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ORIGINAL ARTICLE

Gas Chromatography-Mass Spectrometry Fingerprint and *in vitro* Cytotoxic Studies of *Rubus steudneri* Leaf Fractions against Michigan Cancer Foundation-7 Breast Cancer Cell Line

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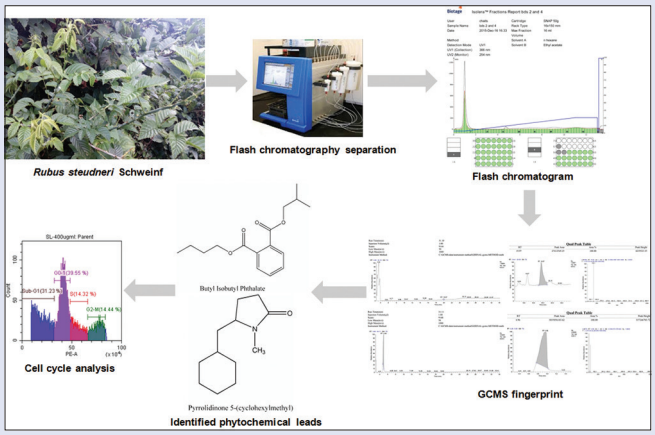
ABSTRACT

Aim: *Rubus steudneri* Schweinf. (*Rosaceae*) is one of three *Rubus* species that grow in Ethiopia. Studies of this species have been restricted to *in vitro* antioxidant, antidiabetic, and nutritional evaluation. Until today, no identification has been made of its phytochemical fingerprints, resulting in an unclear picture of its phytochemical constituents. This study investigated the phytochemical composition of *R. steudneri* and its *in vitro* cytotoxicity against Michigan Cancer Foundation-7 (MCF-7) breast cancer cell lines and Vero cell lines. **Materials and Methods:** The leaf powder was subjected to liquid–liquid fractionation using *n*-hexane, chloroform, ethyl acetate, methanol, and water as solvents and the obtained fractions were subjected to flash chromatography and *in vitro* cytotoxicity studies in MCF-7 cell lines at concentrations from 1 to 1000 µg/mL, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. **Results:** The chloroform fraction proved to be the most promising of the bioactive fractions, with 50% of cytotoxicity inhibition for concentrations of 10 µg/mL on MCF-7 cell lines and 125 µg/mL on Vero cell lines. Two important drug leads, namely butyl isobutyl phthalate and 2-pyrrolidinone 5-(cyclohexylmethyl), were identified in chloroform fraction using gas chromatography-mass spectrometry (GC-MS) and may be responsible for the *in vitro* cytotoxicity. Flow cytometer results indicated that the chloroform fraction arrests cell cycle in the sub-G1 phase at a concentration of 100 µg/mL on MCF-7 cell lines, which proves that metabolites in this fraction may belong to the apoptotic population. **Conclusion:** In this study, butyl isobutyl phthalate and pyrrolidinone 5-(cyclohexylmethyl), which have significant cytotoxic effects and cell cycle arrest, are extracted. Further in-depth research is in progress to prove the anticancer activity of *R. steudneri* in search of new leads for anticancer drugs.

Key words: 2-Pyrrolidinone 5-(cyclohexylmethyl), apoptosis, butyl isobutyl phthalate, cell cycle, flash chromatography

SUMMARY

- This study investigated the phytochemical composition of *Rubus steudneri*, and it contains many important complex fatty acids, phenols, terpenoids, and flavonoids. The butyl isobutyl phthalate and pyrrolidinone 5-(cyclohexylmethyl) showed significant cytotoxic effects and could be exploited as preliminary data for further detailed studies. The majority of the compounds isolated in this study have not been studied, and hence, further in-depth research is necessary to isolate, purify, and confirm the structure along with pharmacological evaluation.



Abbreviations used: GC-MS: Gas chromatography-mass spectrometry; TLC: Thin layer chromatography; CAS: Chemical Abstracts Service; MCF-7: Michigan Cancer Foundation-7; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CTC_{50}: 50% of Cytotoxicity Inhibition; NIST: National Institute of Standards and Technology; DEDM: Dulbecco's modification of Eagle medium.

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INTRODUCTION

Modern medicinal science remains unable to cure multiresistant and challenging diseases, including such threats as tuberculosis, cancer, psoriasis, AIDS, swine flu, and dengue fever.[1] The world is dependent on phytochemicals for the treatment of many serious conditions, such as vinblastine and paclitaxel for cancer, silymarin for liver disease, salicylic acid for inflammation, morphine for pain, and digitoxin for cardiac disease.[2]

Recent interest in plant-based medicine or metabolites also stems from the recognition of multiple drug resistance in pathogens due to synthetic

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drugs.[3] However, most of the research into complementary and alternative treatment is incomplete and still considered to form unvalidated therapies. Hence, there is a pressing need for modern phytochemists and pharmacognosists to validate and document the phytochemicals responsible for the pharmacological activities of the related treatments.[4]

Rubus is a widely distributed genus in the plant kingdom, with more than 740 species, and it is indigenous to six continents. However, few of its species have been explored phytochemically and are not validated in traditional use. In Africa, *Rubus* plant species are indigenous and used in treatments for a range of ailments.[5]

Rubus steudneri Schweinf. (*Rosaceae*) is a wild shrub that grows at around 2500 m above sea level; it has a hairy stem, dorsiventral leaves, and edible fruits.[6] It is commonly known as Gora. The fruits are edible in nature and the plant is utilized as a source of food and medicine.[7,8] The fruits of *R. steudneri* are consumed raw by tribal communities in Arsi zone of Central Ethiopia.[9] However, only the nutritional value,[10] antioxidant potential of leaf[11] and fruits,[12] and antidiabetic[13] activity of *R. steudneri* have been investigated, and there have been no reports on its cytotoxic activity. Moreover, the phytochemical information on this species is not clear.[14] This study was conducted to evaluate the cytotoxic potential of various extracts of the leaves of *R. steudneri* to identify its phytochemical print or what major phytochemical groups are responsible for its cytotoxic activity, as well as checking which phase these fractions are arresting in the cell cycle.

