



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 IC₅₀ 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 quantitated in the aqueous ethanolic extract of *P. incisa* sub. *candolleana* 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

CONTENTS

	Page
List of Contents.....	vii
List of Figures.....	ix
List of Photos.....	xiii
List of Tables.....	xv
List of Abbreviations.....	xviii
Introduction.....	1
Review of Literature.....	4
I. Folk Uses.....	4
II. Biological Activities of Different Extracts of Genus <i>Pulicaria</i>	5
1. Antioxidant Activity	5
2. Cytotoxic Activity.....	10
3. Anti-microbial Activity.....	12
4. Anti-inflammatory Activity.....	19
5. Effect on CNS.....	20
6. Others	21
III. Chemical Review of Different Classes of Active Constituents in Extracts of Genus <i>Pulicaria</i>	24
1. Essential Oil	24
2. Fatty Acids and Other Contents.....	31
3. Flavonoids.....	48
4. Phenolic Compounds.....	51
5. Chalcones	51
6. Coumarins.....	52
7. Terpenoids.....	52
6.1. Monoterpenes	
6.2. Sesquiterpenes	
6.3. Diterpenes	
6.4. Triterpenes	
Taxonomy.....	92
I. Family Asteraceae.....	92
II. Tribe Inuleae.....	95
III. Genus <i>Pulicaria</i>	96
IV. <i>Pulicaria incisa</i>	97
Material, Apparatus and Methods.....	102
Chapter I: Investigation of Essential Oil Contents in the Leaves and Flowers of <i>Pulicaria incisa</i> sub. <i>candolleana</i> & Determination of their Biological Activities.....	117

I.	Extraction of Essential Oils from Leaves and Flowers of <i>Pulicaria incisa</i> sub. <i>candolleana</i>	117
II.	GC/MS analysis of <i>Pulicaria incisa</i> sub. <i>candolleana</i> Essential Oils of Flowers and Leaves.....	117
III.	Biological activities of <i>Pulicaria incisa</i> sub. <i>candolleana</i> Essential Oils from Leaves and Flowers.....	123
1.	Cytotoxic Activity of Leaves and Flowers Essential Oil.....	
2.	Antimicrobial Activity of the <i>Pulicaria incisa</i> sub. <i>candolleana</i> Essential Oils.....	
Chapter II: Phytochemical Investigations of the Ethanolic Extract of <i>Pulicaria incisa</i> sub. <i>candolleana</i>		
I.	Preliminary Phytochemical Screening.....	128
II.	Quantitative Assay of Total Phenolic and Flavonoids Contents of <i>Pulicaria incisa</i> sub. <i>candolleana</i> Using Colorimetric Assay.....	129
III.	HPLC/MS-MS Analysis of the Aqueous Ethanolic Extract of <i>P. incisa</i> sub. <i>candolleana</i>	132
IV.	Fractionation of the 70% Aqueous Ethanol Extract of <i>P. incisa</i> sub. <i>candolleana</i> Leaves.....	142
V.	Isolation and Identification of Compounds.....	145
Chaper III: Biological Investigations of the Ethanolic Extract of <i>Pulicaria incisa</i> sub. <i>candolleana</i> Aerial Parts.....		172
I.	Antimicrobial Activity of <i>Pulicaria incisa</i> sub. <i>candolleana</i> Ethanolic Extract	172
II.	Hepatoprotective Assay of <i>Pulicaria incisa</i> sub. <i>candolleana</i> Ethanolic Extract.....	174
Chapter IV: Molecular Docking Study of the Isolated Phytoconstituents from <i>P. incisa</i> sub. <i>candolleana</i> as Anti-inflammatory Drugs.....		195
Summary		209
References		216
Arabic Summary.....		

