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ORIGINAL ARTICLE

Phytochemicals and Cytotoxicity of *Launaea procumbens* on Human Cancer Cell Lines

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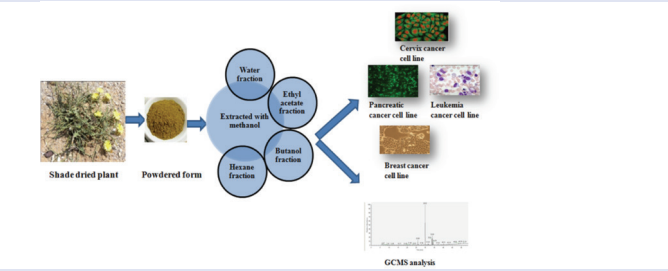
ABSTRACT

Background: The plant *Launaea procumbens* belongs to the family Asteraceae and traditionally used in the treatment rheumatism, kidney, liver dysfunctions and eye diseases. In the present study Phytochemical analysis and fractions of methanolic extract of *L. procumbens* leaves were tested *in vitro* for their cytotoxicity. **Objectives:** Phytochemical analysis and cytotoxic activity of methanolic extract and fractions of *Launaea procumbens* against four cancer cell lines K562, HeLa, MIA-Pa-Ca-2 and MCF-2 by SRB assay. **Materials and Methods:** Powdered leaves of *Launaea procumbens* were extracted sequentially with hexane, ethyl acetate, butanol and water by cold extraction. Phytochemical analysis and cytotoxicity assay were carried out for these fractions using SRB assay against four human cancer cell lines, namely leukemia (K562), cervix (HeLa), pancreatic (MIA-Pa-Ca-2) and breast (MCF-7). **Results:** Ethyl acetate extract exerts potent cytotoxicity against human leukemia (K562), cervix (HeLa) and breast (MCF-7) cell lines IC_{50} value of 25.30±0.50, 19.80±0.10 and 36.90±4.90 µg/ ml respectively. Moderately cytotoxic effect found in hexane extract IC_{50} value of 41±8 and 48.20±0.50 µg/ ml against leukemia (K562), and breast (MCF-7) cancer cell line respectively. The Chemical composition analyzed by GC-MS showed considerable differences in solvent fractions of *Launaea procumbens*. **Conclusion:** This study revealed the cytotoxic potential of ethyl acetate and hexane fractions of *L.procumbens* leaves on different cancer cell lines.

Key words: Cytotoxicity, fractions *Launaea procumbens*, SRB assay

SUMMARY

- Ethyl acetate and Hexane fractions of *Launaea procumbens* plant exhibit cytotoxicity. Among the different fractions Ethyl acetate showed relatively higher cytotoxicity.
- Ethyl acetate found more cytotoxic against leukemia (K 562), cervix (HeLa) and breast (MCF-7) cancer cell lines. Moderete cytotoxicity found in hexane fraction against leukemia (K 562) and breast (MCF-7) cancer cell line.
- GC-MS results showed L. procumbens is a rich source of 1-H- pyrazole, 1-H-imidazole, β-amyrin, α-amyrin and lupeol. These compounds may be attributed for the cytotoxic activity.



Abbreviations used: SRB: Sulforhodamine B assay, MW: Molecular weight

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INTRODUCTION

The family Compositae (Asteraceae) has the distinction of an extremely natural taxon, with its unique floral theme and micro morphological features. It has attracted fascinated and even repelled botanists for over two centuries.[1]

Launaea procumbens is a herb belonging to Compositae (Asteraceae) family commonly known as jangali booti in Hindi and Al-Hewa in Arabic.[2,3] It is found as a weed throughout the plains of India and up to an altitude of 2400 m in the Himalayas.[4] It has been used as a food supplement and as a washing agent[5] in rheumatism and galactogogues.[6] It is used in the folk medicines in the treatment of tumors, skin problems and dysentery.[7] Ayurvedic and herbal preparations of this plant are used in wound healing, longevity,[5] painful urination, and reproductive diseases.[8] It also possesses antipyretic,[9] insecticidal and antifungal properties.[10] Asteraceae family consists of more than 4000 sesquiterpenoids structures with more than 30 different skeletal type. These natural compounds are responsible for wide range of bioactivities, including toxicity for certain cancer cell lines by inhibition of nuclear DNA synthesis.[11]

MATERIALS AND METHODS

Plant material

Plant samples were collected from local area of Lucknow (India) in the month of June, 2014 and identified by Dr. Anand Prakash, Principal Scientist, National Botanical Research Institute (NBRI), Lucknow. A voucher specimen (No. 216343) has been deposited in the herbarium of NBRI.

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Plant extract

The air-dried powdered leaves of *L. procumbens* (580 g) were extracted from methanol. The methanolic extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract was suspended on water and extracted successively with hexane, ethyl acetate and butanol.

Cell lines and culture medium

The cytotoxic activity was performed in Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer, Navi Mumbai. All the cell culture work was performed under sterile conditions and under standard cell culture conditions. Cell cultures were grown in well cultured microtitre plates (RPMI-1640 medium with 2 mM glutamine, pH 7.4 supplemented with 10% fetal bovine serum, 100 µg/mL streptomycin and 100 units/mL penicillin). The targeted human cancer cell lines were grown in a tissue culture flask in carbon dioxide incubator at 37°C and 90% relative humidity to obtain enough number of cells. The cells were harvested by the treatment of trypsin –EDTA and single-cell suspension in complete growth medium.

In-vitro cytotoxicity assay

In-vitro cytotoxic activity against different cancer cell lines was performed using 96-well culture plates in triplicates. To each well of the 96 well microtitre plates 100 µL suspension was added. The cells were allowed to grow at 37°C for 24 h in 5 % carbon dioxide incubator. In the cell suspension, different concentrations of extract were added. The plates were further incubated for 48 h and 25 µL of 50% trichloro-acetic acid added gently to stop cell growth by thin layering of trichloro-acetic acid on test compounds. The 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 distilled water to remove traces of medium, trichloro-acetic 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 (gc–ms)

GC–MS analysis was performed with a Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer instrument using a TR 50MS column (30m x 0.25mm ID x 0.25 µm, film thickness). Constant flow at 1 mL/min of carrier gas (Helium) was used for the analysis. The injector temperature of the instrument was 220°C and oven temperature was started from 50°C, (hold time 5.0 min) to 250°C with ramp of 4°C/ min (hold time 5 min). Sample was injected in split mode (1:50) with injection volume of 1 µL. The ion source temperature was set at 220°C and transfer line temperature was at 300°C. The ionization of the sample was performed in electron impact mode at an ionization voltage of 70 eV. Mass range was used from m/z 50 to 650 amu.

Statistical analysis

The individual data values are presented as the arithmetic mean ± SD (standard deviation). The statistical significance of the results obtained from *in vitro* studies was evaluated by the ANOVA at $P < 0.05$, *using STASTICAL software*.

RESULTS AND DISCUSSION

Cytotoxicity of the extracts

The cytotoxicity study were carried out for different fractions LPH (*n-Hexane*), LPE (Ethyl acetate), LPW (Water) and LPB (*n-Butanol*)