MATERIALS AND METHODS

Collection and identification of the plant

The *R. steudneri* leaf material was harvested during December 2015 from Nekemte, East Wollega Zone, Oromia Region, Ethiopia, at 9°5′N 36°33′E to 9.083°N 36.550°E, with an elevation of 2123 m above sea level. The *R. steudneri* specimen was identified by Plant Taxonomist (Dr. Tesfaye Awas) and authenticated at the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia, based on its characteristics under the Voucher No. 1983.

Extraction procedure

The shade-dried leaf powder was subjected to triple kinetic maceration, using 100% v/v ethanol for 72 h, and was vacuum filtered. The obtained

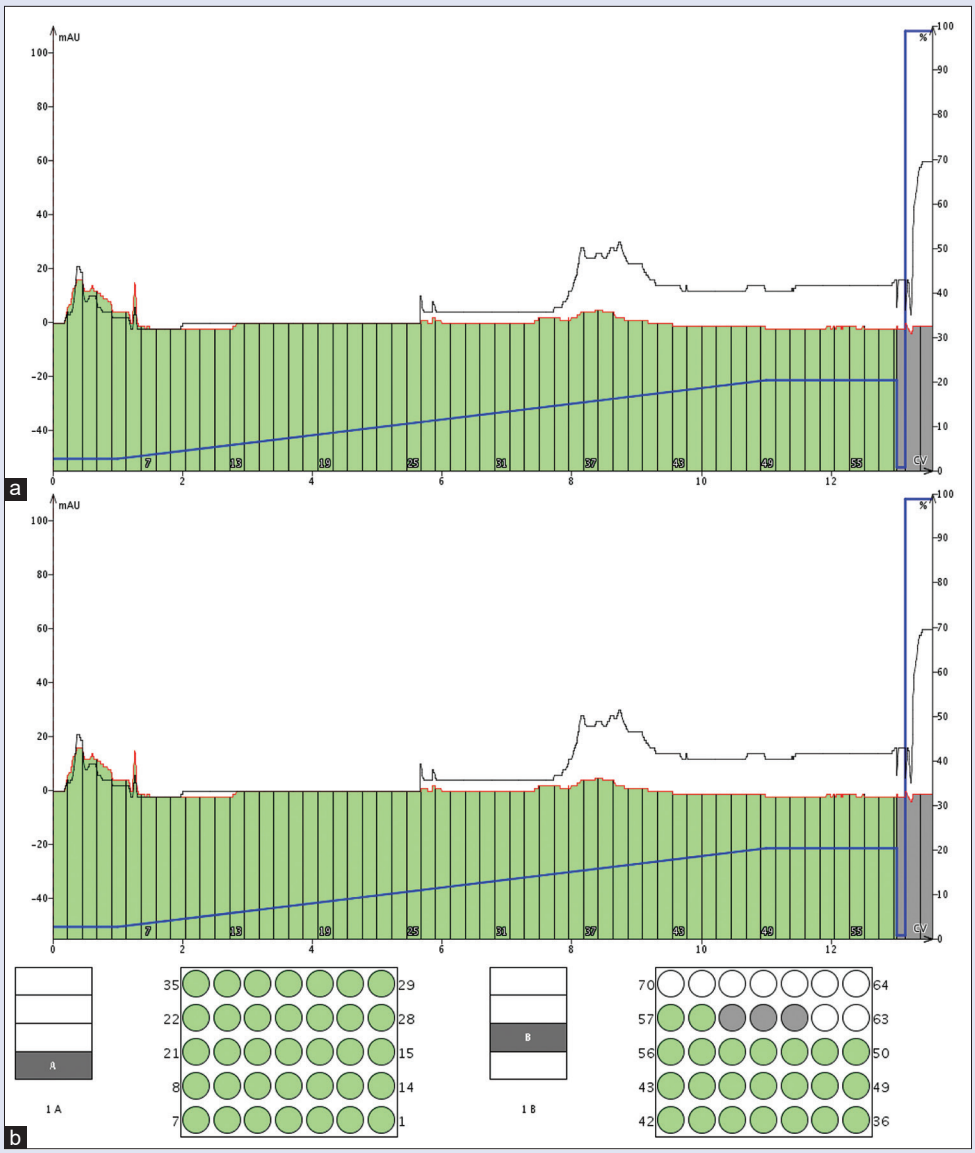

Figure 1: Flash chromatogram of *n*-hexane (a) fraction and (b) fractionation report of *Rubus steudneri* leaf powder extract

filtrate was concentrated in a vacuum using Rotavapor (R-120), and the concentrated mass was kept in a desiccator until a constant weight was obtained. The percentage yield was calculated as 27.5% w/w. An extract of 20 g was dissolved into 50 mL double-distilled water and was filtered. The obtained filtrate was successively fractionated with 150 mL *n*-hexane, 250 mL chloroform, 200 mL ethyl acetate, and 400 mL acetone. The collected fractions were distilled under vacuum using Buchi Rotavapor R-120 to obtain the dried mass of the individual portions.[15] The yield of the portions was calculated and was found to be 4.5% w/w *n*-hexane, 13.5% w/w chloroform, 42% w/w ethyl acetate, 10% w/w acetone, and 30% w/w aqueous fractions.

Isolation and characterization of *n*-hexane fraction using flash chromatography

A 0.5 g hexane fraction was subjected to flash chromatography using hexane:ethyl acetate (80:20) as a mobile phase.[16] A 0.5 g dried fraction was packed onto a 10 g sample holder or samplet. The dried samplet was packed onto the top of a snap 50 cartridge. A gradient method was developed using hexane:ethyl acetate with a flow rate of 15 mL/min, and 58 fractions were collected. Fraction numbers 13–36 were pooled together based on their thin layer chromatography (TLC) similarity [Figure 1]. Because all of the fractions had similar compounds, it was difficult to separate individual compounds from the fraction mass. The obtained mass was subjected to gas chromatography-mass spectrometry (GC-MS) analysis, and it was found to have eight compounds, as presented in the GC-MS report given in Figure 2 and Table 1.

