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ANTIOXIDANT ANTICANCER ACTIVITY OF *Leucas aspera* PLANT EXTRACT AND ITS DNA DAMAGE STUDY ON He-La CELL LINES

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ABSTRACT: The present study has been made to study the phytochemical, antioxidant, anticancer and DNA fragmentation assay for the medicinal plant *Leucas aspera* using water and methanol extracts. DPPH, total flavoinds, FRAP and phenol contrents were estimated. The active fractions were separated using TLC. Cytotoxicity assay was carried out using He La cell line by MTT assay protocol. DNA fragmentation assay was also carried out.

KEYWORDS: Leucas aspera, antioxidant, MTT assay, DPPH, DNA damage, anticancer.

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1.INTRODUCTION

A traditional Indian medical practice using plant drugs called Ayurveda, has been successful from very early times in using these natural drugs and preventing or suppressing various tumours with different lines of treatment [1]. In India, people of different regions adapt their own distinct culture, religious rites, food habit and a rich knowledge of traditional medicine[2]. Herbal medicine was used to cure various diseases. Products from plants have been used to cure thousands of diseases. Egypt, China, India and Greece followed this system from ancient times and a wide variety of modern drugs have been derived from these systems. The first written records on the medicinal uses of plants appeared about 2600 BC from the Sumerians and Akkaidians [3].Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth [4]. Tumour is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) as well as internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Cancer is a significant worldwide

health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind [5].The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity[6]. Over 3000 species of plants with antitumour properties have been reported [7]. Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources [8]. Excessive amounts of free radicals can lead to cell injury and death, which results in many diseases such as cancer, stroke, myocardial infarction, diabetes and major disorders. Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially lead to malignancy [9]. Phenolic compounds such as flavonoids, alkaloids, phenolic acids, stilbenes, lignans, lignin and tannins, found in both edible and nonedible plants, are well known as scavengers of free radicals and have multiple biological effects, including antioxidant activity[10]. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. *Leucas aspera* (Family - Labiatae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The plant is widely found in Bangladesh and its Bengali name is Dondokolos. The juice of the leaves is used as remedies for psoriasis, chronic skin eruptions and chronic rheumatism [11]. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. aspera* leaves are also used as insecticides and mosquito repellent in rural area [12]. The plant extract with honey is a good remedy for stomach pain and indigestion. A great number of *in vitro* and *in vivo* methods have been developed to measure the efficiency of natural anticancer compounds either as pure compounds or as plant extracts. *In vitro* methods like, Tryphan blue dye exclusion assay, LDH (Lactic dehydrogenase) assay, MTT assay, XTT assay and Sulforhodamine B assay are most commonly used for estimating anticancer properties of natural products from medicinal plants. Among all *in vitro* methods MTT and Sulforhodamine B assay most popular for estimating anticancer activity[13]. *Leucas aspera* is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long. Flowers are white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs. Fruit nutlets, 2.5 mm long, oblong,

brown, smooth, inner face angular and outer face rounded. The aim of the present work was to evaluate the antioxidant potential of *L. aspera* extracts using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, total antioxidant capacity and total phenol determination assays to support the pharmacological effects and phytochemical investigation of this plant.

2. MATERIALS AND METHODS

Plant used for the study is *Leucas aspera*, collected from the local farm of Eachanari, Coimbatore District, Tamil Nadu, India.

Preparation of plant extracts

Fresh leaves of *Leucas aspera* were collected and washed under running tap water. It is again washed with distilled water. The sample dried under shade at room temperature. The leaf extracts were prepared by the organic solvents *viz.*, methanol and distilled water. Five grams of leaf samples were soaked in 100ml of methanol and distilled water in two separate conical flasks respectively. The flasks were plugged with cotton wool and are covered with aluminium foils. It is then incubated in orbital shaker for 24hours at 120 rpm. The extracts was filtered through Whatman No. 1 filter paper and stored in air tight container for further study.

Phytochemical Analysis

Qualitative phytochemical tests were carried out for both (aqueous and methanol) the extracts.

Assessment of Antioxidant Activity

DPPH Assay

The free radical scavenging activity of the methanol and aqueous extracts of *Leucas aspera* leaves, based on the scavenging activity of the stable free radical 1,1 diphenyl-2-picrylhydrazyl (DPPH) were determined by mixing of 0.5 ml Crude extract 0.1 ml of 0.1M DPPH in methanol and 400 µl of 50mM Tris HCl solution. The sample were mixed well and incubated for 30 minutes in room temperature. Absorbance is measured at 517nm after incubation. The presence of DPPH activity (µg / ml)were confirmed with standard ascorbic acid.

