Faculty of Agriculture
Minia University

Evaluation of some medicinal and aromatic plants as antioxidants

A Thesis Presented by

Heba kenawy Sayed Mohamed

Faculty of Agriculture, Minia University

B.Sc. of Agricultural Science, Minia University (2012)

M.Sc. of Agricultural Science, Minia University (2016)

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Under Supervision of

Prof. Dr. Mohammady A. Issa
Emeritus Professor of Agricultural chemistry
Agricultural Chemistry Department
Faculty of Agriculture, Minia University

Prof. Dr. Hamadi A. Ismail
Emeritus Professor of Agricultural Chemistry
Agricultural Chemistry Department
Faculty of Agriculture, Minia University

Prof. Dr. Magda E. Mahmoud
Emeritus Professor of Agricultural Chemistry
Agricultural Chemistry Department
Faculty of Agriculture, Minia University

***Prof. Dr.* Emad A. K. Hassan**
Prof. of central Lab. of Organic Agric. (ARC)/ Genetics and plant breeding

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LIST OF ABBREVIATIONS

A549	Human Lung carcinoma
ABTS	2,2'-Azinobis(3-Ethylbenzothiazoline-6-Sulphonic Acid
DMEM	Dulbecco's Modified eagle medium
DPPH	2,2-diphenyl-1-picrylhydrazyl
ERK	Extracellular signal-regulated kinase
FRAP	Ferric Reducing Antioxidant Power
FTIR	Fourier Transform Infrared spectroscopy
G A E	Gallic Acid Equivalent
GC/MS	Gas and Chromatography-Mass Spectroscopy
HCC	hepatocellular carcinoma
HePG2	Human livrer cancer
HPLC	High-performance liquid chromatography
IC_{50}	Inhibitory concentration 50
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCF-7	Human breast cancer cell lines
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
PI3K	Phosphatidylinositol 3-kinase
QE	Quercetin equivalents
ROS	reactive oxygen species
SOD	Super oxide dismutase
TAC	total antioxidant capacity
TBARS	thiobarbituric acid reactive substances
TFC	Total flavonoid content
TG	Triglycerides
TL	Total lipids
TLC	Thin layer chromatography
TPC	Total phenolic content
WHO	World Health Organization

SUMMARY

The leaves of *H. sabdariffa*, *C. citratus* and *L. inermis* were collected from the experimental farm, Fac. of Agric., Minia University. The leaves powder were extracted (1:7 w/v) separately with 80% ethanol, ethyl acetate and n-hexane for 6 hr with constant stirring at room temperature. Suspensions were filtered through Whatman No.1 filter paper.

Qualitative Phytochemical Analysis

The results of the phytochemical constituents of *H. sabdariffa* leaves extract exhibited phenols and flavonoids in 80% ethanol extract, terpenoids in ethyl acetate extract, steroid and tannins in n- hexane ,and emodins in ethyl acetate extract at higher amounts. Terpenoids, anthocyanins , steroids and tannins in 80% ethanol extract, glycosides and emodins in n-hexane extract, phenols and flavonoids in ethyl acetate extract were found at moderate amounts, whereas glycosides, and emodins in 80% ethanol extract, saponins, terpenoids, phenols and flavonoids in n-hexane extract, and anthocyanins, steroids , tannins and glycosides in ethyl acetate at lower amounts.

The result also showed that saponins and fatty acids in 80% ethanol extract, steroids, anthocyanins, and fatty acids in hexane extract, and saponins and fatty acids in ethyl acetate extract were absent. Table (1) showed that *H. sabdariffa* was absent in fatty acid in 80% ethanol, ethyl acetate, and n-hexane extract.

The results of the phytochemical constituents of *C. citratus* extract **(** exhibited the presence of terpenoids, steroids, tannins, phenols and flavonoids in ethyl acetate, 80% ethanol, n- hexane , ethyl acetate and 80% ethanol extract at higher amounts, respectively.

Glycosides and terpenoids in 80% ethanol extract; saponins and phenols in n-hexane extract; steroids, tannins and flavonoids in ethyl acetate extract were found at moderate amounts, respectively, whereas tannins, anthocyanins, and phenols in 80% ethanol extract; fatty acids steroids, terpenoids and glycosides in n-hexane extract; and saponins, glycosides and emodins in ethyl acetate extract were found at low amounts.

The result showed that saponins, fatty acids and emodins in 80% ethanol extract; tannins, anthocyanins and flavonoids and emodins in n-hexane extract and anthocyanins and fatty acids in ethyl acetate extract were absent.