Gas chromatography-mass spectrometry analysis of isolated fractions

The GC-MS analysis of isolated fractions was carried out by using a Thermo Trace 1300 Gas Chromatogram interfaced with Thermo TSQ 8000 Mass Spectrometer equipped with an Xcalibur 2.0 SP1 foundation software, (1 Fisher Pl, Bridgewater Township, NJ 08807, United States), the injector temperature was maintained at 250°C, column used was Restek Rxi-5 ms (Crossbond® 5% diphenyl/95% dimethyl polysiloxane) with length of 30 m, diameter of 0.25 mm, and thickness of 0.25 µm, the pressure was maintained at 100 kPa, the carrier gas used was helium at a flow rate of 1.0 ml/min, and the injection volume is 1.0 µL, and the temperature was started from 70°C for 5 min and increased to 300°C. The mass spectroscopy conditions were as follows: ion source temperature of −250°C, electron energy of −70 eV; interface temperature at 250°C, quadrapole temperature at 150°C, the mass scan range was 50–500 Amu, and detector used was MS TSQ 8000. The isolated fractions and their phytochemical moieties were identified by interpreting the peaks/spectra reported in the National Institute of Standards and Technology (NIST) mass spectral library reported in NIST Standard Reference Database 69: NIST Chemistry web book.[17]

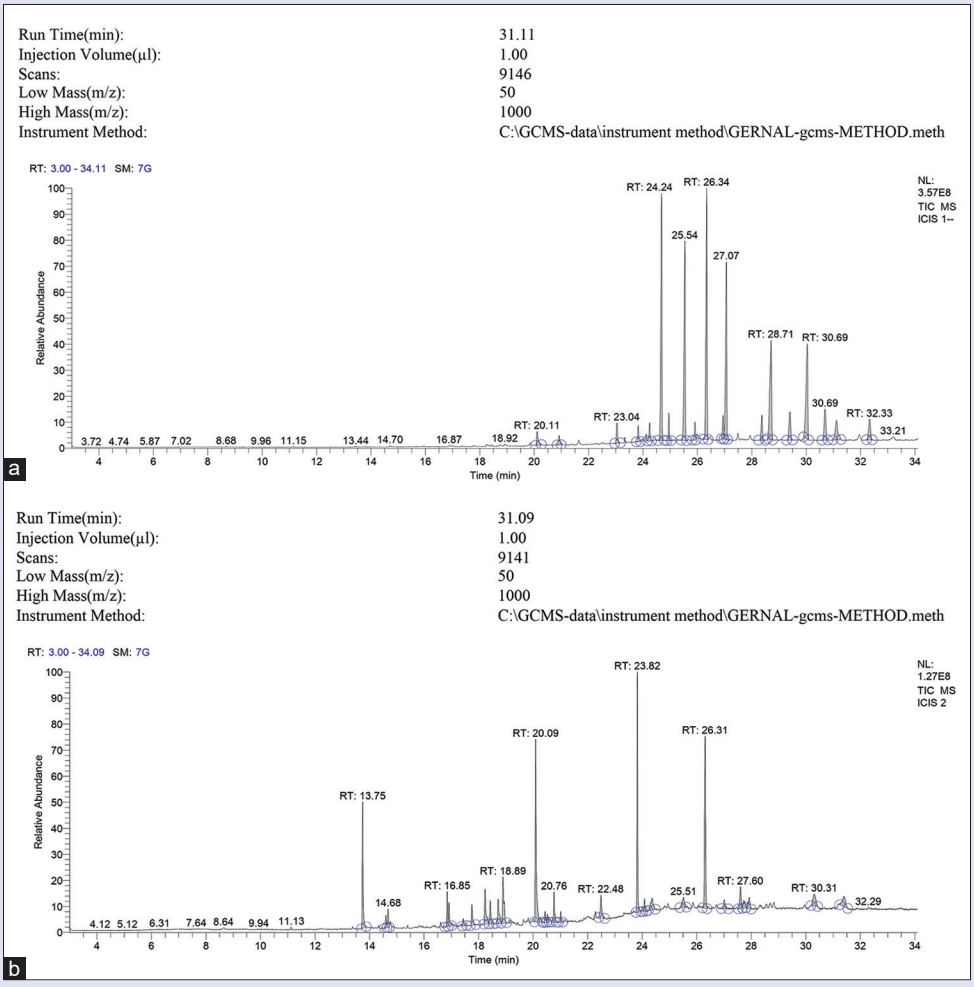

Figure 2: GC-MS chromatogram of acetone (a) fraction and (b) fractionation report of *Rubus steudneri* leaf powder extract

Isolation and characterization of acetone fraction using flash chromatography and gas chromatography-mass spectrometry

A 2 g acetone fraction was purified with Amberlite 50 g of the IR-120 column using chloroform and methanol (50:50). A total of five fractions were produced, with 50 mL per fraction. All fractions were pooled together and evaporated under vacuum, producing brown glittery flakes, and 0.5 g flakes was dried and packed into a 10 g samplet to be subjected to flash chromatography (Biotage, Isolera One) using hexane: ethyl acetate as the mobile phase with a flow rate of 50 mL/min. In all, 54 fractions were collected. Fractions 13–31 [Figure 2] were pooled due to their similar TLC fingerprint analysis and were evaporated under vacuum. The obtained flakes (5 mg) were subjected to GC-MS analysis and found to have phenolics with fatty acids. A total of 16 compounds were identified from the spectral library matching from obtained GC-MS fingerprints [Figures 2 and 3 and Table 1].

