Pharmacogn. Mag.
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ORIGINAL ARTICLE

Chemical Composition and Cytotoxic Activity of Methanol Extract and its Fractions of *Streblus asper* Leaves on Human Cancer Cell Lines

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Submitted: 01-09-2017 Revised: 19-10-2017 Published: 10-04-2018

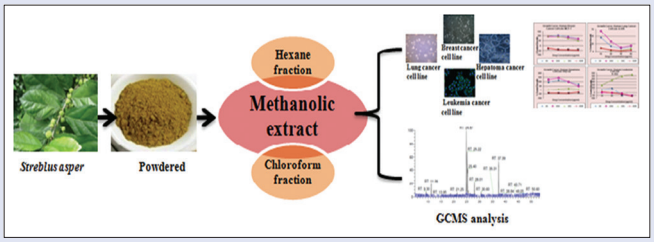
ABSTRACT

Background: *Streblus asper,* family Moraceae is well-known important medicinal plant used in the Indian system of medicine. In Ayurveda, stem bark of *S. asper* is recommended against elephantiasis for which there is still no any other effective medicine in the modern system of medicine. **Objectives:** In the present work, methanol extract (SAM) and its fractions of *S. asper* leave tested for *in vitro* anticancer activity against cancer cell lines (MCF-7, A-549, Hep-G2, and K-562) which claims its folklore importance in cancer and gas chromatography-mass spectrometry identification of extracts was also performed. **Materials and Methods:** Shade dried plant material was extracted with methanol and fractionated sequentially with hexane, chloroform, and butanol. **Results:** All tested extracts found highly effective against human lung cancer cell line (A-549) with IC_{50} <10 μg/mL. On Hep-G2 cancer cell line, only chloroform fraction are highly active with IC_{50} <10 μg/mL. Methanol and hexane fraction showed potent anticancer activity on K-562 cancer cell line with IC_{50} <10 μg/mL. **Conclusion:** Qualitative phytochemical analysis confirmed the presence of fatty acids, phytosterol, triterpenoids, polyol, sugar acid, aldehyde, diterpene, terpene, carboxylic compounds, acid and sugar in *S. asper* leaves extract. Topmost abundant compounds in SAM are α-D-glucopyranoside (10.60%), glycerol (7.96%), myo-inositol (4.90%), and butanedioic acid (3.30%). Hexane consists of the higher amount of hexadecanoic acid (18.07%), octadecanoic acid (7.39%), β-sitosterol (4.50%), and α-D-glucopyranoside (4.03%). Higher component in chloroform extract is lupenyl acetate (11.25%).

Key words: Anticancer activity, cancer cell line, cytotoxic, gas chromatography mass spectrometry, *in vitro*, phytochemicals

SUMMARY

- All extracts of *Streblus asper* found potential anticancer activity against lung cancer cell line (A-549)
- Chloroform fraction is highly active on hepatoma cancer cell line (Hep-G2) whereas methanolic, and hexane fractions have highly cytotoxic potency against leukemia cancer cell line (K-562)
- Methanolic extract of *S. asper* is rich source of glycosides, fatty acids, and phytosterol
- In Gas chromatography-mass spectrometry evaluation of *S. asper* β-stigmasterol, β-sitosterol, lycopene, and lupeol identified as an anticancer agent from previously reported literature.



Abbreviations used: SRB: Sulforhodamine B assay; SAM: Methanol extract; SAH: Hexane extract; SAC: Chloroform extract.

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DOI: 10.4103/pm.pm_391_17

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INTRODUCTION

Natural products in dietary sources are assumed to have anticancer benefits including fruits, vegetables, and spices consists of biologically active[1] components such as phytosterols, isoflavones, saponins, lycopene, triperpenoids, and many others.[2] A number of compounds in these natural products are gaining importance as adjuvant anticancer agents.[3] Many unsaturated fatty acids and a few saturated branched-chain fatty acids have been reported to exhibit anticancer activity.