Total Flavonoids Content

One ml of both the extracts were mixed with 0.1ml of 10 % aluminium chloride. To that 0.1ml of 1M potassium acetate and this were make up to 3ml by adding distilled water. The absorption was read at 415 nm after 30 minutes of incubation. Quercetin used as a control.

Total Phenol Content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. 500 µL of sample were mixed with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by

the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

FRAP Assay

FRAP assay were carried out by the plant extracts in respective solvent were mixed with phosphate buffer (1 ml) and 1% ferric cyanide (1 ml). This mixture was kept at 50°C in water bath for 20 min. After cooling, 2.5 ml of 10% trichloro acetic acid was added and was mixed with distilled water (2ml) and a freshly prepared 0.1% ferric chloride solution (0.25 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. The reducing power was estimated by using ascorbic acid standard.

THIN LAYER CHROMOTOGRAPHY (TLC)

TLC is a chromatography technique used to spread non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic or aluminium foil, which is coated with a thin layer of absorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. Thin –layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance**.** Solvent was prepared by mixing Chloroform, ethyl acetate and formic acid in the ratio 5:5:1. A drop of plant extract was placed on the paper and the paper was kept in the solvent for the separation of components.

ANTICANCEROUS ACTIVITY:

MTT assay

Cytotoxicity of n-hexane, dichloromethane and methanol extracts from *Scrophularia oxysepala* were assessed in MCF-7 cells as well as HUVEC using the MTT reagent (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Roche Diagnostics GmbH, Germany) according to the manufacturer's protocol. This method is based on the ability of viable cells to metabolize yellow tetrazolium salt MTT to purple formazan crystals by mitochondrial dehydrogenases. The cells were seeded in 96-well plates with a density of 104 cells/well/200 µl and incubated for 24 h at 37°C and 5% CO2. The cells were treated with different concentrations of solvent extracts (10, 20, 50, 100, 150, 200, 300, 400 µg/ml) and 0.2 % (v/v) DMSO (Merck, Germany) as a negative control. After 12, 24 and 48 h treatment 10 µl of MTT labeling reagent was added to each well. The plates were incubated at 37°C and 5% CO2 for 4 hours. Then, 100µl of the Solubilization solution was added to each well and followed by incubation overnight at 37°C to dissolve formazan crystals. Finally, absorbance was read using

an ELISA plate reader (Bio Teck, Germany) at a wavelength of 570 nm. The percentage of cytotoxicity and cell viability were calculated using following equation: % Cytotoxicity = 1-[mean absorbance of treated cells/ mean absorbance of negative control] and % Viability

ISOLATION OF DNA

The DNA damage was found by isolationg the DNA and observing it under UV transilluminator. The cultures with aqueous extract is centrifuged at 5000 g for 5 minutes and the pellet is removed. It is washed with Phosphate Buffer Saline (PBS) and centrifuged at 7000rpm for 5 minutes. To the pellet 1X TE buffer of 500µl and was incubated at 37^0C for 10 minutes. It is then added with 10% SDS (200 µl) and incubated at 37^0C for 10 minutes. Supernatant is collected after centrifuging at 10000 rpm. 10 µl of proteinase K is added and incubated at 37^0C for 30 minutes. The pellet is collected after centrifugation and is washed with 70% Ethanol. 1X TE buffer of 20 µl is added and run in 1% Agarose gel.

DNA damage study

The ability of methanol and aqueous extracts to protect super coiled pBR 322 DNA from devastating effects of hydroxyl radicals generated by Fenton's reagent was assessed by the DNA nicking assay described by Lee *et al.* (2002). The reaction mixture contained 1 µl of plasmid DNA. 10 µl Fenton's reagent (30 mM H2O2, 50 mM ascorbic acid and 80 mM FeCl3) followed by the addition of extracts and the final volume of the mixture was brought up to 20 µl using distilled water. The mixture was then incubated for 30 min at 37 ºC and the DNA was analyzed on 1% agarose gel (prepared by dissolving 0.5 g of agarose in 50 ml of 1X TBE Buffer) followed by ethidium bromide

3. RESULT AND DISCUSSION:

DPPH ASSAY

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with a hydrogen donor changes to yellow in color. The DPPH radical scavenging activity of *Leucas aspera* was found to increase with increasing concentration of the extract. This assay was based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) to decolorize in the presence of antioxidants. The Absorbance was measured at 517 nm and the value was found to be 24 µg/ ml in methanol and 12µg / ml for aqueous extract respectively.

Total Flavonoid Content: Flavonoids have demonstrated to exert beneficial effects on some diseases involving lipid peroxidation. Phenolic and flavonoids are the polyphenolic compounds which have been found to have free radical scavenging activity. These compounds donate hydrogen molecules to free radicals and thus act as antioxidants. Our results indicate that the

methanol extract has greater flavonoids content than the methanol extract but both extracts have high flavonoid content. Presence of flavonoids content in *Leucas aspera* is 103 mg / ml for aqueous extract and 184 mg / ml for methanol extract.

Total Phenol Content

Phenolic compounds are commonly found in both edible and inedible plants and plant parts. They have been reported to have multiple biological effects, including antioxidant activity. The content of phenolic compounds (mg/g) in methanol and aqueous extract was determined from regression equation of calibration curve and expressed in gallic acid equivalents (GAE). In determining the total phenol content of aqueous and methanol extract measured for each sample at 765 nm and this were found to be 94 mg/mlfor aqueous extract and 172 mg / ml for methanol extract.

FRAP Assay

FRAP value is based on reduction of the ferric ion by the antioxidants, as they are capable of donating a single electron or hydrogen atom for reduction. Higher FRAP values thus, relate to higher antioxidant capacity. The FRAP assay was done and the absorbance was measured as 64.65 % and 27.88 % for aqueous and methanol extracts respectively.

THIN LAYER CHROMOTOGRAPHY (TLC)

Thin Layer Chromatography (TLC) was done with the methanol extract and the compound was separated. Rf value was estimated as = 0.73 indicated the presence of alkaloids and saponins in the plant extracts.

Fig. 1 TLC study

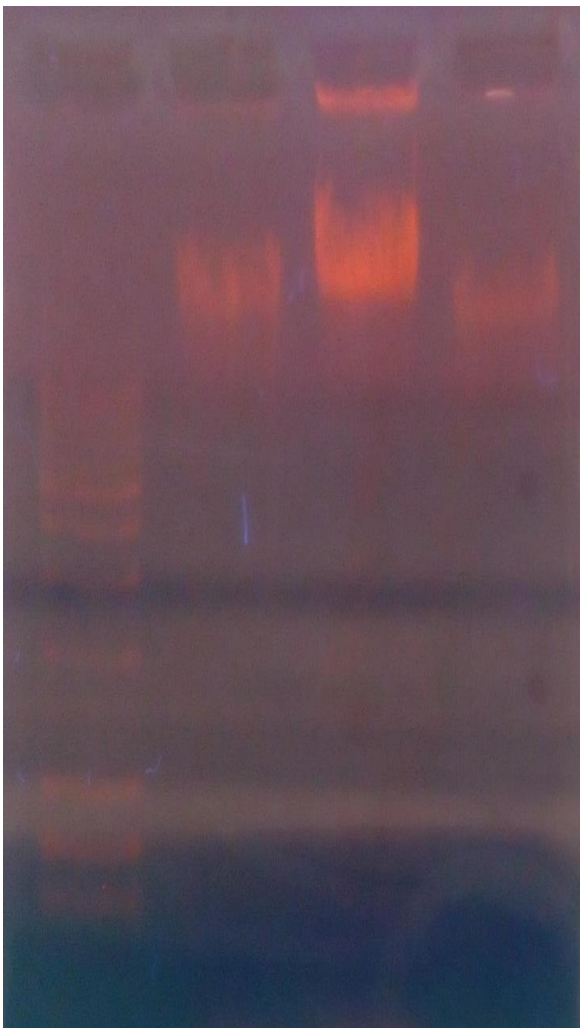
ANTICANCEROUS ACTIVITY

Ant cancerous activity was found by taking different concentrations (10µg/ml to 50µg/ml) of the plant extracts of and the absorbance was estimated at 570nm. The % of cell death was 43.4 % for acqueous extract and 56.7 % for methanol extract.

DNA DAMAGE

The study revealed that the all the nanoparticles damaged the DNA and produced the smeared DNA while running on 1.2% agarose gel . Concluded that the plant extracts were protected the DNA strands.

Fig 2 :



Lane 1 : 1kb marker : Lane 2 : Control DNA ; Lane 3: Water extract ; Lane 4 : Methanol extract

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