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Comprehensive *In-vitro* pharmacological activities of different extracts of *Saussurea lappa*

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ABSTRACT

The current biological study deals with the various activities like Antioxidant, anticancer,cytotoxicity and antimicrobial of the methanolic and chloroform extract of Saussurea lappa. The anti-oxidant assay were carried out using DPPH method and reducing power assay using ascorbic acid as positive control. The study revealed significant activity of the plant when compared to the standards. Anticancer potential of the plant was studied using MTT assay for cell proliferation of DLA cell lines. Cytotoxicity studies and antimicrobial studies were also carried out to determine the Minimum Inhibitory Concentration [MIC] of different extracts of the plant which gave significant results.

Keywords: *Saussurea lappa*,DPPH, MTT Assay, MIC.

INTRODUCTION

Traditional medicines have been used for many centuries by a substantial proportion of the population of India[1].The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs[2], [3]. Pharmacological research on the medicinal properties of phytochemicals has become mandatory, to establish the claimed medicinal properties of herbs [4]. Medicinal plants play an important role in the discovery of novel drugs used in modern medicine[5]

MATERIALS AND METHODS

Plant Collection

The roots of *Saussurea lappa* were collected from local market, Calicut in December2010 and authenticated from Uwin Life sciences, Malappuram. Voucher specimen is preserved in the herbarium of the Institute. The plant material was then shade dried and powdered.

Extraction

The dried and powdered plant materials were extracted separately using methanol[SOL-1] and chloroform [SOL-4]. The extraction was carried out using soxhlet's apparatus. The extracts were then concentrated to dryness and dissolved in respective solvents and the concentration was made upto 100 mg/ml. This extracts were used as the stock for preparing various concentration solutions for various assays.

Antioxidant assay of the different extracts of *Saussurea lappa*

a)DPPH Assay

DPPH was dissolved in 80% ethanol to 200 µM and sonicated for 5 min to obtain the stable free radical DPPH. The test compound was diluted in the ratio 1:1 with the DPPH solution in a 96-wellmicroplate. Appropriate controls were run in each series. Fresh DPPH• solution was prepared daily. Each fruit extract was tested in triplicate at three concentrations, such that a 50% fall in absorbance ofthe DPPH can be calculated. The absorbance of the reaction mixture was measured after 25 min using a microplate spectrophotometer. The IC50 (concentration causing 50% inhibition) value of each extract was determined and compared with the corresponding TEAC. Ascorbic acid was used as the standard[6].

b)Reducing power method

Various concentrations of *Saussurea lappa* methanolic and chloroform extracts (2.5 ml) were mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 _C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed asmean values ± standard deviations. The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as standard[7].

***Invitro* anticancer study**

a)MTT Assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically23-24. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of theviability of the cells[7].The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakhcells/ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37°C in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates are gently shaken to solubilizethe formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm. The percentage growth inhibition was calculated using the formula below:

The percentage growth inhibition was calculated using following formula,

$$\%\text{cell inhibition} = 100-\{(At-Ab)/(Ac-Ab)\}x100$$

Where,
At= Absorbance value of test compound
Ab= Absorbance value of blank
Ac=Absorbance value of control.

Cytotoxicity Study

Short term cytotoxicity studies were done on DLA cells by Trypan blue exclusion method. Cells were aspirated from the peritoneal cavity of tumour bearing mice and washed in PBS twice and counted using a haemocytometer. 1 million cells were taken for cell cytotoxicity studies. Different concentrations of the compound were added to the cells andthen made up to 1 ml with PBS. Cells were incubated for 3hours at 37oC. After incubation, the cell death was evaluatedusing Trypan Blue exclusion method. To the cell suspension,3 drops of Trypan Blue (0.5 % in PBS) was added and thecells were loaded immediately on to a haemocytometer. Thenumber of Dead cells was counted and the percentage ofdead cells was calculated. Viable cells exclude the dye whilenon-viable cells take up the dye and appear blue in colour[8].

