na]$^+$ 869 [M+H]$^+$ 707 [M+H- glu]$^+$ 561 [M+H-glu-rha]$^+$ 429 [aglycone+H]$^+$ 411 [aglycone+H -H2O]$^+$	**31.17**	+	-	868.01	Ruscin	$C_{44}H_{68}O_{17}$	[36]

m/z: mass/charge, Rt (min): retention time (in minutes)

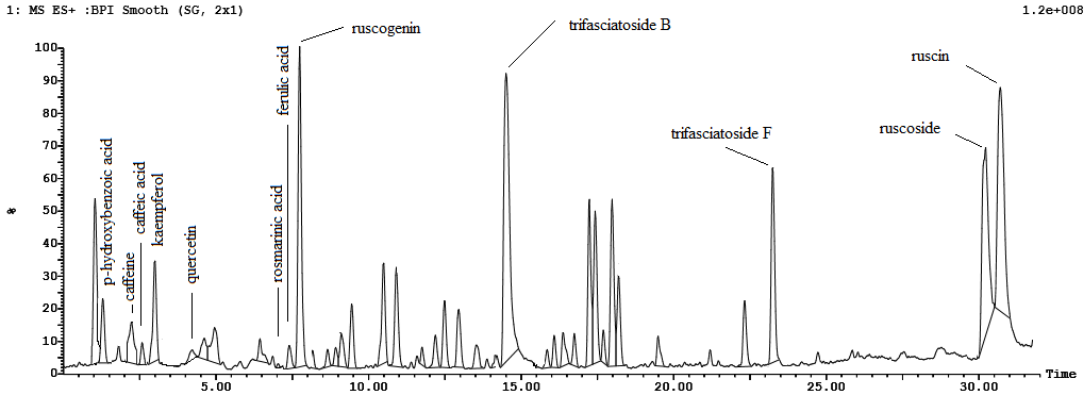


Figure 6. TIC (positive mode) of S. trifasciata Prain.

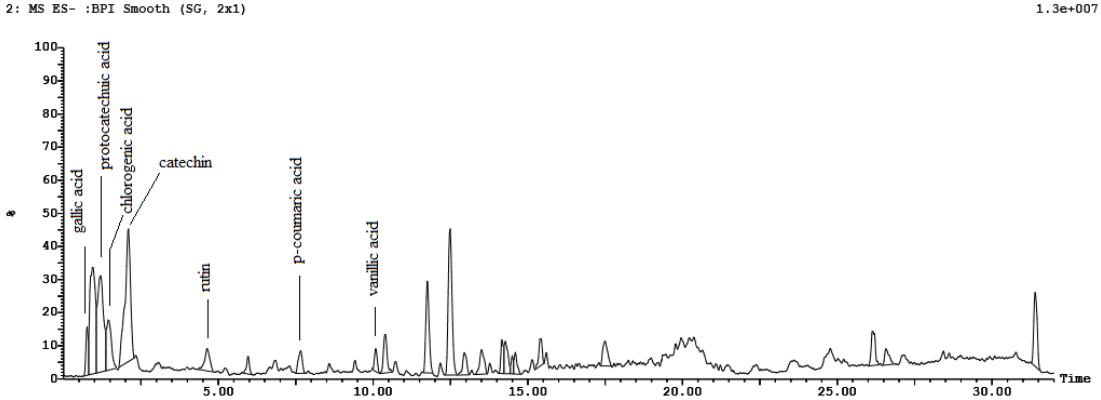


Figure7. TIC (negative mode) of S. trifasciata Prain.

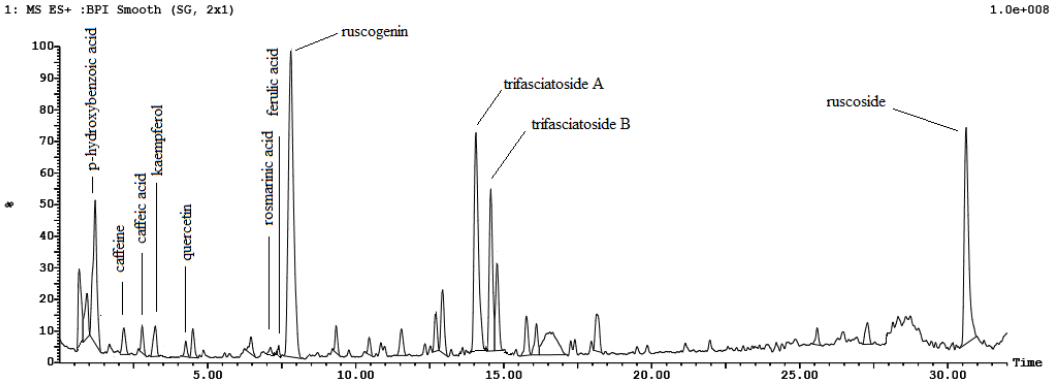


Figure 8. TIC (positive mode) of S. suffruticosa N.E.Br.