List of Figures

No.	Title of Figure	Page
1.	Inflorescence of Compositae.....	99
2.	Disc floret and ray floret of family compositae.....	99
3.	Total ion chromatogram of <i>P. incisa</i> sub. <i>candolleana</i> flowers essential oil.....	122
4.	Total ion chromatogram of <i>P. incisa</i> sub. <i>candolleana</i> leaves essential oil.....	123
5.	Cytotoxic activity of <i>P. incisa</i> sub. <i>candolleana</i> oils against HEPG2.....	125
6.	Standard calibration curve of gallic acid.....	131
7.	Standard calibration curve of quercetin	131
8.	Total ion chromatogram in the negative mode of the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	133
9.	Total ion chromatogram in the positive mode of the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	134
10.	(-) ESI-MS spectrum of identified compound 1 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	137
11.	(-) ESI-MS spectrum of identified compound 2 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	137
12.	(-) ESI-MS spectrum of identified compound 3 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	137
13.	(-) ESI-MS spectrum of identified compound 4 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	138
14.	(-) ESI-MS spectrum of identified compound 5 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	138
15.	(-) ESI-MS spectrum of identified compound 6 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	138
16.	(+) ESI-MS spectrum of identified compound 7 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	139
17.	(-) ESI-MS spectrum of identified compound 8 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	139
18.	(-) ESI-MS spectrum of identified compound 9 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	139

19. (-) ESI-MS spectrum of identified compound 10 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	140
20. (-) ESI-MS spectrum of identified compound 11 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	140
21. (-) ESI-MS spectrum of identified compound 12 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	141
22. (-) ESI-MS spectrum of identified compound 13 in the alcoholic extract of the aerial parts of <i>P. incisa</i> sub. <i>candolleana</i>	141
23. General Fractionation Scheme.....	144
24. ¹ H-NMR of compound 1; Eugenol-1- O- β -glucopyranoside	148
25. APT spectrum of compound 1; Eugenol-1- O- β -glucopyranoside	149
26. HSQC correlations of compound 1; Eugenol-1- O- β -glucopyranoside	150
27. COSY correlations of compound 1; Eugenol-1- O- β -glucopyranoside	151
28. COSY correlations of compound 1; Eugenol-1- O- β -glucopyranoside.....	152
29. (-) ESI-MS spectrum of compound 2; 5-Caffeoylquinic acid	155
30. ¹ H-NMR of Compound 2; 5-Caffeoylquinic acid	156
31. ¹³ C-NMR Spectrum of compound 2; 5-Caffeoylquinic acid	157
32. HSQC Correlations of compound 2; 5-Caffeoylquinic acid	158
33. HMBC Correlations of compound 2; 5-Caffeoylquinic acid.....	159
34. COSY correlations of compound 2; 5-Caffeoylquinic acid	160
35. (-) ESI-MS spectrum of compound 3; 3, 5- Di-O-caffeoyl quinic acid	163
36. ¹ H-NMR spectrum of compound 3; 3, 5- Di-O-caffeoyl quinic acid	164
37. ¹³ C-NMR spectrum of compound 3; 3, 5- Di-O-caffeoyl quinic acid	165
38. HSQC Correlations of compound 3; 3, 5- Di-O-caffeoyl quinic acid	166
39. HMBC Correlations of compound 3; 3, 5- Di-O-caffeoyl quinic acid	167
40. COSY Correlations of compound 3; 3, 5- Di-O-caffeoyl quinic acid	168
41. (-) ESI-MS spectrum of compound 4; Quercetin -3-O- β - glucoside	170
42. ¹ H-NMR of compound 4; Quercetin -3-O- β - glucoside	171
43. Effect of different doses of <i>P. incisa</i> sub. <i>candolleana</i> extract on (AST) , (ALT) and (ALP) levels in methotrexate-induced hepatic dysfunction in rats.....	178