$$\%\text{ of Dead cells} = \text{Dead cells}/\text{ Total cells} \times 100$$

Antimicrobial activity of the different extracts of *Saussurea lappa*

Standard and clinically isolated microorganism strains were used for antimicrobial assays. Bacteria were first grown in LB (Luria-Bertani) broth to an $OD_{600\ nm}$ of 0.8. A 10µlaliquot of the bacteria was then taken and added to 8 ml of fresh LB broth with 0.7% agar and poured over a 90 mm Petri dish containing 25 ml of 1.5% agar in LB broth. After the top agar hardened, a 20 µl aliquot of the test sample filtered on a 0.22 µm Millipore filter was dropped onto the surface of the top agar and completely dried before being incubated overnight at 37^{o}C. If the sample examined had antimicrobial activity, a clear zone would be formed on the surface of the top agar representing inhibition of bacterial growth. Minimal inhibitory concentration (MIC) was determined in liquid LB medium by incubating the bacteria in LB broth with variable amounts of the sample tested[9].

RESULTS

Table1. Detection of antioxidant activity of different plant extracts studied by DPPH and reducing power methods

Sample	IC_{50}DPPH activity [mgml^{-1}]	IC 50 Reducing power method [mgml^{-1}]
SOL1	120.5	12.4
SOL4	78.254	11.5
Ascorbic acid	40	6.8

Table 2.MTT assay for cell proliferation of DLA cell lines using plant extracts under study.

Name of the plant	Extract used for the estimation	%viability (100µg/ml)
Saussurea lappa	SOL1	85± 2
	SOL4	58.5 ± 5.5

Table 3.Cytotoxicity assay for various plant extracts studied (Figure)

Name of the plant	Extract used for the estimation100µg/ml	%Inhibition		IC_{50} (concentration for 50% inhibition)
		DLA cell lines	Normal Mice spleen cells	DLA cell lines
Saussurea lappa	SOL1	22	9	683.2
	SOL4	29	12	580.2

Table4. Results for Antimicrobial assay for plant extracts studied

Name of the plant	Extract used	Diameter of the inhibition zone obtained (mm)						
		Escherichia coli (µg/ml)	*Pseudomonas aeuroginosa (µg/ml)*	*Klebsella pneumonia (µg/ml)*	*Proteus vulgaris (µg/ml)*	*Staphylococcus aureus (µg/ml)*	*Candida albicans (µg/ml)*	*Aspergillus niger (µg/ml)*
Saussurea lappa	SOL1	21± 0.8	23±1.2	23.2±1.4	18±1.1	25± 2	5.5±1	7.1 ±1
	SOL4	24.1±1.5	26.2±0.7	26.2±1.4	22.5± 0.8	28.2±2.1	7.6±1.1	11.4±1.1
Penicillin		22						
Streptomycin		24						

Table 5.MIC obtained for various extracts against the microbes tested (MTCC STRAINS)

Name of the plant	Extract used for the estimation	*Escherichia coli (µg/ml)*	*Pseudomonas aeuroginosa (µg/ml)*	*Klebsella pneumonia (µg/ml)*	*Proteus vulgaris (µg/ml)*	*Staphylococcus aureus (µg/ml)*	*Candida albicans (µg/ml)*	*Aspergillus niger (µg/ml)*
Saussurea lappa	SOL1	250	200	220	225	175.5	425.0	450
	SOL4	154.25	125	117.4	187. 5	90	250	350
Penicillin		20						
Streptomycin		40						
Nystatin		50						

Table.6MIC results obtained against clinical strains tested

Name of the plant	Extract used	*Shigellaflexineri*	*Escherichia coli*	*Klebseilla pneumonia*	*Proteus vulgaris*	*Staphylococu saureus*	*Enterobacter*	*Acenetobacter bauminnii*	*Proteus mirabilis*	*Salmonella Rabis*
Saussurea lappa	SOL1	225.5	222.0	225.0	175	175	175	275	250	450
	SOL4	177.5	156.25	156.25	125	156	125	234	125	375

Table.7.MIC obtained for MDR strain

Name of the plant	Extract used for the estimation	MRSA (µg/ml)
Saussurealappa	SOL1	200
	SOL4	125
Penicillin		40

CONCLUSION

Form the present scientific investigation we revealed all the biological potency of the roots of *Saussurea lappa* using various in-vitro methods.. In most of the cases of the biological activity the chloroform extracts showed much higher activity than the methanol extract. This shows that the slightly polar compounds present in the roots are responsible for the activity than the polar compounds because the chloroform extract will contain more low polar compounds than in the methanol extract in which the majority will be polar compounds.

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