The Use of Medicinal Plant wastes for Production of Chemicals and Pharmaceuticals

Thesis submitted to
Faculty of Pharmacy-Mansoura University
In partial fulfillment of the requirement for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Pharmacognosy)

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2014
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Waste of medicinal plants represents a serious problem as the poor management of these wastes causes risks to public health and the environment. At the same time, the increasing demand for natural resources requires careful planning and highlights the value of using these medicinal plant wastes as a new resource.

Mostly, the previous phytochemical studies were done on *H. sabdariffa* and *A. visnaga* official parts. However, little phytochemical studies were found concerning other parts of the plant. So this research was conducted to isolate the chemical constituents of the unused aerial parts of both plants and evaluate their potential use in pharmacy and medicine.

This study deals with the isolation of bioactive compounds from the unused aerial parts of both *Hibiscus sabdiffera* and *Ammi visinga*. The phytochemical evaluation of extracts using column chromatography, TLC, purification and crystallization leads to isolation of several compounds. The chemical identity of isolated compounds was evidenced by different spectroscopic techniques.
Chapter I

Phytochemical investigation of different extracts of unused aerial parts of *Hibiscus sabdariffa* (F. Malvaceae)

The dried powdered unused aerial parts of the plant were extracted by maceration with MeOH then concentrated to a syrupy consistency which extracted successively with petroleum ether, CH$_2$Cl$_2$ and EtOAc.

**Section 1:**

**Petroleum ether extract**

It was chromatographed on silica gel column gradiently eluted with petroleum ether then ethyl acetate/petroleum ether with different percentage till 100% ethyl acetate. The effluents were monitored by TLC on silica gel plates, and similar fractions were pooled and concentrated. Chromatographic analysis using silica gel TLC plates revealed the presence of 6 major compounds in addition to 4 minor compounds.

The isolated compounds were obtained by purification, silica gel column chromatography and crystallization from different organic solvents. All of the isolated compounds were identified using different spectral data as well as comparison with reference authentic samples and comparing the results with previously published results including:
English Summary

1- Oleic acid (50 mg).
2- Lupeol (10 mg).
3- β-sitosterol (800 mg).
4- Betulinic acid (10 mg).
5- Oleanolic acid (25 mg).
6- 5α, 8α-Epidioxyergosta-6,22-dien-3β-ol (15 mg).

All of compounds (except β-Sitosterol) were isolated for the first time from *H. sabdariffa*. Both Oleanolic acid and 5α, 8α-Epidioxyergosta-6,22-dien-3β-ol were isolated for the first time from genus *Hibiscus*.

**Section 2:**

**Methylene chloride extract**

It was chromatographed on silica gel column gradiently eluted with mixture of methylene chloride/petroleum ether then the elution was continued using methanol/methylene chloride with different percentage till 100% methanol. The effluents were monitored by TLC on silica gel plates, and similar fractions were pooled and concentrated. Chromatographic analysis using silica gel TLC plates revealed the presence of 3 major compounds.

The isolated compounds were obtained by purification, silica gel column chromatography and crystallization from different organic solvents. All of the isolated compounds were identified using
different spectral data as well as comparison with reference authentic samples and comparing the results with previously published results including:

1- 5'-methoxy propacin=jatrocin B (7 mg).
2- Aquillochin=Cleomiscosin C (36 mg).

These two compounds were isolated for the first time from *H. sabdariffa*.

3- β-sitosterol 3-O-D-glucoside (130 mg): It was isolated before from *H. sabdariffa*.

**Section 3:**

**Ethyl acetate extract**

It was chromatographed on silica gel column gradiently eluted with mixture of ethyl acetate/petroleum ether then the elution was continued using methanol/ethyl acetate with different percentage till 100% methanol. The effluents were monitored by TLC on silica gel plates, and similar fractions were pooled and concentrated. Chromatographic analysis using silica gel TLC plates revealed the presence of 3 major compounds in addition to 3 minor compounds. The isolated compounds were obtained by purification, silica gel column chromatography and crystallization from different organic solvents. All of the isolated compounds were identified using different spectral data as well as comparison with reference authentic
samples and comparing the results with previously published results including:

1- 5, 8 dihydroxy dodeca-5, 7-dienedioic acid (20 mg): It was isolated for the first time from genus *Hibiscus*.

2- Gallic acid (10 mg).

3- Kaempferol-3-O- -D-(6``-E-p-coumaroyl) glucopyranoside 

(*Trans* Tiliroside) (6 mg).

Both of these compounds were isolated for the first time from *H. sabdariffa*.

**Chapter II**

**Phytochemical investigation of different extracts of unused aerial parts of Ammi visnaga (F. Apiaceae)**

The dried powdered unused aerial parts of the plant were extracted by maceration with MeOH then concentrated to a syrupy consistency which extracted successively with petroleum ether, CH₂Cl₂ and EtOAc.

**Section 1:**

**Petroleum ether extract**

It was chromatographed on silica gel column gradiently eluted with petroleum ether then ethyl acetate/petroleum ether with different percentage till 100% ethyl acetate. The effluents were monitored by TLC on silica gel plates, and similar fractions were pooled and
concentrated. Chromatographic analysis using silica gel TLC plates revealed the presence of 5 major compounds in addition to 5 minor compounds.

