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In Vitro Pharmacognostic Evaluation of Laurus Nobilis Against Human Cancer Cells

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Abstract

In vitro antimitotic, and antioxidant activities of bay leaf extracts of *Laurus nobilis* were evaluated as a preliminary study for anticancer activity using the MTT assay. The crude Methanol, Ethyl acetate, Petroleum ether subsequent solvent fractions were employed in the study. y. In antimitotic assay, the methenol fraction showed strongest antimitotic activity with mitotic index. The antioxidant activity of *Laurus nobilis* and the isolated compounds was evaluated in vitro methods based on the free-radical scavenging properties of antioxidants. The Trolox equivalent antioxidant activity assay, in which *Laurus nobilis* showed the antioxidant activity in all extract but the best results, was found in aqueous extract as compared to others. The study concluded that the bay leaf extracts of *Laurus nobilis* can be used as a source of biologically active compounds with antimitotic, antiproliferative and antioxidant activities.

Keywords: Anticancer Antimitotic Antiproliferative Antioxidant, *Laurus nobilis.*

Introduction

One of the most dreaded illnesses of the 20th century, cancer is continuing to spread and is becoming more common in the 21st. Every fourth individual has a lifelong risk of cancer since the situation is so concerning. Over 11 lakh new instances of cancer are reported in India year; globally, over 14 million cases are reported. Is it possible to cure cancer? The quick response to this query is "Yes." Actually, if detected early enough, all tumors are treatable. Cancer cells proliferate until one of four things happens: Radiation therapy is one option; (2) chemotherapy or another cancer-specific drug, such as hormone therapy; (3) surgical removal of the diseased mass.[1] We have presented age-standardized cancer incidence and mortality rates for the years 2003-2007 using the CANCER Mondial clearing house maintained by the International Agency for Research on Cancer. Additionally, we provide mortality through 2012 and incidence trends through 2007 for a few chosen nations across five continents. All sites combined, as well as lung, colorectal, breast, and prostate cancer, have the greatest incidence rates in high-income countries (HIC) however certain low- and middle-income nations (LMIC) are currently among the leading rate-setters. [2] The range of diseases associated with obesity is becoming more and more prevalent, making it a public health issue. In terms of population attributable fraction, the global cancer burden attributed to obesity is 13.1% for women and 11.9% for men. Excess weight is linked to a higher risk of cancer at least 13 anatomic sites (endometrial, esophageal, renal, and pancreatic adenocarcinomas, gastric cancer, hepatocellular carcinoma, meningioma, multiple myeloma, colorectal, postmenopausal breast, ovarian, gallbladder, and thyroid cancers).[3] It is also necessary to investigate epigenetic

modifications, the gene expression of malignant cells, and to use techniques that allow us to reduce or reverse these alterations.[4] Laurus nobilis is also referred to as the lovely bay leaf, the real Roman or Turkish laurel, among other names used in commerce. a little evergreen tree of the Lauraceae family. Sesquiterpene lactones such as 10 epigazaniolide, Gazaniolide, spirafolide, costunolide, Reynosin, santamarine, flavonoid glycosides, and others have been found to be present in its chemical makeup. a fragrant material Wound- healing, neuroprotective, antioxidant, anticonvulsant, antiulcerogenic, antimutagenic, antiviral, anticholinergic, antibacterial, and antifungal actions have all been documented for it. Laurusnobilis is anaromatic evergreen tree or large shrub with green, glabrous (smooth) leaves, variable in size and sometimes reaching 7–18 m (23–59 ft) tall. It is in the flowering plant family Lauraceae. It is native to the Mediterranean region and is used as bay leaf for seasoning in cooking.Its common names include baytree (esp. United Kingdom), baylaurel, sweetbay, truelaurel, Grecianlaurel, or simply laurel. The origin of bay leaf is most probably South Asia, from where it spread to Asia Minor and all over the world. Laurus nobilis figures prominently in classical Greco-Roman culture. The genus Laurus has a range of 24,00 to 25,00 species, and their varieties are native to the Southern Mediterranean region, the subtropics and tropics of Eastern Asia, South and North America, the Balkans, and Asia Minor. The great variability among species is largely attributed to the uncertainty in the exact number ofspecies. Due to the morphology, flower color, growth habitat, leaves, stems, andchemical composition, variability .Two Laurel species are traditionally found: Laurus azorica and L. nobilis. There arenumber of plants outside the genus Laurus with the common name bay laurel, including bay rum tree, or simply bay (Pimenta racemosa). Laurus nobilis is known by different names. In Urdu, it is known as teejh pat. In English, it is typically called bay leaf or sweet bay A few pharmacological and phytochemical characteristics of Laurus nobilis were discovered in this review, which the researcher might employ in further research.

Materials and methods

2.1 Collection and extraction of leaves materials

Collection of Laurus Nobilis (bayleaf) plant part was collected from Apollo collage of Pharmacy Anjora, Durg. C.G. The leaves was homogenized into a fine powder after being dried out of direct sunlight. Using methanol as a solvent, the Soxhlet extraction technique was used to get crude extracts. To produce crude methanol extracts, the obtained extract was concentrated in a rotary evaporator at low temperature and pressure. The crude extract was fractionated using several solvents with polarity ranging from non-polar to low polar, medium polar, and highly polar in that order for the first separation of phytoconstituents. Sequentially, n-hexane, dichloromethane, ethyl acetate, and n-butanol were the solvents utilized. Every fraction was stored and Concentrated and kept for later use at 4 °C.

2.2 chemicals

a-naphthol, Ethanol Concentrated, sulphuric acid, sodium citrate, Sodium carbonate, Copper(II)Sulfate, penta hydrate, Sodium potassium, artrate Sodium hydroxide, Nitric acid Copper(II)Sulfate, Sodium hydroxide Sudan III solution, Mercuric chloride, Potassium iodide, Iodine Bismuth nitrate, Acetic acid, Sodiumbi carbonate, Diluted ammonia, Gelatine solution, Lead acetate solution, Ferric chloride solution, Chloroform, Glacial acetic acid.

2.3 preparation of extract

Using the completely dried residue obtained after the aqueous extraction perform methanolic extraction. Place the residue in a thimble prepared by rolling the filter paper sheet and then load the thimble into the

extractor arm of the Soxhlet extractor. Fill the reservoir of the extractor with 250 ml of methanol, Ethyl acetate, Petroleum ether and heat it at a constant temperature of 35via heating mantle. Extract the sample with methanol for the complete Soxhlet cycles and then filter the suspension through Whatman no.1 filter paper. Remove the thimble from the extract or arm and keep the residue at 40^{o}c for drying in an oven for further extraction.

2.4. Phytochemical analysis Preliminary phytochemical analysis

Test for the presence or absence of selected phytochemical constituents was carried out according to the standard methods

Alkaloids: To 1 mL of each extract were added 2–3 drops of Dragendorff's reagent. The orange red color confirmed the presence of alkaloids.

Anthraquinones: Each plant extract (0.5 g) was boiled with 10% HCl and filtered. The filtrate was cooled and mixed with equal volume of chloroform. Few drops of 10% ammonia were added to the mixture and heated. Appearance of rose pink color confirmed the presence of anthraquinones.

Flavonoids: To detect flavonoids 1 mL of 5% AlCl3 was added to equal volume of plant extract. Appearance of yellow color confirmed the presence of flavonoids.

Cardiac glycosides: To 5 mL of each plant extract were added one drop of FeCl3 solution, 1 mL of concentrated H2SO4 and 2 mL of glacial acetic acid. Cardiac glycosides were confirmed by the formation of a brown ring at the junction of the two layers.

Phenolics: To detect the presence of phenolics 2 mL of plant extract was mixed with equal volume of 5% FeCl3 solution. A bluish black color indicated the presence of phenolics.

Phlobatannins: About 10 mg of each plant extract was boiled in 1% HCl. Appearance of red precipitates confirmed the presence of phlobatannins.

Saponins: The oil emulsion formation property of saponins was used for their detection. The crude extracts and various solvent fractions (20 mg) were dissolved in 20 mL of distilled water and boiled for 5 min. To 10 mL of the filtrate 5 mL of distilled water were added and vertex to develop froth. The froth was mixed with olive oil to check the development of emulsion that confirmed the presence of saponins.

Steroids: Equal volumes of plant extract and chloroform were mixed together followed by addition of H2SO4 A reddish brown color at the interface indicated the presence of steroids.

Tannins: To detect the presence of tannins 50 mg of each plant extract was mixed with 20 mL of distilled water and boiled. To the above mixture were added few drops of 0.1% FeCl3. A blue-black or brownish green color confirmed the presence of tannins.

Triterpenes: Triterpenes were detected by using Salkowski's test. For this purpose two mL of different solvent extracts were mixed with few drops of chloroform and filtered. To the filtrates were added 3–4 drops of concentrated H2SO4. The contents were shaken, and allowed to stand for 5 min. A golden yellow color indicated the presence of triterpenes.

