Journal of Traditional and Complementary Medicine Vo1. 4, No. 4, pp. 253-257

Journal of Traditional and Complementary Medicine
Journal homepage http://www.jtcm.org

Evaluation of Anticancer, Antioxidant, and Possible Anti-inflammatory Properties of Selected Medicinal Plants Used in Indian Traditional Medication

Rafik Shaikh[1,2], Mahesh Pund[1], Ashwini Dawane[1], Sayyed Iliyas[2]

[1]School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded - 431 606, Maharashtra, India.
[2]Department of Botany, Poona College, Camp, Pune - 411 001, Maharashtra, India.

ABSTRACT

The present study was carried out to evaluate the anticancer, antioxidant, and possible anti-inflammatory properties of diverse medicinal plants frequently used in Indian traditional medication. The selected botanicals such as *Soymida fembrifuga* (Roxb.) A. Juss. (Miliaceae), *Tinospora cordifolia* (Willd.) Miers. (Menispermaceae), *Lavandula bipinnata* (L.) O. Ktze. (Lamiaceae), and *Helicteres isora* L. (Sterculiaceae) extracted in different solvents were evaluated for their *in vitro* anticancer and antioxidant activities. The results obtained indicate that *H. isora* has potent cytotoxic activity toward the selected cancer cells such as HeLa-B75 (34.21 ± 0.24%), HL-60 (30.25 ± 1.36%), HEP-3B (25.36 ± 1.78%), and PN-15 (29.21 ± 0.52%). Interestingly, the selected botanicals selectively inhibited cyclooxygenase-2 (COX-2) more than (COX-1), which are the key enzymes implicated in inflammation. COX-2 inhibition was observed to be in the range of 19.66-49.52% as compared to COX-1 inhibition (3.93-19.61%). The results of the antioxidant study revealed that the selected plants were found to be effective 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl (OH), and superoxide radical (SOR) scavenging agents. High-performance thin layer chromatography (HPTLC) fingerprint of flavonoids was used as a measure of quality control of the selected plant samples. The results of the present findings strengthen the potential of the selected plants as a resource for the discovery of novel anticancer, anti-inflammatory, and antioxidant agents.

Key words: Anticancer, Anti-inflammatory, Antioxidant, Medicinal plants

INTRODUCTION

Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases.[1] It is estimated that 12.5% of the population dies due to cancer (WHO, 2004). The disease is widely prevalent, and in the West, almost a third of the population develops cancer at some point of time during their life. Although the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the biology of the disease at the molecular level.[2]

Breast cancer is the most common form of cancer in women. The incidence of breast cancer is the highest in Pakistan among the South-Central Asian countries. It is the most frequent malignancy in women and accounts for 38.5% of all female cancers. About half (43.7%) of all breast cancers are detected in an advanced stage.[3] Colon cancer is the second most common cause of cancer

Correspondence to:
Dr. R. U. Shaikh, Department of Botany, Poona College, Camp, Pune - 411 001, Maharashtra, India. Tel: 020- 26454240; Fax: 020-26453707;
E-mail: rafikbot@gmail.com

DOI: 10.4103/2225-4110.128904

deaths in the US. Prostate cancer is the most frequently diagnosed cancer among men in the US, and ranks second to skin cancer, with an estimated 180,000 new cases and 37,000 deaths expected to occur by the American Cancer Society each year.[4]

Moreover, it is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, aging, and inflammatory diseases.[5,6] All cells are exposed to oxidative stress, and thus, oxidation and free radicals may be important in carcinogenesis at multiple tumor sites.

The enzymes cyclooxygenase-1 and -2 (COX-1 and -2) are the key enzymes involved in recruiting inflammation. Nevertheless, the proinflammatory cytokines play a crucial role in the initiation and progression of various cancers.[7] Besides the key role of COX in the initiation and progression of inflammation, overexpression of COX has been considered as one of the culprits in the formation of carcinogenic state in the body.[8] It is this molecular attribute of the COX upregulation that has made it an attractive target for the design and development of anticancer agents also. Free radical induced oxidative stress and its relevance with inflammation and carcinogenesis is well established.[9] Therefore, inflammation, free radicals, and carcinogenesis are closely related with one another. The drug candidates having anti-inflammatory and free radical scavenging activities are more appreciated as anticancer agents.

Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects of anticancer drugs, cancer can be a cause of death. Therefore, efforts are still being made to search for effective naturally occurring anticarcinogens that would prevent, slow, or reverse cancer development. Medicinal plants have a special place in the management of cancer. It is estimated that plant-derived compounds in one or the other way constitute more than 50% of anticancer agents.[10,11] Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents. Taking into consideration the above facts, an attempt has been made to evaluate the anticancer, anti-inflammatory, and antioxidant activities of selective medicinal plants used in Indian traditional medicine system.

MATERIALS AND METHODS

Materials

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The COX assay was performed by using colorimetric COX (ovine) inhibitor screening assay kit (catalog no. 760111; Cayman Chemical Company, USA). 1,10-Phenanthroline, phenazine methosulfate (PMS), and nitroblue tetrazolium (NBT) were obtained from SD Fine Chem. (Mumbai, India). Nicotinamide adenine dinucleotide (NADH) was purchased from Spectrochem, Pvt Ltd (Mumbai, India). All other chemicals and reagents used were of AR grade and were obtained from commercial sources.

Collection, identification, and authentication of the selected medicinal plants

The selected plants, *Soymida fembrifuga* (Roxb.) A. Juss. (SRTH-08), *Tinospora cordifolia* (Willd.) Miers. (SRTH-54), *Lavandula bipinnata* (L.) O. Ktze. (SRTH-24), and *Helicteres isora* L. (SRTH-61), were collected from the nearby regions of Nanded district (Maharashtra) during September 2009. The plants were identified and authenticated by RNG, Head, Department of Botany, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India.[12] Voucher specimens of the collected plants were deposited in the herbarium center of the host institute. The shade-dried and powdered plant samples were preserved for further experiments.

Sequential extraction of the plant samples

The shade-dried, powdered plant samples (10 g) were sequentially extracted in hexane, ethanol, and water as per their boiling points which are (69°C), (79°C), and (100°C), respectively, up to 8 h using Soxhlet's apparatus. The extracted samples were evaporated under reduced pressure at room temperature. The yield of the individual plant extract was measured and the dried extracts were preserved at 4°C in a refrigerator for further analysis.

HPTLC analysis

High-performance thin layer chromatography (HPTLC) analysis was performed using the instrument from CAMAG (Germany). Thin layer chromatography (TLC) plates (silica gel >60 F254, 20 cm × 10 cm; Merck) were prewashed with methanol. The plates were activated in an oven at 100°C for 10 min. Ten microliters of individual plant extracts (1 mg/ml) was spotted onto the precoated plates using Linomat 5 application system. Rutin hydrate (50, 100, 200 μg/ml) was used as the marker flavonoid. The flavonoids were separated using ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:27) as the mobile phase. Natural product (NP) reagent was used as the flavonoid derivatizing agent, and the spots developed were visualized under CAMAG UV cabinet (366 nm) and digitized using CAMAG photodocumentation system.

Cell lines and culturing

Human cancer cell lines HeLa-B75, HL-60, HEP-3B, PN-15, and normal liver cell lines were obtained from National Center for Cell Sciences, Pune, Maharashtra. >All cell lines were propagated in Minimum Essential Medium (Eagle) with 2 mM l-glutamine and Earle's BSS (Balanced Salt Solution) adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate 90%, and 10% fetal calf serum. All cell lines were grown in a humidified incubator at 37°C.

MTT cytotoxicity assay for in vitro anticancer study

The cytotoxicity assay was performed according to the microculture MTT method with slight modifications.[13] The cells were harvested (1.5×10^4 cells/well) and inoculated in 96-well microtiter plates. They were washed with phosphate-buffered saline (PBS)