44. Effect of different doses of <i>P. incisa</i> sub. <i>candolleana</i> extract on hepatic tissue content of oxidative stress marker (MDA) in methotrexate-induced hepatic dysfunction in rats.	181
45. Effect of different doses of <i>P. incisa</i> sub. <i>candolleana</i> extract on hepatic tissue content of reduced (GSH) and (SOD) activity in methotrexate-induced hepatic dysfunction in rats.....	183
46. Effect of different doses of <i>P. incisa</i> sub. <i>candolleana</i> extract on hepatic tissue content of (TNF- α) and s (MPO) activity in methotrexate-induced hepatic dysfunction in rats.....	186
47. 2D interaction of redocked pose of ibuprofen with the key amino acids in COX-2 binding site.	196
48. 2D representation (a) & 3D representation (b) of the superimposition of the co-crystallized (green) and the redocked pose (red) of ibuprofen in the COX-2 binding site with RMSD of 0.47Å.....	197
49. 2D diagram (a) 3D representation (b) of eugenol-1-O- β -glucoside in COX-2 binding site.	198
50. 2D diagram (a) 3D representation (b) of chlorogenic acid in the COX-2 binding site.	198
51. 2D diagram (a), 3D representation of 3, 5-Dicaffeoylquinic acid in the COX-2 binding site.	199
52. 2D diagram (a) 3D representation (b) of quercetin-3-O- β -glucoside in COX-2 binding site.....	200
53. 2D interaction diagram showing the redocked pose interactions with the key amino acids and calcium in S-PLA2 binding site.....	202
54. 2D representation (a), 3D representation (b) of the superimposition of the co-crystallized ligand (green) and the redocked pose (red) in the PLA-2 binding site with RMSD of 0.486Å.	203
55. 2D diagram (A) and 3D representation (B) of eugenol-1-O- β - glycoside in S PLA-2 binding site.	204

56. 2D diagram (A) and 3D representation (B) of chlorogenic acid in the S-PLA-2 binding site.	205
57. 2D diagram (A) and 3D representation in (B) of 3,5-dicaffeoyl quinic acid in the S-PLA-2 binding site.	206
58. 2D diagram (A) and 3D representation (B) of quercetin-3- <i>O</i> - β - glycoside in S PLA-2 binding site.	207

List of Photos

No.	Title	Page
1.	<i>Pulicaria incisa</i> (Lam.) DC. blooming in desert.....	100
2.	<i>Pulicaria incisa</i> (Lam) DC.	100
3.	Leaves of <i>P. incisa</i> (Lam.) DC.	101
4.	<i>Pulicaria incisa</i> subsp. <i>candolleana</i>	101
5.	<i>Silybum marianum</i>	174
6.	Liver of rats in normal control group (Group 1) showing normal histological structure of the central vein and surrounding hepatocytes in the parenchyma.....	188
7.	Liver of rats in methotrexate control group (Group 2) showing severe dilatation and congestion on central vein with degeneration in surrounding hepatocytes and fatty changes.....	188
8.	Liver of rats in methotrexate control group (Group 2) showing magnification of photo 7 to identify the dilated and congested central vein.....	189
9.	Liver of rats in methotrexate control group (Group 2) showing magnification of photo 7 to identify the fatty change in hepatocytes surrounding the central vein.	189
10.	Liver of rats in methotrexate control group (Group 2) showing congestion in portal vein.	190
11.	Liver of rats in methotrexate control group (Group 2) showing few inflammatory cells infiltration in portal area with fatty change in hepatocytes....	190
12.	Liver of rats treated by 100 mg extract of <i>P. incisa</i> sub. <i>candolleana</i> (Group 3) showing mild congestion in central vein, few inflammatory cells infiltration in portal area with few micro fat vacuoles in cytoplasm of hepatocytes.....	191
13.	Liver of rats treated by 100mg extract of <i>P. incisa</i> sub. <i>candolleana</i> (Group 3) showing magnification of photo 12 to identify inflammatory cells infiltration in portal area and the few micro fat vacuoles in cytoplasm of hepatocytes.....	191
14.	Liver of rats treated by 250 mg extract of <i>P. incisa</i> sub. <i>candolleana</i> (Group 4) showing almost normal hepatocytes, mild dilatation in central vein with few micro fat vacuoles in hepatocytes.....	192