Streblus asper family Moraceae is an ancient medicinally important plant[4] commonly known as sheora, siamese rough brush, toothbrush tree and koi. It is widely found in Southern Asia such as India, Sri Lanka, Bangladesh, Thailand, Philippines, and Malaysia.[5] *S. asper* is well known for its healing properties and used for specific ailments.[6] It is used traditionally in leprosy, piles, diarrhea, elephantiasis, dysentery,[7] menorrhagia,[8] epilepsy, and inflammatory swelling.[9] Root juice of *S. asper* in Marma tribe in Bangladesh has been used to treat irregular menstruation and to improve delayed menstruation.[6] The leaves are chewed with salt as an anthelmintic.[10] *S. asper* finds a place in the Ayurvedic Pharmacoepia of India[11] and has also been described in some monographs.[12] Its bark extract is useful in the treatment of fever, dysentery, gingivitis, and toothache. The leaf extract consists of insecticidal activity toward mosquito larvae, antibacterial effect, and preventive effect on oral and dental diseases.[13] *S. asper* consists of high amount of cardiac glycosides.[14] Several cardiac glycosides are

Cite this article as: Rawat P, Kumar A, Singh TD, Pal M. Chemical composition and cytotoxic activity of methanol extract and its fractions of *streblus asper* leaves on human cancer cell lines. Phcog Mag 2018;14:141-4.

toxic to human cancer cell lines.[15] Two cardiac glycosides of this plant strebloside and mansonin and volatile oil have been reported to have cytotoxic activity.[16]

MATERIALS AND METHODS

Plant materials

S. asper was collected from local area of Lucknow (India) in June 2015 and authenticated by Taxonomy Division of the CSIR-National Botanical Research Institute (NBRI), Lucknow. A voucher specimen (No. LWG-82) has been deposited in the herbarium of NBRI.

Extraction of plant

The shade dried crushed leaves of *S. asper* were extracted thrice with methanol at room temperature. The methanol extract (SAM) was evaporated in a rotatory evaporator and dried by vacuum pump. The SAM was suspended on water and fractionated successively with hexane, chloroform, and butanol.

Culturing of cell lines

The *in vitro* anticancer activity was performed in Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer, Navi Mumbai. All the procedure was performed under sterile condition. The cells were grown in medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% nonessential amino acids in tissue culture plates with Roswell Park Memorial Institute-1640 medium with 2 mM glutamine. The culture plates were incubated in a carbon dioxide incubator at 37°C and 90% relative humidity for maximum growth of cells. The cells were trypsinized by the treatment of trypsin- ethylenediaminetetraacetic acid and single cell suspension in complete growth medium.

In vitro cytotoxicity assay

Anticancer activity was performed using dye in replicates against four human cancer cell lines. To each well of the 96 tissue culture plates 100 µL suspensions was added. The cells were grown at 37°C for 24 h in 5% carbon dioxide incubator. Extracts of different concentration were added in cell suspension. The plates were further harvested for 48 h and 25 µL of 50% trichloroacetic acid. Trichloroacetic acid was added gently to stop cell growth by thin layering of trichloroacetic acid on test compounds. Then plates were further incubated at 40°C for 1 h to fix the cells attached to the bottom of the wells. The plates were washed five times with distill water to remove traces of medium, trichloroacetic acid, sample, serum proteins, and then air dried. The cell growth in air-dried plates was measured by staining with sulforhodamine B dye. The unbound dye was removed by dissolving Tris-base buffer (100 µL/well, 0.01M, pH 10.4) and plates were stirred for 5 min on a mechanical shaker. The optical density was measured at 540 nm on ELISA reader.

Gas chromatography-mass spectrometry

The samples of *S. asper* were analyzed by gas chromatography-mass spectrometry (GC-MS) (Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer) instrument using a TR 50MS column (30 mm × 0.25 mm ID × 0.25 µm, film thickness). Constant flow at 1 mL/min of carrier gas (Helium) was used for sample analysis. The injector temperature of the instrument was programmed at 220°C. Oven temperature was started from 70°C to 290°C with ramp of 5°C/min withhold time 5 min. The sample was injected in a split mode (1:50) with an injection volume of 1 µL. Temperature of the ion source was set at 220°C, and transfer line temperature was at 300°C and ionization of the sample was performed in electron impact mode at an ionization voltage of 70 eV with mass range used from m/z 50–650 amu.

Statistical analysis

The parameters of experiments were conducted and results are presented as mean ± standard deviation. The statistical significance of the data obtained from *in vitro* studies was evaluated by the ANOVA at $P < 0.05$ using Statistical software (SPSS 13).

