Phytochemical and Biological Studies on *Pulicaria incisa* subspecies candolleana Family Asteraceae

A Thesis Submitted By

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To the soul of my Grandfather...

Abstract

Phytochemical and Biological Studies on *Pulicaria incisa subspecies candolleana* Family Asteraceae

Pulicaria incisa sub. candolleana E-Gamal Eldin (Asteraceae) is one of the neglected wild plants growing in Egypt. This study includes in-depth phytochemical and biological investigations on *P. incisa* sub. candolleana essential oils and aqueous ethanolic extract in addition to *in-silico* molecular docking study of the isolated compounds as anti-inflammatory drugs on COX-2 and S-PLA-2. The composition of the essential oils extracted from leaves and flowers was analysed by GC-MS where 49 and 68 compounds were identified accounting for 86.69% and 84.29%, respectively of the total detected constituents. The cytotoxic activity of both essential oils was evaluated against hepatocellular carcinoma cell line HEPG-2, using MTT assay and vinblastine as a reference where leaf oil showed higher activity with IC_{50} 11.4 µg/mL compared with 37.4 µg/mL for flower oil. Their antimicrobial activity was also evaluated using agar well diffusion method. The MIC of both essential oils against the tested bacterial and fungal strains was obtained in the range of 0.49 – 15.63 µg/mL.

The phenolics and flavonoids were quantitatified in the aqueous ethanolic extract of *P. incisa* sub. canolleana then the main phenolic constituents were identified using HPLC-MS/ MS of the extract where the results allowed tentative identification of thirteen phenolic compounds. Four compounds were isolated from the ethanolic extract using semi-preparative HPLC including; eugenol-1-O-*β*-glucoside, 5-O-caffeoylquinic acid, 3, 5-Di-O-caffeoylquinic acid, quercetin-3-O-*β*-glucoside. Their structures were elucidated using different spectroscopic analysis methods including 1D and 2D-NMR as well as by ESI/MS.

In-vitro study exhibited significant antimicrobial activity of the extract against some of the tested bacterial and fungal strains with MICs in the range of 1.9-7.81 µg/mL while showed a weak activity against others with MICs of 62.5 and 15.63 µg/mL. Moreover, *In-vivo* biological investigations showed the safety of the extract at dose 250 mg/kg as anti-hepatotoxic agent in methotrexate induced rats when compared to Silymarin with high antioxidant and anti-inflammatory activities. These results were further confirmed using *in-silico* screening of the isolated compounds as anti-inflammatory drugs through binding with COX-2 and S-PLA-2. Quercetin-3-O-*β*- glucoside showing the best docking energy score -19.12 kcal/mol against COX-2 and 3,5- Di-O-caffeoylquinic acid (-5.68 kcal/mol) was the most active one against S-PLA-2

Key words; *Pulicaria incisa* sub. canolleana, essential oil , GC/MS, Quantitative determination, HPLC/MS, phenolics, antimicrobial , hepatoprotective, anti-inflammatory, molecular docking, COX-2 , PLA-2

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