Isolation and characterization of chloroform fraction using flash chromatography and gas chromatography-mass spectrometry

The chloroform fraction was subjected to liquid–liquid subfractionation with *n*-hexane and water. The obtained hexane fraction was evaporated under vacuum and the obtained 0.5 g thickly viscous fraction was subjected to flash chromatography (Biotage, Isolera One). The compound was isolated by gradient elution using hexane:ethyl acetate with a flow rate of 50 mL/min. In all, 54 fractions were collected. Fractions 1–19 were pooled together and evaporated to obtain a thick yellow sticky resinous compound (CF-1) and fractions 20–54 were pooled together to get a pale brown (CF-2) [Figure 4].

Both isolates were subjected to GC-MS analysis and the results are given in Figures 5 and 6 and Table 1. The isolated compound CF-1 [Figure 5] was found to be 1,2-benzene dicarboxylic acid butyl 2 methyl propyl ester, and the isolated compound CF-2 [Figure 6] was found to be 2-pyrrolidinone 5-(cyclohexylmethyl).

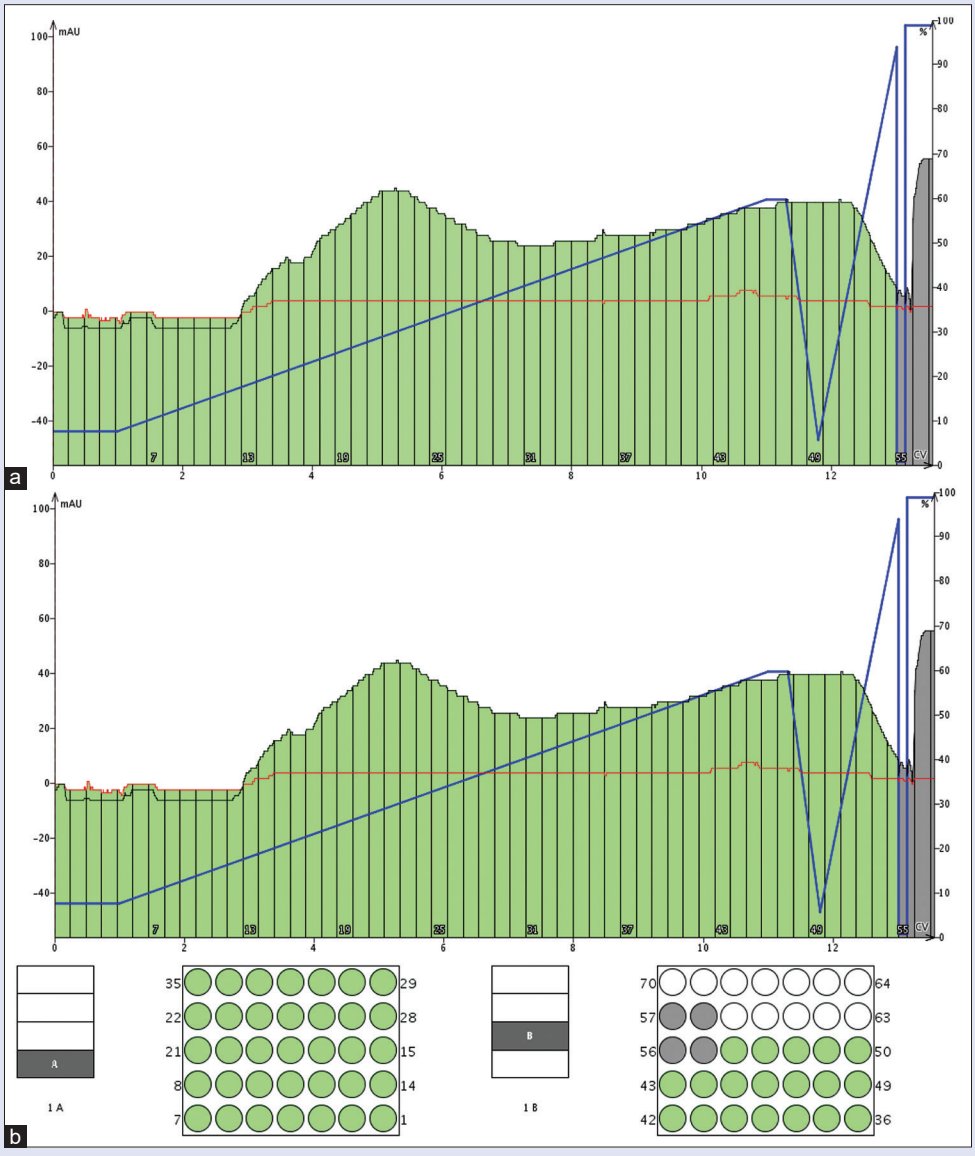

Figure 3: Flash chromatogram of (a) n hexane and (b) acetone fraction from *Rubus steudneri leaf* powder extract