2.5 MTT assay

Aseptically add 1.2 ml of cell-based assay buffer in MTT vial and completely dissolve the powder. MTT powder dissolves slowly in the buffer.Vigorous vertexing is needed to dissolve the powder completely. Concentration of the resulting solution is 5mg/ml. MTT solution should appear bright yellow in colour. Seed the freshly harvested cells in a cell culture flask or 96-well plate in an amount appropriate for the assay and incubate at 37in a 5% CO2 environment. Allow the cells to grow for upto 24hours or till confluence is reached. Harvest the cells and use for assay. Adjust the cell density to 1×10^{6}cells/ml. Serially dilute the cell suspension from 1×10^{6}to1×10^{3}cells/ml using appropriate culture medium. Seed

100µl of each dilution in 96-well micro liter plate duplicate. Add medium control in duplicate .Incubate the cells under appropriate conditions depending on the cell in under study.Add10µl of MTT to each well including controls. Wrap the plate with aluminum foil to avoid exposure to light. Return the platet the incubator for 2 to 4 hours. Observe the cells at periodic intervals under an inverted microscope for presence of needle-shaped crystals. Slow growing cell lines require longer time to develop formazan crystals. After incubation period, add 100 µl of solubilization solution to each well. Stirgently on ratory shaker to enhance dissolution of the crystals. Read the absorbance on wavelength 650 nm using a micro titre plate reader and the viability (%) was calculated.

2.6 Apoptosis and fluorescence microscopy

In this work, apoptosis was analyzed using a fluorescent microscope. Ethidium bromide (EtBr) (100 mg/mL) and acridine orange (AO) (100 mg/mL) were produced and combined. 1 mL of dye was extracted from this combination, and 0.9 mL of cancer cell lines were applied to cover slips under aseptic conditions. Following this preparation, the cancer cells were stained with AO/Et Br staining solution and then rinsed with phosphate buffered saline (pH 7.2). After being incubated for 120 seconds, it was twice cleaned with PBS and examined at 400 magnification using a fluorescence microscope. Similarly, the cancer cell lines were plated and stored on cover slips in DAPI (10 lL). At last, the cells were examined under a fluorescent microscope with microtitre plate and treated.

Results

In this it is concluded that Laurus nobilis is a good source of some primary metabolites and secondary metabolites which showed various health benefits, the Soxhlet apparatusis a good source, beneficial and advantageous for the phytochemical screening. Cell cytotoxicity effects were related to their secondary metabolites content. In which Laurus nobilis showed the cell cytotoxicity in an ethyl acetate extract, which had the ability to inhibit growth of cells. The degree of cytotoxicity of these test compounds towards the cell lines were determined using MTT assay. In the current study, the cytotoxicity of ethyl acetate extract of *Laurus nobilis* was determined using MTT assay cell lines exposed to 0.16,0.70,0.72,02.18,2.4,1.41,1.33,.17,0.38 and 5mg/ml of the extracts at incubation period of 24 h.

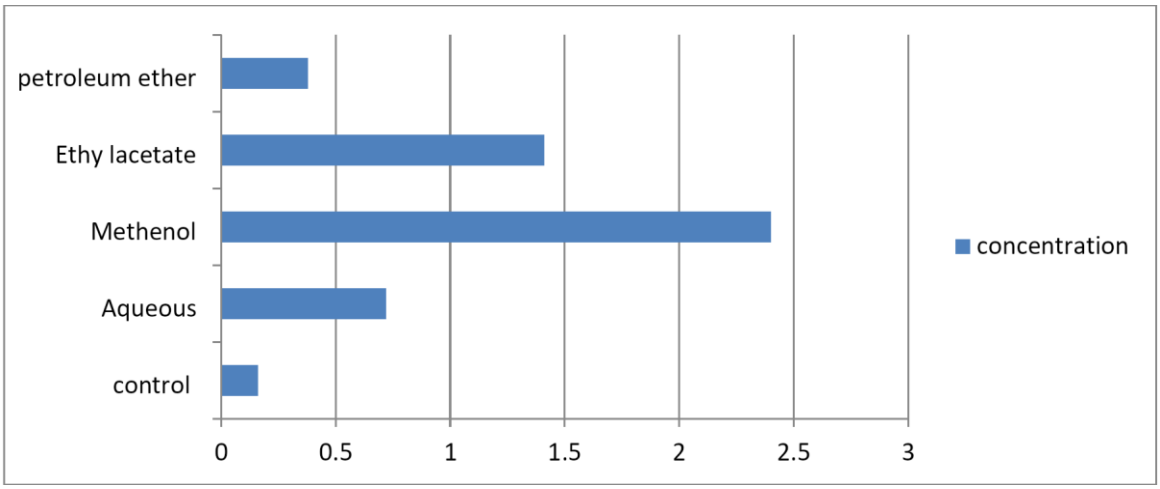

Figure.1 The effect of increasing concentration of Laurus nobilis extract in cell line.each point represent mean of data experiments.

Declaration

The overall results of the study indicate that the leaves material of L. nobilis a good source of biologically active compounds with antioxidant and anticancer potentials. However, It is recommended to conduct more research on the in vivo examination of these extracts utilizing animal models and cancer cell lines to see how they affect animal cells.

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