SUMMARY

Ambrosia L. is the largest genus of flowering plants in Egypt, being represented by (25.000:33.000) species. The genus *Ambrosia* is of great importance due to diversity of phytochemical constituents, including sesquiterpene lactones, flavonoids and terpenoids. *Ambrosia maritima* L. is a widely distributed in southern parts of Egypt, Sudan and neighboring countries.

This study inlcudes three parts:

Part I: Phytochemical studies including:

1) Preliminary phytochemical screening.
2) Quantitative determination of total phenolic and total flavonoid contents.
3) Investigation of *n*- hexane fraction.
4) Investigation of dichloromethane fraction.
5) Investigation of ethylacetate and *n*-butanol fractions.

Part II: Biological studies

1) Cytotoxicity.
2) Anti-inflammatory activity.
3) Evaluation of the anti-microbial activity.
4) Anti-oxidant activity.

Part III: Molecular docking studies

1) Docking of the five sesquiterpene lactones into the active site of wild and mutant Src kinase.
2) Docking of the five sesquiterpene lactones into the active site of COX-1 and COX-2.

Part I: PHYTOCHEMICAL STUDIES

Preliminary phytochemical screening:

The preliminary phytochemical screening of the total ethanolic extract of the aerial parts of *Ambrosia maritima* showed the following:

1. Presence of tannins, carbohydrates and/or glycosides, alkaloid and/or nitrogenous bases, flavonoids as well as sterols and /or triterpenes.
2. Absence of saponins, anthraquinones and cardiac glycosides.

Preparation of Ethanolic Extract and Its Fraction and TLC Investigation

The weight of residue left after the extraction of aerial parts of *Ambrosia maritima* with 70% ethanol 430.0 g, while the residues left after fractionation with *n*-hexane, CH_2Cl_2, EtOAc and *n*-butanol were 30.0, 50.0, 26.6 and 46.4 g, respectively. The organoleptic characters of the different extracts were recorded. The extract was also screened by TLC. Based on their chromatographic profiles the *n*-hexane, dichloromethane, ethyl acetate and butanol fractions of *Ambrosia maritima* were selected for isolation of their major constituents.

Total Phenolic and Total Flavonoid Contents

Using Folin-Ciocalteu reagent and $AlCl_3$, respectively for estimation of the total phenolic and total flavonoid contents in total alcoholic extract of the aerial parts of *Ambrosia maritima* and the results revealed that, the total phenolic content of the aerial part of *Ambrosia maritima* showed 214.67 µg GAE/g. While the total flavonoid content in the aerial part of *Ambrosia maritima* was 179.82 µg rutin/g RE/g; relative to total phenolic.

Investigations of The *n*-Hexane Fraction of The Aerial Parts of *Ambrosia maritima*

GC-MS analysis of the unsaponifiable (USM) and saponifiable (FA) lipoidal matters.

The *n*- hexane extract of the aerial part of *A. maritima* (10 g) was saponified. The USM (80%) and FA (20%) were separated. The fatty acids were methylated to yield the fatty acid methyl esters (FAME). GC-MS analysis for both revealed the following:

1. The number of identified components in the unsaponifiable matter of the aerial parts of *A. maritima* was 48, representing 97.99 % of the total hydrocarbons, sterols and triterpenes content.
2. The percentage of the total hydrocarbons and oxygenated hydrocarbons were (78.08 %) and Phytol was the major identified hydrocarbon (27.85 %).
3. Total sterols and triterpenes content was (19.91%). α-amyrin was identified as the major triterpene (11.25 %). *β*-sitosterol (3.32%) and stigmasterol (3.20%) were the major triterpenes detected in the aerial parts of *Ambrosia maritima*.
4. The identified components of FAME were 30 in number representing 99.72 % of the total fatty acid composition in the aerial parts of *Ambrosia maritima*.
5. The percentage of total identified as methyl esters of saturated and unsaturated fatty acids were (33% and 66.72%, respectively. Hexadecanoic acid, methyl ester was the major identified saturated fatty acid (22.32 %). 9,12-Octadecadienoic acid, methyl ester was the predominant unsaturated fatty acid (18.48 %).

Investigation of The *n*-Hexane Fraction of The Aerial Parts of *Ambrosia maritima*

The *n*-hexane fraction was subjected to a silica gel column using eluents of increasing polarity to give one main fraction. This fraction was purified by successive column chromatography to give one main compound; **C1 (***β*-sitosterol-3-*O*-*β*-D-glucoside).