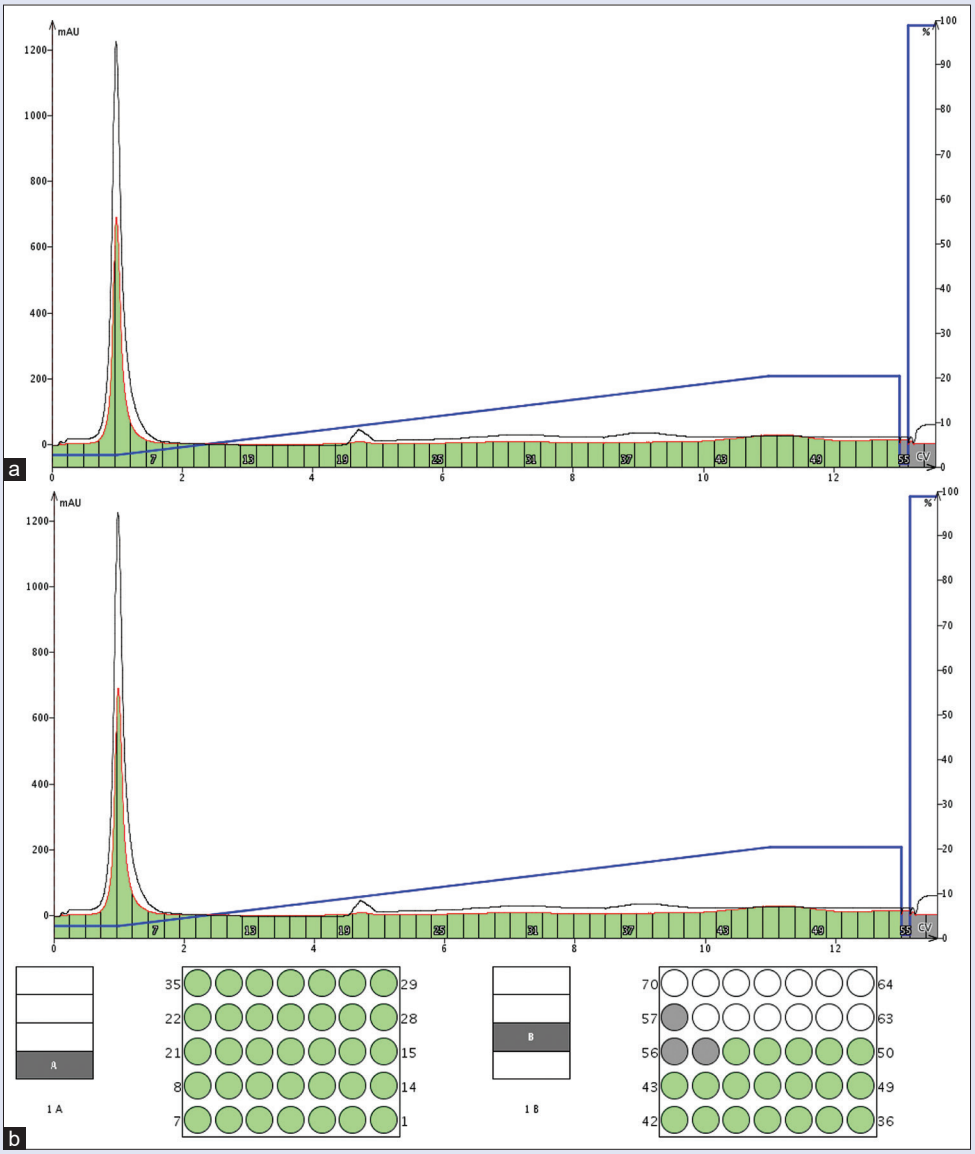

Figure 4: Flash chromatogram of chloroform (a) fraction and (b) fractionation report of *Rubus steudneri* leaf powder extract

In vitro cytotoxic studies of isolated fractions using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay on Michigan Cancer Foundation-7 and Vero cell line

The human breast cancer cell line (Michigan Cancer Foundation-7 [MCF-7]/182R-6 [ECACC 16022506]) and African green monkey kidney cell line (Vero C1008 [Vero 76, clone E6, Vero E6]) were purchased from the European Collection of Cell Cultures (Merck, USA) for this research. The MCF-7 cell lines were grown in Eagle's minimum essential medium (Sigma-Aldrich Chemicals, 56416C, USA) supplemented with 10% v/v of fetal bovine serum (Sigma-Aldrich Chemicals, F4135, USA) and 0.01 mg/mL bovine insulin (Sigma-Aldrich Chemicals, I1882, USA), and the Vero cell line were grown in Dulbecco's modification of Eagle medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich Chemicals, F4135, USA). All of these cell lines were incubated at 37°C in a 5% CO_2 atmosphere in an incubator (Remi Elektrotechnik Ltd., India).

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using DMEM containing 10% FBS. Then, 100 µL diluted cell suspension was added to each well in a 96-well microtiter plate. After the formation of a partial monolayer in the plates, the supernatant was discarded and the monolayer was washed with DMEM. Finally, 100 µL different concentrations for all fractions were added to per well in triplicate, and it was incubated at 37°C for 48 h in 5% CO_2 atmosphere with examinations being conducted every 24 h microscopically.

After 48 h, the sample solutions were discarded and 20 µL 3-(4,5 dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich Chemicals, USA) was added to each well. The plates were gently shaken in the shaking incubator for 5 h at 37°C in a 5% CO_2 atmosphere. The supernatant was removed, 50 µL isopropanol was added, the formed formazan was solubilized with gentle agitation, and the absorbance was measured under a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated following the method described by Kumar *et al.*[18]