The results of the phytochemical constituents of *L. inermis* extract **(**T exhibited the presence of steroids, tannins in 80% ethanol extract, terpenoids, phenols and flavonoids in ethyl acetate extract were found at high amounts. Terpenoids, anthocyanins, flavonoids and phenols in 80% ethanol extract; Tannins in n-hexane extract; glycosides and steroids in ethyl acetate extract were found at moderate amounts, whereas glycosides, and emodins in 80% ethanol extract; anthocyanins, terpenoids, steroids phenols, flavonoids, and emodins in n-hexane extract; Tannins and emodins in ethyl acetate extract were found at low amounts.

Quantitative analysis of some Phytochemicals

The result of the terpenoids, steroids and tannins of three plant leaves in different solvents. *H. sabdariffa* of n-hexane extract had the highest tannins content (1220.02± 65.45) mg /g, whereas *H. sabdariffa* had lowest tannins content (18.96± 1.59) mg /g in ethyl acetate. *H. sabdariffa* had lowest steroids content (12.82±1.60) mg /g in ethyl acetate. *H. sabdariffa* had lowest terpenoids content (11.28± 1.45) mg /g in n-hexane. It can be noted that there is a slight variation of terpenoids concentration between all extracts of both *L. inermis* and *H. sabdariffa*, whereas *C. citratus* had

lowest terpenoids concentration. The steroid concentrations in the 80% ethanol and ethyl acetate extracts of *L. inermis* were (310±4.44 and 280.76±8.87) mg /g, respectively, which is more than that of *H. sabdariffa* and *C. citratus*. Among all plant extracts, *L. inermis* of 80% ethanol extract had the highest tannins content (4573.28±214.47), followed by n-hexane extract (4150.69±389.08) and ethyl acetate extract (3973.37±231.7) mg /g.

Ethyl acetate extracted was the highest Total phenolic content (TPC) and total flavonoids content (TFC) in *L. inermis* (141.84 ± 6.7 and 29.56 ± 1.63) mg /100g, respectively. On the other hand, TPC and TFC in 80% ethanol extract obtained from *H. sabdariffa* were recorded to be (83.91±6.58 and 34.09±2.11 05mg /100g), respectively higher than other extracts. Ethyl acetate extracted the highest TPC (66.8 ± 3.6 mg /100g) in *C. citratus* and 80% ethanol extracted the highest sTFC (37.23± 2.05mg /100g) when compared to other extracts.

DPPH Radical Scavenging Assay

The expression IC_{50} (efficient concentration value) is applied to explain the results from the DPPH assay which define as the concentration of substrate that cause 50% loss of the DPPH activity. In this study, *C. citratus* leave extract antioxidant activity showed for 80% ethanol extracts of an IC_{50} of 68.78 ± 2.3 µg/ml in DPPH radical scavenging assay to be significantly higher than that of ethyl acetate and n-hexane extract (296.9± 10.8 and above 1000 mg /g, respectively**).**

On other hand, IC_{50} value of 80% ethanol extract of both *L. inermi*s and ***H. sabdariffa*** were lower (69.49 ± 3.1 µg/ ml and 110.4 ± 7.35 µg/ ml, respectively), which showed that the plant leave extracts antioxidant activity was to be effective in these solvent extracts. Among all extracts studied, the n-hexane leaf extract of *L. inermis and H. sabdariffa* extract

had higher IC_{50} values (above 1000 and 823.8± 30.2 µg /ml), respectively, which indicated insufficient scavenging activity.

ABTS Radical Scavenging Assay

The relative antioxidant capacity was estimated by calculating the ability to scavenge $ABTS^{\cdot+}$ of a specific compound against standard Trolox. Potassium persulphate was utilized to form $ABTS^{\cdot+}$ in the stable form. After achieving a steady optical density, the extract was allowed to react with the prepared solution, and the antioxidant capacity was assessed by decolorization. The ABTS was the highest for ethyl acetate leaf extract of *L. inermis* (799.63±11.5 µM TE/mg). The lowest value was observed for n-hexane leaf extract of both *C. citratus* and *L. inermis* (30.13±3.8 and 69.5±6.3 µM TE/mg), respectively. Thus 80% ethanol extract for all plants displayed the maximum antioxidant capability in ABTS radical scavenging assay.

Ferric-Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant powers of all extracts of *C. citratus, L. inermis and H. sabdariffa* are shown represented by µM Trolox Equivalents/mg . The obtained values pointed out the reducing ability of 80% ethanol extract of *C. citratus*, *L. inermis* and *H. sabdariffa* was the highest among the other extracts (630.22±25.7, 746.69±54.49 and 546.70±20.3 µM TE /mg), respectively. The ethyl acetate extract of *L. inermis* leaves had more reducing ability than *H. sabdariffa and C . citrates* (729.26 ± 52.02, 171.31± 17.28 and 135.44 ± 12.50) respectively, While n-hexane extract had the lowest reducing ability than the rest of the extracts under this study.