RESULTS

Anticancer activity of the extracts

These extracts tested against breast (MCF-7), lung (A-549), hepatoma (Hep-G2), and leukemia (K-562). These cell lines revealed different level of sensitivity to different extracts. Results are tabulated in Table 1 and shown in Figure 1. The 50% inhibitory activity with IC_{50} value was below 10 µg/ml on A-549, Hep-G2, and K-562 cell lines. The IC_{50} value revealed that A-549 cells are highly sensitive toward all tested extracts, Hep-G2 cells are toward chloroform fraction and K-562 cells

Table 1: Cytotoxicity of *Streblus asper* extract and its fractions toward sulforhodamine B assay

Fractions and standard	Breast (MCF-7)	Lung (A-549)	Hepatoma (Hep-G2)	Leukemia (K-562)
Methanolic extract	>80	<10	>80	16.8
Hexane fraction	>80	<10	>80	<10
Chloroform fraction	>80	<10	<10	>80
Adriamycin	>80	<10	<10	<10

Table 2: Chemical constitutent identification of different fractions of *Streblus asper* leaves by gas chromatography mass spectrometry

Compound name	Chemical formula	Molecular weight	Area (%)		
			SAM	SAH	SAC
Butanoic acid	$C_4H_8O_2$	88	0.62	0.04	-
Glycerol	$C_3H_8O_3$	92	7.96	1.94	0.76
Phosphoric acid	C_3PO_4	97	0.03	-	-
Glyceric acid	$C_3H_6O_4$	106	1.20	-	-
Benzoic acid	$C_7H_6O_2$	122	-	-	0.25
Butanedioic acid	$C_4H_6O_5$	118	3.30	0.04	0.43
Pentanoic acid	$C_5H_{10}O_2$	102	1.66	-	-
D-pinitol	$C_7H_{14}O_6$	194	1.46	-	-
Benzaldehyde	C_7H_6O	106	-	-	0.53
Azelaic acid	$C_9H_{16}O_4$	188	-	1.31	-
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	-	1.39	-
Myo-inositol	$C_6H_{12}O_6$	180	4.90	1.99	1.15
Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.60	18.07	7.61
Cinnamic acid	$C_9H_8O_2$	148	-	-	0.48
Phytol	$C_{20}H_{40}O$	296	-	0.52	1.29
Heptadecanoic acid	$C_{17}H_{34}O_2$	270	-	0.90	3.10
Octadecanoic acid	$C_{18}H_{34}O_2$	298	0.61	7.39	-
9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280	-	1.18	0.49
α-linolenic acid	$C_{18}H_{30}O_2$	278	-	0.05	0.10
α-D-glucopyranoside	$C_7H_{14}O_6$	194	10.60	4.03	0.96
1-monopalmitin	$C_{19}H_{38}O_4$	330	-	0.85	0.59
Lupenyl acetate	$C_{32}H_{52}O_2$	468	-	-	11.25
Stigmasterol	$C_{29}H_{48}O$	412	-	1.48	0.40
β-sitosterol	$C_{29}H_{50}O$	414	-	4.50	1.27
α-amyrin	$C_{30}H_{50}O$	426	-	1.95	-
β-amyrin	$C_{30}H_{50}O$	426	-	1.67	-
Lupeol	$C_{30}H_{50}O$	426	-	1.78	-
Lycopene	$C_{40}H_{56}$	536	-	0.03	0.84

SAM: Methanol extract; SAH: Hexane extract; SAC: Chloroform extract

are towards methanol and hexane fraction. MCF-7 cells are resistant towards all tested extracts.

