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Research Article

BIOACTIVITY-GUIDED PHYTOCHEMICAL INVESTIGATIONS OF *ARTEMISIA MARITIMA:* ISOLATION AND CHARACTERIZATION OF CHEMICAL CONSTITUENTS

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ABSTRACT

Objective: In the present study, the extracts (petroleum ether and methanolic extract) of plant *Artemisia maritima* were subjected to bioactivity evaluation and compound isolation and characterization.

Methods: Antioxidant activity was carried out using ferric reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The anticancer activity was evaluated by MTT assay using three different human cancer cell lines: Embryonic kidney cancer cell (HEK), lung adenocarcinoma epithelial cell (A-549), and human colon cancer cell (HCT), and isolated compounds were characterized using nuclear magnetic resonance (^{1}HNMR), ^{13}CNMR, DEPT, infrared, and mass spectroscopic techniques.

Results: The petroleum ether extract of the plant displayed significant antioxidant and cytotoxic effects, which on phytochemical analysis led to the isolation of two bioactive sesquiterpene lactone compounds. These phytochemicals were identified using different spectral techniques in the light of literature. All the compounds displayed significant cytotoxic activity; however, compound-1 exhibited potent anticancer activity with inhibitory concentration value of 17.3 µg/mL. The isolated compounds also displayed significant antioxidant potential.

Conclusion: *Artemisia maritima*, a rich source of sesquiterpene lactone which may be responsible for significant anticancer potential and it also possess remarkable antioxidant activity and hence may be of immense importance to food Chemistry.

Keywords: *Artemisia maritima*, Phytochemical investigation, Isolation, Cytotoxicity, Antioxidant activity.

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INTRODUCTION

The genus *Artemisia* (family: Asteraceae) is one of the most widely distributed and largest genus comprising around 500 species which are distributed mainly in the temperate zones of Europe, Asia, and North America. Of these, 32 species occur in India. *Artemisia* species possess pharmacological properties that are used for medical purposes worldwide. These species are known for their chemical constituents that are extensively used in food and pharmaceutical industry [1-3]. Literature reveals the isolation of artemin, 1-Keto-6β, 7α, 11β-4(5)-en-6, 12-olide, vulgarin, and maritimin from the aerial parts of *Artemisia maritime* [4,5]. Camphor and 1,8-cineole have found to be the main constituents of essential oil of this plant and also the antibacterial, antifungal, mosquito biting deterrent, and larvicidal activities of this oil have been evaluated [6]. The significance of the present study lies in the fact that bioactivity-guided isolation of aerial parts of *A. maritima* was carried out and it was found that pet ether extract was rich in phytochemicals exhibiting significant antioxidant and cytotoxic effects. Therefore, it was subjected to column chromatography and led to the isolation of two bioactive compounds. These isolated compounds were evaluated 1st time for the cytotoxic effect against HEK, HCT, and A-549 cancer cell lines. The isolated compounds were also subjected to antioxidant evaluation using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing assay.

METHODS

Plant material collection

Aerial parts of *A. maritima* were collected in July 2015 from high altitude area of Minjhe, Kargil Region of J and K (India) and were properly authenticated by Prof. A. R. Naqshi and curator Akhter Ahmed Malik of Taxonomy Department, University of Kashmir. A specimen under accession no. 2326-(KASH) was deposited in the herbarium of the institute.

Preparation of extracts

The plant material was finely chopped into small pieces, shade dried and powdered. The powdered material (2.340 g) was extracted with different solvents (hexane, chloroform, ethyl acetate, and methanol) of their increasing polarity using Soxhlet apparatus. Solvent was changed once a clear solution was obtained indicating that everything gets solubilized in that particular solvent. Solvent was removed from the extracts using rotary evaporator to afford crude extract. Preliminary antioxidant and cytotoxic screening of extracts revealed that petroleum ether extract to possess better antioxidant and cytotoxic effects, and subsequently, used for isolation of active chemical constituents.

Isolation of compound

77 g of pet ether extract of the plant material was subjected to column chromatography using silica gel (60–120 mesh) to afford compounds using hexane-EtOAc as eluent with increasing polarity of 1%, 2%, 5%, and so on. Two compounds were obtained: Compound-1 and compound-2.

Compound 1

Light orange solid; mp: 123–126°C; infrared (IR) νmax cm^{-1}: 1779, 1709, 1654 (C=O), 1604, 1500, 1457, 902;^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.51 (1H, d, *J* = 12.0 Hz, H-6), 2.41 (1H, dq, *J* = 4.5, 8.0 Hz. H-12), 2.12 (3H, s, H-15), 1.90 (2H, m, H-9), 1.85 (1H, m, H-7). 1.82 and 1.51 (1H each, m, H-8), 1.34 (3H, s, H-14), 1.29 (3H, d, *J* = 8.0 Hz, H-13);^{13}C NMR (101 MHz, $CDCl_3$): δ 213.50, 178.46, 129.63, 127.00, 81.80, 52.94, 48.87, 41.01, 36.00, 34.83, 31.90, 23.93, 23.48, 19.80, 12.33.

Compound 2: Santonin

Colorless crystalline solid; mp: 171–174°C; IR (KBr) νmax cm^{-1}: 1710, 1685 (C=O), 1610, 1385, 1278, 820; 1H NMR (CDCl3, 400 MHz) δ: