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Original Article

Assessment of anticancer activity: A comparison of dose–response effect of ethyl acetate and methanolic extracts of *Pergularia daemia* (Forsk)

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ABSTRACT

Background: Oral cancer is the sixth most common malignancy worldwide and is thus an important cause of death and morbidity. Although several drugs have been developed for the treatment of oral cancer, naturally occurring phytochemicals have been recommended by nutritionists and health experts to treat various types of cancer. *Pergularia daemia* is a well-known herb distributed across India, finding many uses in medicine.

Aim: The present study was mainly designed to determine the dose–response effect of the whole plant extract of *P. daemia* on experimental oral carcinogenesis.

Materials and methods: Male golden Syrian hamsters were painted with 0.5% of 7,12-dimethylbenz[a]anthracene (DMBA) in liquid paraffin on the left buccal pouch to induce oral cancer, thrice a week for 14 weeks. The anticancer effect of the ethyl acetate and methanolic extracts of *P. daemia* was observed to be dose dependent (100, 200, and 300 mg/kg body weight (bwt)) by assessing the bwt, tumor incidence, changes in oxidant/antioxidant levels, and histological alterations of control and experimental animals.

Results: Our results showed that of the three doses used, the high dose (300 mg/kg bwt) exhibited excellent antioxidant activity in comparison to the other two doses. Moreover, our findings suggest that the methanolic extract significantly inhibited cancer development to a greater extent than the ethyl acetate extract.

Conclusion: Thus, it could be concluded that the whole plant of *P. daemia* presents an easily accessible source of natural antioxidants, and regular consumption can reduce the incidence of oral cancer.

1. Introduction

The significance of reactive oxygen species (ROS) and free radicals has been increasingly studied over the past decade. These molecules accelerate cellular injury and aging [1]. ROS are continuously produced during normal physiologic events, which can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. However, these compounds are removed by antioxidant defense mechanisms. In living organisms, a balance is maintained between the generation of ROS and inactivation of ROS by the antioxidant system. Under pathological conditions, ROS are produced in excess due to inadequate endogenous antioxidant defense, resulting in oxidative stress. The imbalance between ROS and antioxidant defense mechanisms leads to life-threatening diseases, including cancer [2].

Oral cancer is the sixth most fatal malignancy, belonging to a group of cancers commonly referred to as head and neck cancers. In India, oral cancer accounts for 22.9% of cancer cases. This figure is significantly higher on a global scale, with >450,000 new cases being reported annually. Oral cancer typically begins as a focal clonal overgrowth of altered stem cells near the basement membrane, expanding upward and laterally, ultimately replacing the normal epithelium [3]. The major risk factors of oral cancer include smoking, betel quid and Areca nut chewing, alcohol use, consumption of smokeless tobacco products, and human papillomavirus (HPV) infection. Although various treatment regimens are available

Abbreviations: PDEAE, *Pergularia daemia* ethyl acetate extract; PDME, *Pergularia daemia* methanolic extract; DPPH, diphenyl picryl hydrazyl.

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for oral cancer, including surgery, radiotherapy, and chemotherapy, severe side effects are still observed [4]. Therefore, prognosis and treatment must be performed before the cancer develops further.

Chemoprevention refers to the use of natural, synthetic, or biological substances to reverse, suppress, or prevent the development of cancer [5]. The chemoprotective potential of naturally occurring phytochemicals in food or medicinal plants continues to be a major area of scientific interest [6]. It is generally accepted that the consumption of fruits and vegetables may reduce the risk of human cancers [7]. Plants and plant products have long been used in medicine especially by nonindustrialized societies [8]. In certain African countries, up to 90% of the population still rely on medicinal plants for their primary health-care needs [9]. Thus, various medicinal plants have been studied using modern scientific approaches to identify various biological components of these plants. The medicinal properties of plants can be attributed to the presence of certain substances known as bioactive polyphenolic compounds, which may be stored in plant parts such as roots, leaves, stem bark, fruits, and seeds. These compounds can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [10].

Pergularia daemia (Forsk) (family Asclepiadaceae) is a fetid-smelling laticiferous twiner that grows in plains of the hotter regions of India, ascending to an altitude of 1000 m in the Himalayas. *P. daemia* is known as "Veliparuthi" in Tamil, "Uttaravaruni" in Sanskrit, and "Utranajutuka" in Hindi. Traditionally, *P. daemia* is used as an anthelmintic, laxative, antipyretic, and expectorant, as well as to treat infantile diarrhea and malarial intermittent fevers. The excellent antifertility [11], antidiabetic [12], hepatoprotective [13], anti-inflammatory [14], and cardiovascular effects [15] of this plant have been reported in folk and Ayurvedic medicine. Phytochemical investigations of this plant have demonstrated the presence of alkaloids, terpenoids, flavonoids, and steroids [16]. The curative properties of medicinal plants can be attributed to the presence of various complex chemical substances of different compositions, which occur as secondary metabolites. Thus, the present study aimed to investigate the chemopreventive effect of the ethyl acetate and methanolic extracts obtained from the whole plant of *P. daemia* (Forsk) on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis in a dose-dependent manner.