Investigation of The Methylene Chloride Fraction of The Aerial Parts of *Ambrosia maritima*

The dichloromethane fraction was subjected to a silica gel column using eluents of increasing polarity to give three main fractions. These fractions were purified by successive column chromatography to give five compounds, **C2** (damsin), **C3** (ambrosin), **C4** (hymenin), **C5** (damsinic acid) and **C6** (maritimolide). Their structures were identified based on physico-chemical properties, spectral data and comparison with previously published data**.**

Investigation of Ethyl Acetate Fraction of The Aerial Parts of *Ambrosia maritima*

The ethyl acetate fraction was fractionated on silica gel column to give two main fractions **(E1-E2)**. These fractions were purified by repeated column chromatography to give three compounds; **C7** (apigenin), **C8** (Luteolin) and **C9** (kaempferol-3-*O*-*β*-D-glucoside). Their structures were identified based on physico-chemical data, spectral data and comparison with previously published data.

Investigation of *n*-Butanol Fraction of The Aerial Parts of *Ambrosia maritima*

The butanol fraction was subjected to a polyamide column to give one main fraction. The latter was purified by successive column chromatography to give one compound, **C10** (quercetin 3-*O*-*β*-D-glucoside). The structure of **C10** was identified based on physico-chemical properties, spectral data and previously published data**.**

PART II: BIOLOGICAL STUDIES

Cytotoxic activity

In vitro cytotoxic activity of the isolated sesquiterpene lactones was determined against three tested cell lines: HCT-116 cells (Human colon carcinoma cell line), A-549 cells (Human lung carcinoma cell line) and MCF-7 cells (Human breast carcinoma cell line). Three compounds have high cytotoxic activity (hymenin (C4), ambrosin (C3) and damsin (C2)). Hymenin (C4) show the highest activity against the tested three cell lines. Followed by ambrosin (C3) with high cytotoxic effect on HCT-116 cells and MCF-7 cells with IC_{50} values of 7.74 and 17.9 µg/ml, respectively then damsin (C2), while damsin (C2) has higher cytotoxic effect in comparison with ambrosin (C3) on A-549 cells with IC_{50} value of 7.38 µg/ml. Then damsinic acid (C5) show weak cytotoxic activity with IC_{50} values of 29.1, 47.3 and 95.3 µg/ml on A-549 cells, MCF-7 cells and HCT-116 cells, respectively. Maritimolide (C6) show the less cytotoxic activity with IC_{50} values of 145, 187 and 194 µg /mL on A-549 cells, MCF-7 cells and HCT-116 cells, respectively.

Structure activity relationship:

The structure-activity relationship revealed that α-methylene-γ-lactone moiety enhances the cytotoxic activity, OH group at C-1 increase activity of hymenin (C4). However, the reduction of

the double bond at C-2 as in damsin (C2) resulted in a significant decrease in activity against HCT-116 and MCF-7 cells.

Anti-inflammatory Activity

In vitro anti-inflammatory activity of the isolated sesquiterpene lactones was determined by COX-1, COX-2 and nitric oxide inhibitor. The results showed that the hymenin (C4), ambrosin (C3), damsin (C2), maritimolide (C6) and damsinic acid (C5) have IC_{50} values 18.21, 49.73, 86.54, 136.36 and 570.17 µg/ml, respectively for COX-1, while, damsin (C2), damsinic acid (C5), maritimolide (C6), hymenin (C4) and ambrosin (C3) have IC_{50} values 33.97, 36.80, 58.75, 107.99 and 165.01 µg/ml, respectively for COX-2 compared to celecoxib ®(IC_{50}=91.75 ± 6.04 and 2.79 µg/ml for COX-1 and COX-2, respectively). Hymenin (C4) showed potent selectivity against NO with IC_{50} 18.19 µg/ml while Ascorbic acid the positive control has IC_{50} 18.73 µg/ml.

Antimicrobial activity:

All fractions and isolated flavonoids **(C7-C10)** were investigation for their antimicrobial activity against *E.coli* ATCC 8739, *Staphylococcus aureus* ATCC 33592, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6051, *Mycobacterium smegmatis* ATCC 19420, *Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 7644 and *Candida albicans* ATCC 90028. Luteolin **(C8)** and apigenin **(C7)** has MIC of 1.25 mg/ml against *Micrococcus luteus* and *Bacillus subtilis*, while dichloromethane and ethylacetate extracts have MIC of 625 µg/ml against the same pathogens. Kaemferol 3-*O*-glucoside **(C9)** has activity against *Listeria monocytogenes* with MIC of 156 µg/ml.

Antioxidant Activity

In vitro anti-oxidant activity for the total ethanolic extract of *Ambrosia maritima* was estimated using DPPH and RP.

The results of DPPH assay revealed that the antioxidant activity of the 95% ethanol extract of *aerial part of A. maritima* was calculated to be 233% mg AEAC/g. The RP of extract was higher than those found for control (rutin and ascorbic acid). Significant correlations were also evaluated between extract, rutin and ascorbic acid (r = 1.720, 0.063 and 0.738, respectively).