Gas chromatography-mass spectrometry analysis of extracts

To identify the compounds that were responsible for the anticancer activity of the *S. asper* extracts, the trimethylsilyl derivatives of all tested extracts were made and subjected to GC-MS analysis. Interpretation of GC-MS data was performed using the data of National Institute Standard and Technology. These identified compounds fall in the category of fatty acids, carboxylic acid, triperpenes, sugar, alcohol and phytosterol shown in Table 2 and Figure 2. Identified fatty acids are butanoic acid (0.04%–0.62%) hexadecanoic acid (1.60%–18.07%), octadecanoic acid (0.61%–7.39%), heptadecanoic acid (0.90%–3.10%), tetradecanoic acid (1.39%), 9,12-octadecadienoic acid (0.49%–1.18%), α-linolenic acid (0.05%–0.10%), and 1-monopalmitin (0.59%–0.85%). Butanedioic acid (0.04%–3.30%), pentanoic acid (1.66%), azelaic acid (1.31%), benzoic acid (0.25%), and cinnamic acid (0.48%) chemically are carboxylic acid. Phytosterols are β-stigmasterol (0.40%–1.48%) and β-sitosterol (1.27%–4.50%). Triterpene is β-amyrin (1.95%) and α-amyrin (1.67%). Phytol (0.52%–1.29%) is diterpene. Lupenyl acetate (11.25%) and lupeol (1.78%) are triterpenoid. Lycopene (0.03%–0.84%) is a terpene. α-D-glucopyranoside (0.96%–10.60%) is a sugar moiety. Glycerol (0.76%–7.96%), D-pinitol (1.46%) and myo-inositol (1.15%–4.90%) belong to polyol group. Glyceric acid (1.20%) is a category of sugar acid. Benzaldehyde (0.53%) belongs to aldehyde group. Phosphoric acid (0.03%) is a mineral acid. Based on the abundance top major compounds in methanolic extracts are α-D-glucopyranoside (10.60%), glycerol (7.96%), myo-inositol (4.90%), and butanedioic acid (3.30%). Hexane extract consists of high amount of hexadecanoic acid (18.07%), octadecanoic acid (7.39%), β-sitosterol (4.50%), and α-D-glucopyranoside (4.03%). In chloroform, extract higher components are lupenyl acetate (11.25%).

DISCUSSION

Our present data show that *S. asper* is a potent anticancer plant. In medical practice, an impressive number of natural products have been introduced for lead and model molecules for structure optimization

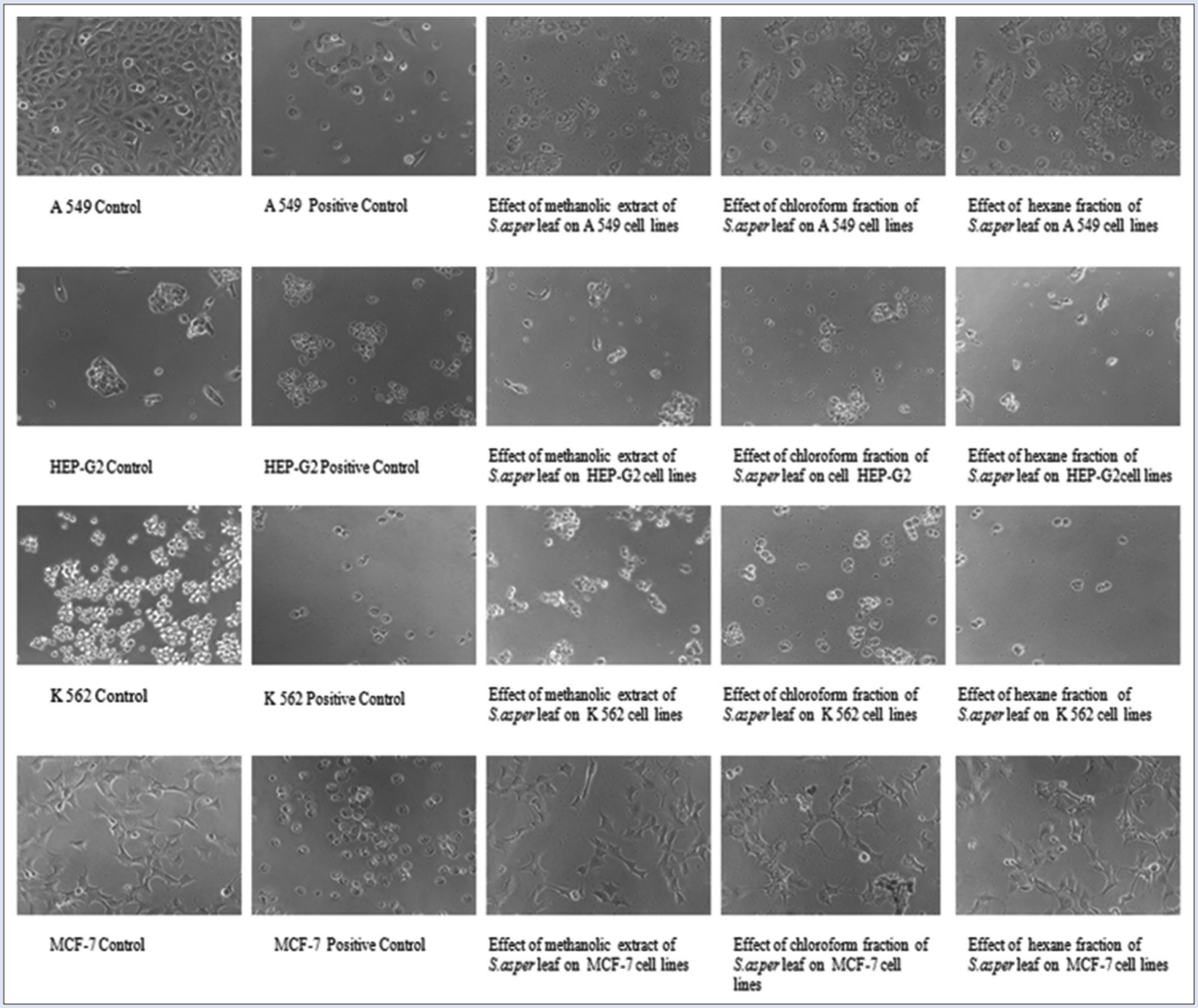

Figure 1: Effect of extracts of *Streblus asper* on different cancer cell lines by Sulforhodamine B assay

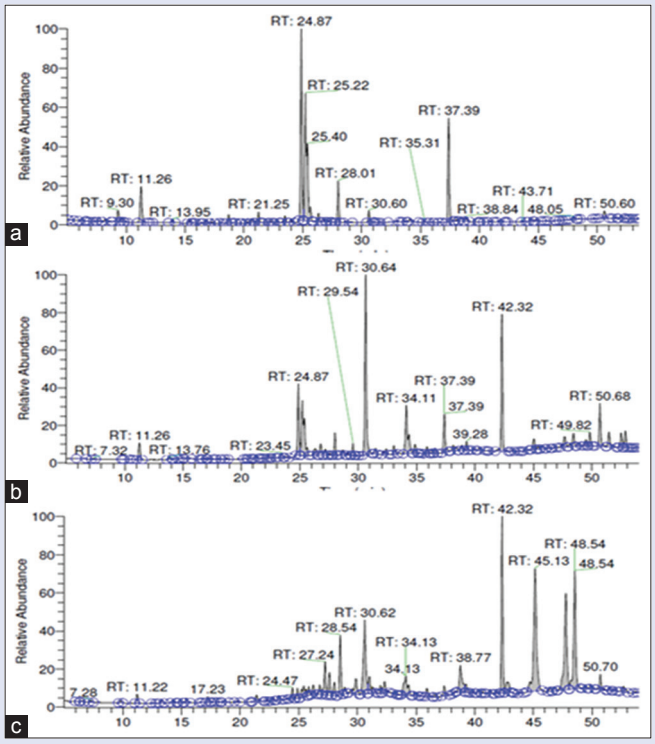

Figure 2: Gass chromatography mass spectrometry spectra of *Streblus asper* (a) methanolic extract (b) hexane extract (c) chloroform extarct

and for the development of more potent and or better-tolerated drugs.[17] Triterpenoids including lupeol enlisted in our study being highly multifunctional measured by their ability to block kappa B activation (nuclear factor), inhibit signal transducer, apoptosis, transcription, and angiogenesis.[18] Phytosterols are known to have various biological properties including anticancer activity. It is indicated that phytosterol rich diet may reduce cancer risk by 20%. Phytosterols enabling anticancer responses by enhancing immune responses recognition of cancer and also have properties including cell cycle progression, apoptosis induction and tumor metastasis inhibition.[19] Hence, phytosterols could be incorporated in diet potentially prevent cancer development. Many fatty acids such as omega-3-fatty acids (α-linolenic acid) were shown to induce apoptosis in a variety of human cancer cell lines and hence is important nutritional adjuvant therapeutics in the prevention of various human cancer diseases.[20] Recently, triterpenes are possibly an alternative method for curing cancer.[21]

CONCLUSION

Our study represents a step forward in the preclinical evaluation of this important medicinal plant. Many traditional medicinal plants are used by tribes for treatments of different diseases.[22] Extracts of *S. asper* leaves expressed potential anticancer agent. The anticancer activity of *S. asper* leaves might be due to the synergistic action of bioactive compounds present in it that are associated with its chemical composition. These effects may be related to the anticancer compounds contained in this, especially stigmasterol, β-sitosterol, lycopene, and lupeol.

Acknowledgements

Authors are thankful to the University Grant Commission, New Delhi, India for the financial support. The authors are also thankful to the Director, CSIR-National Botanical Research Institute, Lucknow, India for facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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