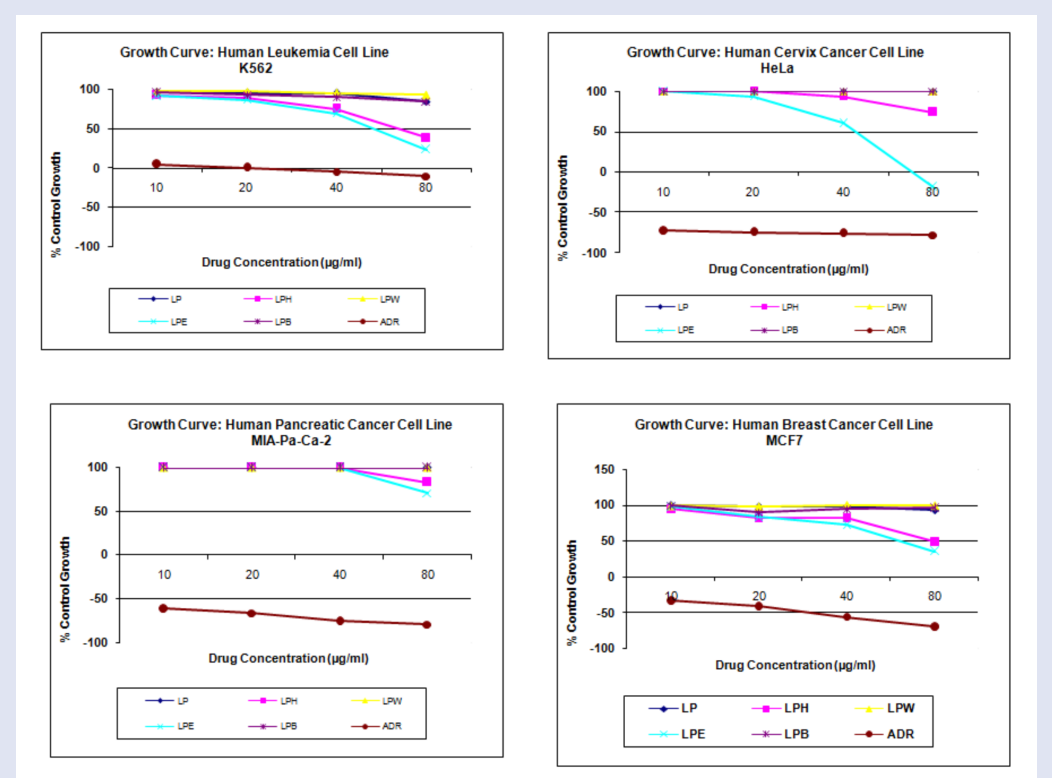

Figure 1: Effect of methanolic extract and its solvent fractions of *L. procumbens* leaf on human cancer cell lines