Part III: MOLECULAR DOCKING STUDIES

The molecular docking studies were conducted in an attempt to understand the results of the previous study on the effect of some of the separated compounds (sesquiterpene lactones) from the *Ambrosia maritima* under study inhibiting COXs/ Src kinase in treating cancer and its relationship to its anti-inflammatory function. These studies revealed good fitting of the isolated sesquiterpene lactones into the active sites of COX-1/2. In addition, the docking study into human Src kinase showed high binding affinities for the isolated sesquiterpene lactones. These results recommended that both COXs/ Src kinase inhibition could participate even partially to the overall mechanism of cytotoxic activity of the isolated sesquiterpene lactones.

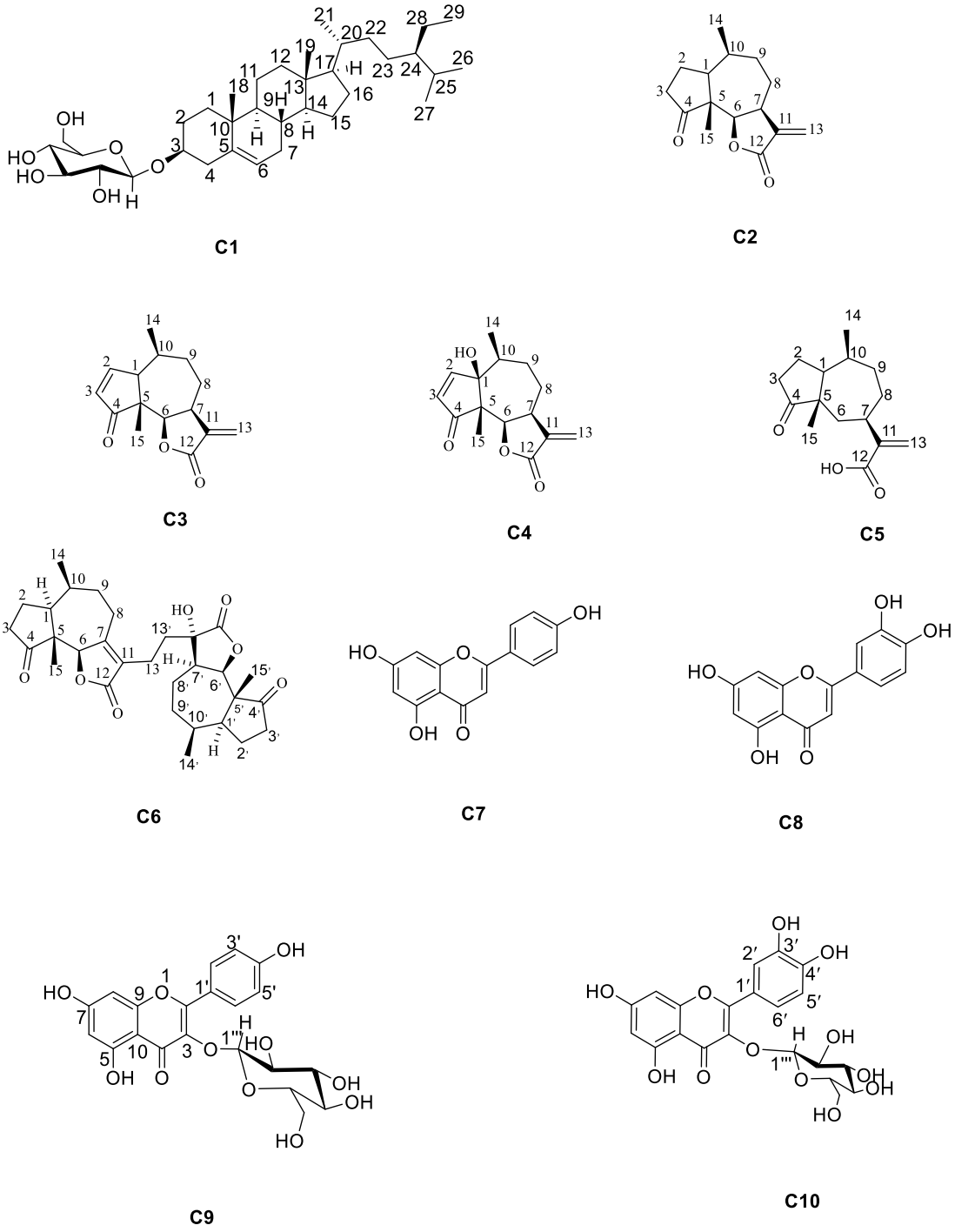

Figure 64: Chemical structure of isolated compounds **(C1-C10)** from aerial parts of *Ambrosia maritima*.