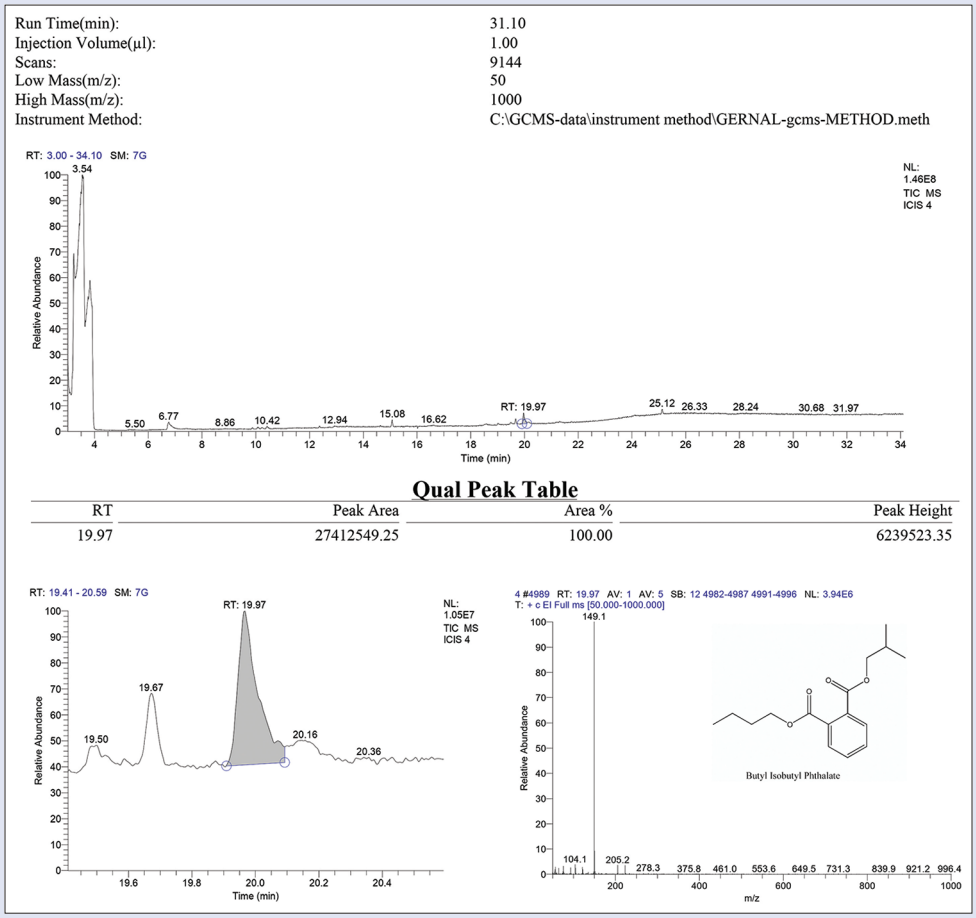

Figure 5: Gas chromatography-mass spectrometry fingerprint analysis of isolated fraction (CF-1) from chloroform fraction of *Rubus steudneri* leaf powder extract

Cell cycle analysis

The fraction showing the highest cytotoxicity was subjected to cell cycle analysis, following the method described by Chaitanya *et al.*[19] using the propidium iodide (Sigma-Aldrich Chemicals, USA) assay technique. After 24 h, the 0.3×10^7 cells in each well plate were treated with different concentrations of chloroform fraction, and after 48 h, the cells were collected and fixed with cool isopropanol (70%) for 14 h at 3°C and the cells were stained with propidium iodide (Sigma-Aldrich Chemicals, USA). The results were analyzed using a flow cytometer (FACS Aria III).

RESULTS AND DISCUSSION

The hexane fraction was subjected to a gradient flash separation system (Isolera One, Biotage, Switzerland) using an *n*-hexane:ethyl acetate mixture as eluent. A total of 58 fractions were obtained and fractions numbered 13–36 were pooled together based on TLC purity matching [Figure 3]. After the pooled concentrate was subjected to GC-MS, it was shown to contain eight important fatty acid moieties with long-chain hydrocarbons [Figure 7]. All of the isolated compounds were long-chain hydrocarbons with different side chains and constituents. These included octacosane, tetratriacontane, 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaenoic, triacontane, nonacosane, tetracosane, methyl octacosanoate, and octacosyl acetate. These eight hydrocarbons thus are the main long-chain fatty acids present in the leaves of the plant *R. steudneri*. These compounds are non-polar and lipid soluble; they have different melting and boiling points and some have racemic mixtures.

The 13–31 flash isolates from the acetone fraction were subjected to GC-MS and 16 phytochemical similarities were found, including polar fatty acids (phthalic acid) and phenolics (phenol 2,4, bis (1,1dimethylethyl)) [Figure 7]. These phytochemicals were reported for their antioxidant activities (Li, 2017). Hexadecane is a squalene type of plant metabolite, commonly found in the oils of *Piper nigrum* L. (*Piperaceae*), with a melting point of 18°C and a boiling point of 287°C. However, its pharmacology and uses remain unreported.[20] The octadecane is a fatty acid moiety with a boiling point of 316°C and a melting point of 28.2°C, reported to be present in alcoholic beverages prepared from hops. There are no clear pharmacological reports available on it.[21] The acetone fraction proved to have many interesting unexplored fatty acids and phenolic polar moieties. There is no available pharmacological evidence on these chemicals, so there is wide scope for researchers to work in this area. Our team is pursuing investigations here for possible future publication.

Of all the fractions, the chloroform fraction of *R. steudneri* leaf extract showed an excellent *in vitro* cytotoxic inhibition with 50% of cytotoxicity inhibition (CTC_{50}) values of 10 µg/mL on MCF-7 cell lines, compared to *n*-hexane fraction, with 55.5 µg/mL; ethyl acetate fraction, with 352.5 µg/mL; acetone fraction, with 225.2 µg/mL; water fraction, with 721 µg/mL; and chloroform fraction, with CTC_{50} values of 125 µg/mL on Vero cell lines. This made researchers to explore the phytochemical nature of this fraction through GC-MS as a part of the initial research and are two phytochemical similarities were found,