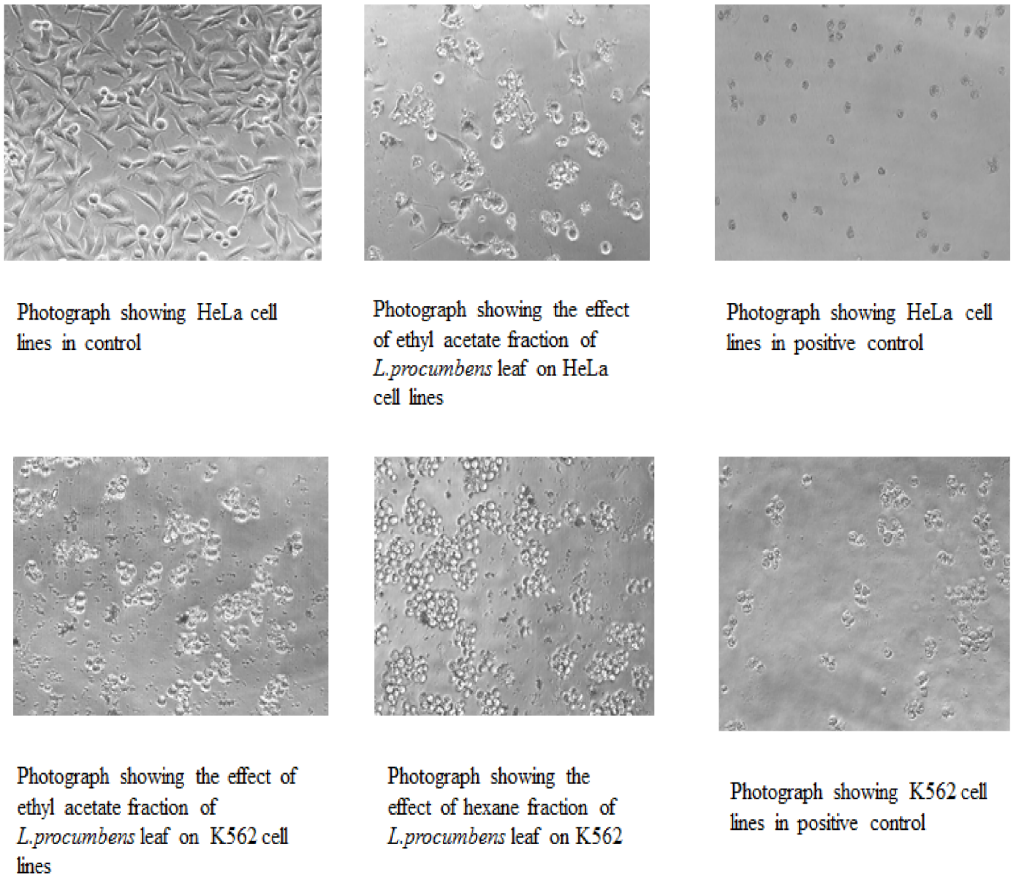

Figure 2: Effect of *L. procumbens* fractions on different human cancer cell lines at 10 µg/mL by SRB

prepared from methanolic extract of *L. procumbens*. These extracts tested against Leukemia (K 562), Cervix (HeLa), Pancreatic (MIA-Pa-Ca-2) and Breast (MCF-7) human cancer cell lines at different concentrations to determine the IC_{50} value by SRB assay. SRB assay was done in triplicate to evaluate the cytotoxic activity of this plant. The results are presented in Table 2. Adriamycin drug has been used as a standard in all above human cell lines. Among the different extracts only ethyl acetate and hexane fraction exhibited the property to inhibit the growth of cancer cell lines (Figure 1 and Figure 2) except pancreatic cell line. Ethyl acetate fraction was found active against cervix (HeLa), leukemia (K562) and breast (MCF-7) cancer cell lines with IC_{50} value of 42, 56.70 and 64 µg/mL, respectively. Cytotoxic activity was found in hexane extract against leukemia (K562) cell lines with IC_{50} of 69.10 µg/mL. The pancreatic cell line (MIA-Pa-Ca-2) was found resistant against these extracts. Water and butanolic fractions were not found to be active against these human cancer cell lines.

Phytochemical analysis

Phytochemical analysis of different solvent fractions prepared from methanolic extract of *L. procumbens* was performed and identified compounds have been listed in Table 1. The major compounds in hexane fraction are 1-H-pyrazole (40.55%), β-amyrin (17.40%), α-amyrin (10.23%), lupeol (7.35%), and 1-H-imidazole (3.19%). The major compounds in ethyl acetate are 1-H-pyrazole (25.25%), β-amyrin (13.21%), α-amyrin (5.53%), and lupeol (4.05%). Butanol fraction contains major components as 1-H-pyrazole (30.95%) and D-glucose (5.50). The major compounds in water fraction are inositol (38.91), 1-H-pyrazole (33.17%) and fructose (7.34%). Most often, a particular biological activity is not due to one constituent but mostly a mixture of bioactive plant compounds is responsible for the activity. Many scientists have tested different moieties for cytotoxicity such as pyrazole as pyrazole hydrazoles,[12] Pyrazolo [3,4,5- kl] acridines, anthrapyrazoles,[13] ester coupled bisanthrapyrazole derivatives, 3-(I H- indole-3-yl)- 1H-pyrazole-5-carbohydrazide derivatives 32, 1-aryl-4-(4,5-dihydro-1H-imidazole-2-yl)-1H-pyrazoles and 5-amino-1-aryl-4-(4,5-dihydro-1H-imidazole-2-yl)-1H-pyrazoles. Imidazole being a heterocyclic compound is generally known as anticancer compound. These have toxic effect on cell division. This is why they have major role in chemotherapy in cancer such as imidazole-(benz) azole and imidazole piperazine derivatives.[14] Terpenoids are also known for anticancer activity.[15] Presently, triterpenes are considered an alternative method for curing cancer because of their cytotoxic and chemotoxic properties.