2. Materials and methods

2.1. Chemicals

DMBA and other chemicals such as reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide (NADH), ethyl acetate, methanol, and diphenyl picryl hydrazyl (DPPH) were purchased from Sigma–Aldrich Chemicals Pvt Ltd (Bangalore, India). All other chemicals used were of analytical grade.

2.2. Plant materials

Matured *P. daemia* (Forsk) was collected from the riverbank of the Pudukkottai District, Tamil Nadu, India. The plant was identified by Dr. V. Venkatesalu, Professor, Department of Botany, Annamalai University. A voucher specimen (ACC: 196) was deposited in the herbarium of the Department of Botany, Annamalai University.

2.3. Preparation of plant extracts

The shade-dried plant materials (root, stem, leaves, flower, and barks) of *P. daemia* (1000 g) were subjected to size reduction to form a coarse powder. The powdered plant material was defatted using petroleum ether (60–80 °C) and then extracted with ethyl acetate and methanol using a Soxhlet apparatus for approximately 72 h at 40 °C. The sediment was subsequently filtered with Whatman No. 1 filter paper (Whatman Ltd, Maidstone, UK). Both the ethyl acetate and methanolic extracts of *P. daemia* (PDEAE and PDME) were further concentrated under vacuum using a rotary vacuum evaporator (Buchi R-V120, Flawil, Switzerland) at 40 °C, then reconstituted in a minimum amount of dimethyl sulfoxide (DMSO), and stored at 4 °C for further use [17]. The percentage yield of the ethyl acetate and methanolic extracts were found to be 4.5% (w/w) and 8.1% (w/w), respectively.

2.4. In vitro antioxidant assays

2.4.1. Hydroxyl radical-scavenging assay

The incubation mixture consisted of 0.1 mL of buffer, varying concentrations of PDEAE and PDME (100, 200, 300, 400, and 500 μg/mL), 0.2 mL of ferric chloride, 0.1 mL of ascorbic acid, 0.1 mL of ethylenediaminetetraacetic acid (EDTA), 0.1 mL of H_2O_2, and 0.2 mL of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min; then, 1 mL of thiobarbituric acid (TBA) and 1 mL of trichloroacetic acid (TCA) were added. All the tubes were kept in a boiling water bath for 30 min. Ascorbic acid was used as a positive control for the purpose of comparison. The absorbance of the supernatant was read using a spectrophotometer at 535 nm with a reagent blank containing water in place of PDEAE and PDME. The decreased absorbance of the reaction mixture indicated increased hydroxyl radical-scavenging activity. The percentage scavenging was calculated using the following formula [18]:

$$\%\ \text{of hydroxyl radical-scavenging activity} = \left\{\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}}\right\} \times 100,$$

where A_{control} is the absorbance of the control and A_{test} is the absorbance of the sample extract/standard.

2.4.2. Superoxide anion-scavenging activity

The reaction mixture contained 1 mL of nitro blue tetrazolium (NBT), 1 mL of NADH solution, and varying volumes of PDEAE and PDME (100, 200, 300, 400, and 500 μg/mL), which were mixed thoroughly. The reaction was initiated by the addition of 100 μL of phenazine methosulfate (PMS). Then, the reaction mixture was incubated at 30 °C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Water was used as the blank and ascorbic acid was used as a reference for comparison. The decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. The percentage scavenging was calculated as follows [19]:

$$\%\ \text{of superoxide anion-scavenging activity} = \left\{\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}}\right\} \times 100,$$

where A_{control} is the absorbance of control, A_{test} is the absorbance of sample extract/standard.

2.4.3. DPPH$^{\bullet}$ radical-scavenging assay

The reaction mixture contained 1 mL of DPPH and varying concentrations of PDEAE and PDME (100, 200, 300, 400, and 500 μg/mL), which was made up to 3 mL with water. The tubes were incubated for 10 min at 37 °C. The reaction was performed in a dark room. A blue-colored chromophore was formed, the absorbance of which was measured at 517 nm. Ascorbic acid was used as the standard for the comparison [20]:

$$\%\ \text{of scavenging} = \left\{\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}}\right\} \times 100,$$