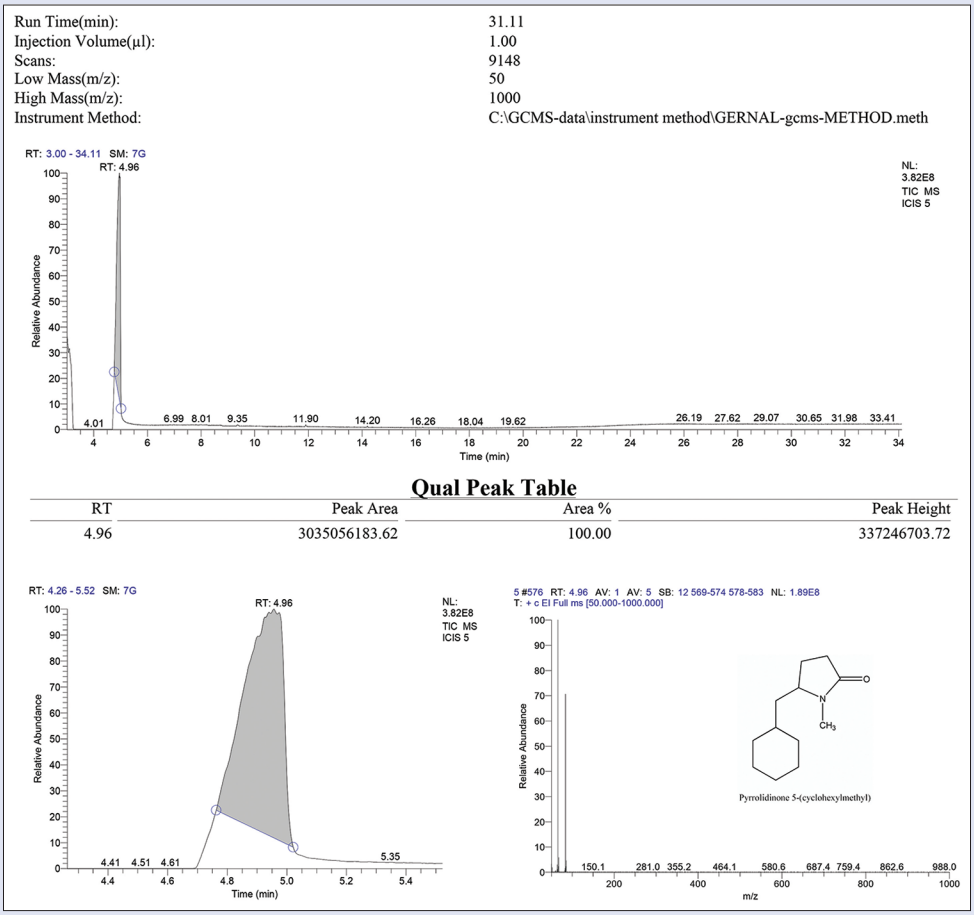

Figure 6: Gas chromatography-mass spectrometry fingerprint analysis of isolated fraction (CF-2) from chloroform fraction of *Rubus steudneri* leaf powder extract

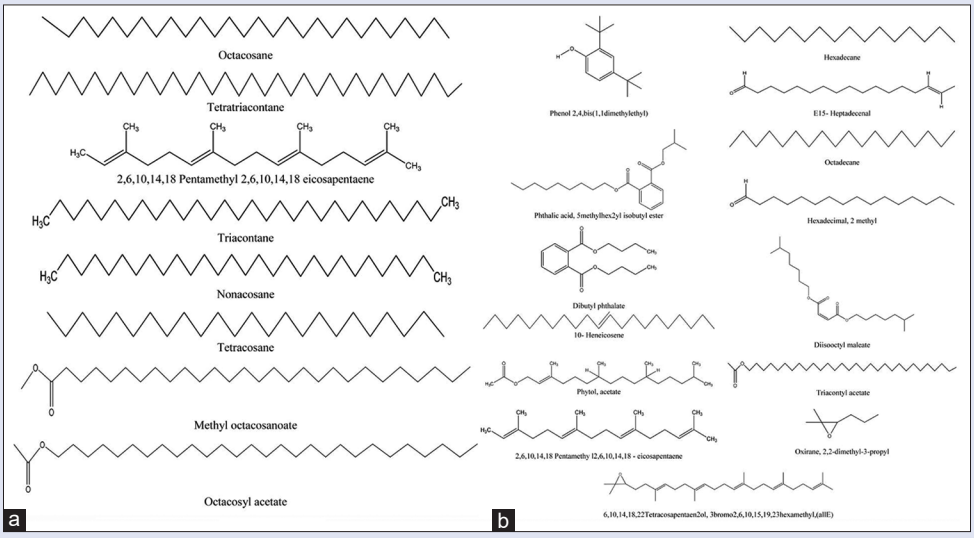

Figure 7: Identified phytochemical parent similarities from (a) *n*-hexane and (b) acetone fraction of *Rubus steudneri* leaf powder extract

namely 1,2-benzene dicarboxylic acid butyl 2 methyl propyl ester and 2-pyrrolidinone 5-(cyclohexylmethyl), which were separated using flash chromatography [Figures 5 and 6].

To observe the cell cycle arrest pattern, the chloroform fraction was subjected to cell cycle analysis. It was observed that this fraction arrested the cell cycle at 100–400 µg/mL at the sub-G1 phase and this value increased from 4.75% to 31.23%, with a decrease of the G01 phase from 65.83% to 39.55% [Figure 8]. This shows that the phytochemical population present in this fraction may be apoptotic. To confirm this, our research team is conducting other assays.

Butyl isobutyl phthalate is also known as phthalic acid, and it has a molecular weight of 278.3 g/mol and a monoisotopic mass of 278.1 g/mol. The molecular formula for the compound is $C_{16}H_{22}O_4$. It is acidic and has a pKa of 5.7, with a pH of 3.0.

Butyl isobutyl phthalate is polar and dissolves in water to form a purple solution. It is an essential extract from plants, such as from the leaves and twigs of *Clerodendrum inerme* (L.) Gaertn. (*Lamiaceae*). According to Chemistry of Biomolecules, 2017, this chemical is of great importance because it is a tissue antioxidant and a potent alpha-glucosidase inhibitor, which can be used in the management of type II diabetes mellitus.[22]

Pyrrolidinone 5-(cyclohexylmethyl) has a molecular weight of 181.3 g/mol, and it has one hydrogen donor and one hydrogen acceptor. It is monoisotopic, with an approximate monoisotopic mass of 181.5 g/mol).[23]

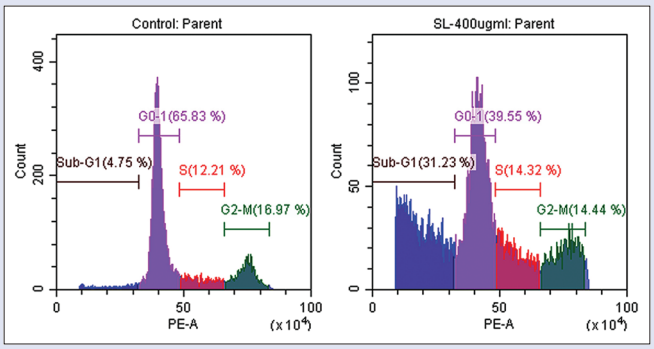

Figure 8: Chloroform fraction containing drug leads induces cell cycle arrest in Michigan Cancer Foundation-7 cell lines

Hence, butyl isobutyl phthalate and pyrrolidinone 5-(cyclohexylmethyl) are ideal subjects for research to develop isolation techniques and cytotoxic molecular mechanisms.