CONCLUSION

Our results indicate that ethyl acetate and hexane fractions prepared from methanolic extract of *L. procumbens* leaves were found active against specified human cancer cell lines. On the basis of literature pyrazole, imidazole and triterpenes show activity against cancer. Thus, anticancer potency in specified fractions against tested cell lines may be due to one of these or mixture of these as our study revealed high amount of these in plants.

Table 1: Chemical composition identified from different fractions prepared from methanolic extract of *L. procumbens* leaves

Fractions	Chemical composition	Molecular formula	MW	%
Hexane fraction	1-H-Pyrazole	$C_3H_4N_2$	68	40.55
	1-H-Imidazole	$C_3H_4N_2$	68	3.19
	Inositol	$C_6H_{12}O_6$	180	1.49
	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.02
	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.56
	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.68
	Octadecanoic acid	$C_{18}H_{34}O_2$	298	1.56
	Stigmasterol	$C_{29}H_{48}O$	412	0.46
	β-Amyrin	$C_{30}H_{50}O$	426	17.40
	α-Amyrin	$C_{30}H_{50}O$	426	10.23
	Lupeol	$C_{30}H_{50}O$	426	7.35
Ethylacetate fraction	1-H-Pyrazole	$C_3H_4N_2$	68	25.25
	1-H-Imidazole	$C_3H_4N_2$	68	0.09
	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.60
	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	1.29
	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.04
	Stigmasterol	$C_{29}H_{48}O$	412	0.60
	β-Amyrin	$C_{30}H_{50}O$	426	13.21
	α-Amyrin	$C_{30}H_{50}O$	426	5.53
	Lupeol	$C_{30}H_{50}O$	426	4.05
Butanolic fraction	1-H-Pyrazole	$C_3H_4N_2$	68	30.95
	1-H-Imidazole	$C_3H_4N_2$	68	1.78
	Xylitol	$C_5H_{12}O_5$	152	0.08
	Ribitol	$C_5H_{12}O_5$	152	0.44
	D-Glucose	$C_6H_{12}O_6$	180	0.95
	Inositol	$C_6H_{12}O_6$	180	5.50
	Stigmasterol	$C_{29}H_{48}O$	412	0.06
	β-Amyrin	$C_{30}H_{50}O$	426	2.01
	α-Amyrin	$C_{30}H_{50}O$	426	0.75
	Lupeol	$C_{30}H_{50}O$	426	0.41
Water fraction	1-H-Pyrazole	$C_3H_4N_2$	68	33.17
	1-H-Imidazole	$C_3H_4N_2$	68	1.86
	Proline	$C_5H_9NO_2$	115	0.01
	Xylitol	$C_5H_{12}O_5$	152	0.40
	Arabitol	$C_5H_{12}O_5$	152	2.64
	Mannitol	$C_5H_{12}O_5$	182	0.43
	Fractose	$C_5H_{12}O_5$	180	7.34
	Inositol	$C_5H_{12}O_5$	180	38.91
	Trehalose	$C_{12}H_{22}O_{11}$	342	0.01
	Galactinol	$C_{12}H_{22}O_{11}$	342	0.10
	3-α-Mannobiose	$C_{12}H_{22}O_{11}$	342	1.43
	Octadecanoic acid	$C_{18}H_{34}O_2$	298	2.29
	Stigmasterol	$C_{29}H_{48}O$	412	0.06
	β-Amyrin	$C_{30}H_{50}O$	426	0.41
	α-Amyrin	$C_{30}H_{50}O$	426	0.05
	Lupeol	$C_{30}H_{50}O$	426	0.03

Table 2: The IC_{50} µg/mL values of fractions prepared from methanolic extract of *L. procumbens* leaves on different cancer cell lines

Fractions and Standard	Leukemia K562	Cervix HeLa	Pancreatic MIA-Pa-Ca-2	Breast MCF-7
Methanolic extract	>80	>80	>80	>80
n-Hexane fraction	69.10	>80	>80	>80
Ethyl Acetate fraction	56.70	42	>80	64
n-Butanol fraction	>80	>80	>80	>80
Water fraction	>80	>80	>80	>80
Adriyamycin	<10	<10	<10	<10

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Conflicts of interest

There are no conflicts of interest

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