***In vitro* cytotoxic activity of different extracts of plants on hepatocellular carcinoma (HepG2) Cell line**

The cytotoxic effects of *H. sabdariffa, C. citratus and L. inermis* leaves extracts were investigated against HepG2 cell line. Cell viability was

assayed using SRB assay. The IC_{50} was detected for all the plant extracts which have showed cytotoxic activity.

The percentage of cell viability of 80% ethanol, ethyl acetate and n-hexane extract from *H. sabdariffa* on HepG2 cell line were 89.03%, 91.72 % and 93.41% at the concentration of 10 µg/ml, respectively; 86.01% 88.89% and 66.02% at the concentration of 100 µg/ml, respectively and 34.08%, 1.11% and 0.20% at the concentration of 1000 µg/ml, respectively.

The percentage of cell viability of 80% ethanol, ethyl acetate and n-hexane extracts from *C. citratus* on HepG2 cell line were 89.80%**,** 86.73% and 89.64% at the concentration of 10 µg/ml, respectively; 86.55% 41.22% and 71.72% at the concentration of 100 µg/ml, respectively and 2.09%, 3.86% and 12.03% at the concentration of 1000 µg/ml, respectively.

The percentage of cell viability of 80% ethanol, ethyl acetate and n-hexane extract from *L. Inermis* on HepG2 cell line were 87.62%, 92.84 % and 87.48% at the concentration of 10 µg/ml, respectively; 72.18% 89. 91% and 73.58% at the concentration of 100 µg/ml, respectively and 3.23%, 0.78% and 1.05% at the concentration of 1000 µg/ml, respectively.

<u>*In vitro* cytotoxic activity of different extracts of plants on breast cancer (MCF-7) cell line</u>

The percentage of cell viability of *H. sabdariffa* of 80% ethanol, ethyl acetate and hexane extract on MCF-7 cell line were 91.01%**,** 93.87% and 98.80% at the concentration of 10 µg/ml, respectively**;** 79.57%, 89.91% and 64.70% at the concentration of 100 µg/ml, respectively and 31.28%, 0.78% and 0.86% at the concentration of 1000 µg/ml, respectively.

The percentage of cell viability of *C. citratus* of 80% ethanol, ethyl acetate and n-hexane on MCF-7 cell line were 91.01 %, 95.60 % and 96.55% at the concentration of 10 µg/ml, respectively; 79.57%, 66.24%

and 77.16% at the concentration of100µg/ml, respectively **a**nd 31.28 %, 5.021% and 15.14% at the concentration of 1000 µg/ml , respectively.

The percentage of cell viability in 80% ethanol, ethyl acetate and n-hexane extract from *L. Inermis* on MCF-7 cell line were 93.72%, 95.08% and 94.61% at the concentration of 10 µg/ml, respectively; 91.42% 91.50% and 87.10% at the concentration of 100 µg/ml, respectively and 6.42%, 1.11% and 1.02% at the concentration of 1000 µg/ml, respectively.

In vitro cytotoxic activity of different extracts of plants on lung cancer (A549) cell line

The percentage of cell viability of *H. sabdariffa* of 80% ethanol, ethyl acetate and hexane on A549 cell line were 92.17%, 90.87% and 93.61% at the concentration of 10 µg/ml , respectively; 88.22%, 89.08% and 35.99% at the concentration of 100µg/ml , respectively and 23.22%, 15.05% and 4.60% at the concentration of 1000 µg/ml, respectively.

The percentage of cell viability of *C. citratus* of 80% ethanol, ethyl acetate and hexane extract on A549 cell line were 95.26%**,** 97.3% and 94.51% at the concentration of 10 µg/ml , respectively**;** 72.34%, 64.09% and 70.58% at the concentration of 100µg/ml , respectively and 1.84 %, 12.47 % and 33.44% at the concentration of 1000 µg/ml , respectively.

The percentage of cell viability of *L. inermis* of 80% ethanol, ethyl acetate and hexane extract on A549 cell lines were 92.72%, 94.87% and 94.46% at 10 µg/ml, respectively; 98.42% , 80.08% and 64.43% at the concentration of 100µg/ml, respectively and 1.42%, 2.48 % and 4.21% at the concentration of 1000 µg/ml , respectively.