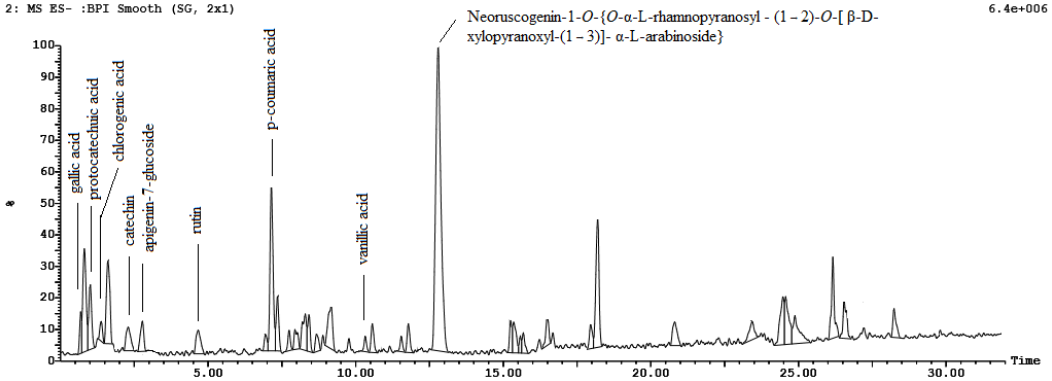


Figure 9. TIC (negative mode) of S. suffruticosa N.E.Br.

Table 3. Scores of docked compounds in the active sites of the three targets

Name	Docking Energy (kcal/mol)		
	EGFR (1M17)	**VEGFR2 (4ASD)**	**RAGE (2MOV)**
Co-crystalized Ligand	**-7.1 (erlotinib)**	**-12.0 (sorafenib)**	**-4.9 (L-ornithine derivative)**
Caffeine	-5.7	-5.6	-4.5
Neoruscogenin-1-*O*-{*O*-α-L-rhamnopyranosyl - (1→2)-*O*-[β-D-xylopyranoxyl-(1→3)]- α-L-arabinoside}	-8.3	-5.4	-6.2
Gallic_Acid	-5.7	-5.6	-4.7
Kaempferol	-8.2	-8.3	-5.6
Quercetin	-8.5	-8.2	-4.7
Apigenin-7-glucoside	-9.4	-9.2	-6.7
Caffeic_acid	-6.0	-6.8	-5.4
Catechin	-7.9	-8.4	-5.7
Chlorogenic acid	-8.0	-8.3	-6.1
p-hydroxybenzoic acid	-6.1	-5.8	-4.5
Protocatechuic acid	-6.0	-6	-4.7
p-coumaric acid	-5.7	-7.3	-4.8
Rosmarinic acid	-7.4	-8.6	-5.9
Ruscin	-8.4	-6.7	-5.9
Ruscogenin	-9.6	-8.6	-7.3
Ruscoside	-9.0	-7	-6.6
Rutin	-9.5	-8.4	-6.3
Trifasciatoside A	-7.9	-5	-5.1
Trifasciatoside B	-8.0	-3.8	-5.7
Trifasciatoside F	-8.0	-3.8	-4.8
Vanillic acid	-6.1	-5.9	-5.0
Ferulic acid	-5.2	-6.3	-4.7

5. Conclusion

From this comparative study, it was found that there is direct relation between cytotoxic activity and phenolic content. The cytotoxic activity of the alcoholic extract of *S. suffruticosa* N.E.Br. was higher than that of *S. trifasciata* Prain. against colon cancer cells CACO2 and lung cancer cells A-549 which is related to the higher phenolic and flavonoid contents in *S. suffruticosa* N.E.Br. than that in *S. trifasciata* Prain. and also, to the presence of apigenin-7-glucoside only in *S. suffruticosa* N.E.Br. Alcoholic extract of *S. suffruticosa* N.E.Br. appeared to be attractive material for further studies leading to possible drug development of cytotoxic potentiality.

6. Conflicts of interest

The authors declared that there is no conflict of interest.

7. Acknowledgement

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