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Evaluation of some medicinal and aromatic plants as antioxidants

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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 IC₅₀ (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 IC₅₀ 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, IC₅₀ value of 80% ethanol extract of both *L. inermis* 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 IC₅₀ 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⁺ of a specific compound against standard Trolox. Potassium persulphate was utilized to form ABTS⁺ 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 IC₅₀ 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.

In vitro cytotoxic activity of different extracts of plants on breast cancer (MCF-7) cell line

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 of 100µg/ml, respectively and 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.