The isolated compounds were obtained by purification, silica gel column chromatography and crystallization from different organic solvents. All of the isolated compounds were identified using different spectral data as well as comparison with reference authentic samples and comparing the results with previously published results including:

1- Tetracosanoic acid=Lignoceric acid (12 mg).
2- β-sitosterol (750 mg)

These two compounds were isolated for the first time from *A. visnaga*

3- Visinadine (500 mg).
4- Khellin (900 mg).
5- β-sitosterol 3-O-β-D-glucoside (270 mg).

These three compounds were isolated before from *A. visnaga*

**Section 2:**

**Methylene chloride extract**

It was chromatographed on silica gel column gradiently eluted with mixture of ethyl acetate/petroleum ether then the elution was continued using methanol/ethyl acetate with different percentage till
100% methanol. The effluents were monitored by TLC on silica gel plates, and similar fractions were pooled and concentrated. Chromatographic analysis using silica gel TLC plates revealed the presence of 5 major compounds in addition to 3 minor compounds. The isolated compounds were obtained by purification, silica gel column chromatography and crystallization from different organic solvents. All of the isolated compounds were identified using different spectral data as well as comparison with reference authentic samples and comparing the results with previously published results including:

1- Norkhellol (7 mg): It is isolated for the first time from *Ammi* genus.

2- Khellol (39 mg)

3- Rhmnazin (6 mg)

These two compounds were isolated before from *A. visnaga*

4- Cimifugin (13 mg): It is isolated for the first time from *Ammi* genus.

5- *Cis*-khellactone glucoside (9 mg): it was isolated before from *A. visnaga*
Chapter III

Biotransformation of khellin

Biotransformation of khellin using Aspergillus niger ATCC 10549 resulted in the production of khellol. Antioxidant and acetylcholine esterase inhibitory assays of the transformed product and khellin were performed. It was found that khellol exhibited a higher degree of antioxidant and acetylcholine esterase inhibitory activities compared to khellin. This is the first report on the biotransformation of khellin by microorganisms and the first evaluation of the neuroprotective activity of either khellin or khellol. It is worth to mention that khellol was also isolated from the unused part of A. visnaga, proving that the plants and fungi exhibited similar metabolic pattern and enzyme cascade that can transform khellin to khellol.

Chapter IV

Molecular modeling and preparation of oleanolic acid derivatives as potential topoisomerase inhibitors

Inhibition of topoisomerases is one of the important mechanisms of anticancer drugs. Oleanolic acid competes with DNA for topoisomerase binding through direct interaction with the enzyme preventing topoisomerase-DNA complex formation in both topoisomerases. Based on these consequences, oleanolic acid which has been isolated from the unused parts of H. sabdariffa suggested as
backbone for rational design of specific topoisomerase inhibitors. In this study ten rationally designed oleanolic acid derivatives (S1-S10) using Molegro virtual Docker, nine of them are new, were synthesized based on docking studies and tested for their topoisomerase I and IIα inhibitory activity.

The structural elucidation of the new compounds was determined by spectroscopic methods (1D-, 2D-NMR and MS). Some of the prepared compounds act as dual inhibitors of topoisomerase I and IIα enzymes including S2, S3, S5 and S7.

Chapter V

Biological study

1- Antibacterial assay:

Some of the isolated compounds from both *H. sabdariffa* and *A. visnaga* were tested for their antibacterial activity. Among the evaluated compounds, tiliroside showed the strongest antimicrobial effect against *S. aureus* and a moderate activity against *E.coli* in comparison with the positive control (sorbic acid) while other tested compounds nearly show no activity. MIC of tiliroside against *S.aureus* was 200 μg/mL while MBC was higher than 200 μg/mL.
2- **B16 melenoma cell line assay:**

Khellin (20 µg/mL) displayed a melanin synthesis inhibitory activity (~37%) and of low cytotoxicity (~1%). However, khellol (40 µg/mL) exhibited ~28% inhibition and ~20% cytotoxicity. Visnadine at concentration of 40 and 20 µg/mL showed cytotoxicity on B16 melenoma cells rather than melanin formation inhibition but at a concentration of 10 µg/mL, it showed about 8% melanin inhibition with less toxicity. Also, cimifugin at concentration of 40 µg/mL showed cytotoxicity on B16 melenoma cells rather than melanin formation inhibition. β-sitosterol and β-sitosterol glucoside had no effect on melanin inhibition.

3- **ABTS antioxidant assay:**

The methylene chloride and ethyl acetate extracts for the unused parts of *H. sabdariffa* and *A. visnaga* showed good antioxidant activity, while hexane fractions for both showed low antioxidant activity. Results showed that phenolic compounds (flavonoids, coumarinolignan and gallic acid) have higher antioxidant activity, while steroids, triterpenes, fatty acids and pyranocoumarins have lower activity comparing to ascorbic acid.
4- Neuroprotective assay:

The highest inhibition among the compounds was observed for β-sitosterol, β-sitosterol glucoside, aquillochin and 5'-methoxy propacin, which was 47.0%, 55.6%, 50.2% and 68.2% respectively, at concentration of 38.46 μg/mL.

5- Antilipase activity:

From the results of the antilipase activity inhibitory assay, the strongest significant inhibitions for lipase enzyme were recorded for oleanolic acid followed by visnadine then khellin. To the best of our knowledge that this is the first study for indication of components from either genus of Hibiscus or Ammi for its effect against obesity by direct inhibition of the lipase enzyme.