Earlier research has asserted the importance of *R. steudneri* as an antioxidant and lipid peroxidation inhibitor, due to the presence of phenols and flavonoids in them.[11,12] This study showed that there are fatty acid moieties in plant extract, the major part of which is present as phthalate ester or fatty acid ester, identified as butyl isobutyl phthalate. This compound is a commercially available metabolite in its viscous oil form (CAS No. 17851-53-5). It has been identified as a volatile oil constituent in ligraine extracts from different parts of *C. inerme*, reported for its antioxidant property. Recent evidence supports the claim that butyl isobutyl phthalate is being synthesized due to its economic significance as a potential α-glucosidase inhibitor for the treatment of type II diabetes.[24] Phthalates have attracted attention in polymers as plasticizers, and these phthalates are toxic at various concentrations, depending on their specific structures. Many phthalate metabolites have been identified as secondary metabolites in plants, animals, and microbes in various protective and defensive mechanisms.[25]

There have been no pharmacological reports on pyrrolidinone 5-(cyclohexylmethyl), but its chemical structure indicates that it belongs to the polar alkaloid group. This study provoked the suspicion that the *in vitro* cytotoxic potentiality of the chloroform fraction of this plant is also due to the presence of the alkaloid moiety pyrrolidinone 5-(cyclohexylmethyl). However, further in-depth *in silico* molecular studies are required to prove cytotoxicity here. Some research appears

Table 1: Gas chromatography-mass spectrometry fingerprinting of *n*-hexane, acetone and chloroform (chloroform fraction-1 and chloroform fraction-2) flash chromatography isolate from *Rubus steudneri* leaf

Identified similar chemical entities from GC-MS	RT	Molecular formula	CAS number
***n*-hexane fraction**			
Tetratriacontane	23.04	$C_{34}H_{70}$	14167-59-0
Octacosane	24.24	$C_{28}H_{58}$	630-02-4
Supraene	24.68	$C_{30}H_{50}$	7683-64-9
Tetracosane	24.95	$C_{24}H_{50}$	646-31-1
2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	26.34	$C_{25}H_{42}$	75581-03-2
Nonacosane	27.07	$C_{29}H_{60}$	630-03-5
Octacosanoic acid, methyl ester	30.69	$C_{29}H_{58}O_2$	55682-92-3
Octacosyl acetate	31.11	$C_{30}H_{60}O_2$	18206-97-8
Acetone fraction			
Phenol, 2,4bis (1,1dimethylethyl)-	13.75	$C_{14}H_{22}O$	96-76-4
Hexadecane	14.68	$C_{16}H_{34}$	544-76-3
E15Heptadecenal	16.85	$C_{17}H_{32}O$	--
Octadecane	16.91	$C_{18}H_{38}$	593-45-3
Hexadecanal, 2-methyl-	17.44	$C_{17}H_{34}O$	55019-46-0
Phthalic acid, 5-methylhex-2-yl isobutyl ester	17.75	$C_{19}H_{28}O_4$	--
Dibutyl phthalate	18.72	$C_{16}H_{22}O_4$	84-74-2
Diisooctyl maleate	20.44	$C_{20}H_{36}O_4$	1330-76-3
10-Heneicosene (c,t)	20.76	$C_{21}H_{42}$	95008-11-0
Phytol acetate	21.01	$C_{22}H_{42}O_2$	--
Triacontyl acetate	24.35	$C_{32}H_{64}O_2$	41755-58-2
2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	26.31	$C_{25}H_{42}$	75581-03-2
2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	27.60	$C_{29}H_{48}O$	--
6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-	27.73	$C_{30}H_{51}BrO$	65746-05-6
Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	27.93	$C_{30}H_{50}O$	7200-26-2
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	30.31	$C_{20}H_{40}O$	102608-53-7
CF-1 and CF-2			
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	19.97	$C_{16}H_{22}O_4$	17851-53-5
2-Pyrrolidinone, 5-(cyclohexylmethyl)	4.96	$C_{11}H_{19}NO$	14293-08-4

CAS: Chemical abstracts service; CF: Chloroform fraction; GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

to have shown that pyrrolidine derivatives such as pyrrolidine dithiocarbamate are good anticancer drug leads having cytotoxicity against lung cancer cell line (SCLC cell line).[26] Hence, the pyrrolidinone 5-(cyclohexylmethyl) molecule should be studied in depth to confirm its cytotoxic potentiality.

This study is focused on examining the *in vitro* cytotoxic potentiality of this plant extracts and fractions, and their phytochemical nature, which is responsible for this cytotoxic potentiality, was identified using GC-MS. Additional in-depth research is underway, focusing on the isolation of the individual metabolites.[27]

CONCLUSION

R. steudneri is a storehouse of both non-polar and polar cytotoxic phytochemicals. In this study, butyl isobutyl phthalate and pyrrolidinone 5-(cyclohexylmethyl), which have significant cytotoxic effects and cell cycle arrest, are extracted. The cytotoxic activity of the majority of the compounds isolated in this study through phytochemical fingerprints has not been studied in depth. Thus, the investigation described in this report produced a valuable foundation for a potential anticancer drug through a primary initiation. Further, in-depth research, some of which is already in progress, is needed to isolate, purify, and confirm the structure of these drug leads. This study indicated that the leaf powder of *R. steudneri* contains many important complex fatty acids, phenols, terpenoids, and flavonoids. The major cytotoxic compounds found in this study were butyl isobutyl phthalate and pyrrolidinone 5-(cyclohexylmethyl) derivatives. Further work is in progress to determine its unknown moieties and pharmacological properties.

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Conflicts of interest

There are no conflicts of interest.

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