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Phytochemical Screening and Antioxidant Activity of some Egyptian Medicinal Plants

Heba K. Sayed[1*], Mohammady A. Issa[2], Hamadi A. Ismail[2], Emad A. Hassan [1], Magda E. Mahmoud[2]

[1]Central Lab of Organic Agriculture. Agri. Res. Center.
[2]Agric. Chem. Dept., Fac. of Agri. Minia Univ. Egypt.
*Corresponding author : hebakenawy85@yahoo.com ;Tel: +01007827641.

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Abstract

Extracts of three leaves: *Cymbopogon citratus, Hibiscus sabdariffa, and Lawsonia inermis* were screened for phytochemicals in ethanol (80%), hexane, and ethyl acetate. The potential of plant extracts to scavenge free radicals such as DPPH, ABTS and FRAP (Ferric Reducing Antioxidant Power) was investigated.

For all extracts, phytochemical analysis indicated the presence of terpenoids, steroids, tannins, anthocyanins, phenols and flavonoids with absence of fatty acids in ethanol 80% and ethyl acetate.

Ethyl acetate extract of *L. inermis* achieved a high percentage of TPC and TFC, but it was higher in 80% ethanol of *H. sabdariffa* than the other extracts under study. Ethyl acetate extracted the highest TPC (66.8 ± 3.6 mg/100g) in *C. citratus* and 80% ethanol extracted the highest TFC (37.23± 2.05mg/100g).

Ethanolic extract (80%) of the three plants' leaves showed a higher IC_{50} value than both ethyl acetate and hexane extracts. This indicates that 80% ethanol was the best solvent for extracting phenolic compounds and flavonoids.

The "ABTS radical" scavenging activity was the highest for ethyl acetate extract of *L. inermis.* The lowest value was observed for hexane extract of both *C. citratus* and *L. inermis.*

This study exhibited potential of ethanolic extracts (80%) of all plants for removing free radicals and their effectiveness to be a powerful antioxidant.

Introduction

Cymbopogon citratus (lemon grass), a Poaceae plant with 1 to 2 percent essential oil on dry weight and a large variety of chemical compositions depending on genetic diversity, habitat, and agronomic treatment of culture is a plant with one to two percent essential oil on dry weight. Many scientific studies have announced the benefits of lemongrass oil such as various antimicrobial activities [1, 2], astringent, anti-inflammatory [3], insecticidal [4], carminative [5], antioxidant and antifungal [6].

Laswsonia inermis L., family Lythraceae, known as henna, contains numerous biological active constituents such as "Lawson, quinone, xanthone, gallic acid, triterpenoids" which are found to show "anti-neoplastic, anti-inflammatory, anti-hemorrhagic, hypotensive activity etc." Also, it can be used as astringents in the treatment of leprosy, nervous disorders, as well as for the treatment of boils and burns [7].

Hibiscus sabdariffa L. Family (Malvaceae) has long been known as a dicotyled annual herbaceous plant with its English name Roselle or Sorrel [8, 9]. It is widely used in making jellies, jams and beverages [10], and many studies have recommended the use of diverse regions within the herb as a natural recipe for many diseases such as high blood pressure, fever, liver disorders and as an antidote for poisoning chemicals (acids, alkalis, pesticides) and poisonous mushrooms [11,12]. Many active compounds were isolated from this plant and identified their composition, including anthocyanins, flavonols and protocatechuic acid (PCV) [13, 14].

The major goal of this work was to examine phytochemicals qualitatively and quantitatively in various solvent extracts of medicinal plants *C. citratus, H. sabdariffa and L. inermis* leaves, as well as to evaluate their antioxidant activities in vitro.

Materials and Methods

Plant sample collection and preparation of extracts

The leaves of *H. sabdariffa*, *C. citratus* and *L. inermis* were collected from nursery Fac. of Agric., Minia University. The fresh and healthy leaves were cleaned carefully and rinsed well with running tap water and air dried, away from the sun, at ambient condition for 4 weeks and ground with an electric grinder to a fine powder. The powdered leaves were kept in a sealed container. One hundred grams of powdered sample was transferred into the round bottom flask (1000 mL) mixed with a magnetic stirrer and 700 mL solvents (80% ethanol, ethyl acetate and hexane) added with constant stirring for 6 hrs. The extract was then collected and filtered off using Whatman No.1 filter paper.

Phytochemical Screening:

Photochemical screening tests of different crude extracts were done for leaves of *L. inermis*, *H. sabdariffa* and *C. citratus* constituents. Steroids were tested the before method of [15], flavonoids by [16], terpenoids [17], tannins[18], saponins [19], glycosides [16], anthocyanins [20], emodins [21] and fatty acids[16] were tested by standard procedures.

Quantitative Determination of Phytochemical

Total Steroids Content:

Steroids were estimated according to procedure described by [22]. One mL of each extract placed into 10 mL volume bottles. "H_2SO_4 (4 N, 2 mL)" and "$FeCl_3$ (0.5% w/v, 2 mL)" solution were added, followed by "$K_3[Fe(CN)]_6$ (0.5% w/v, 0.5 mL)" solution. The combination was heated for half an hour in a water bath at 70±2°C with intermittent shaking, then adjusted to the desired concentration using deionized H_2O. The optical density (OD) was estimated at 780 nm compared to the blank with the aid of a spectronic 21D.

Total Terpenoids Content:

Terpenoid content of leave extract was attained by technique reported by [23]. Crude extracts (0.01 ml of each) were dehydrated at 55-60°C in a steam bath, half ml of recently formulated (5% w/v) "glacial CH_3COOH-vanillin" then one ml of $HClO_4$ were mixed, thereafter, the specimens were agitated and heated for 10 minutes in a steam bath at 55-60°C. The samples were then immediately cooled to an ambient condition in iced water. Subsequently, 10 ml of "glacial CH_3COOH" was mixed and the optical density (OD) of the specimen against blank at 544 nm was estimated after half an hour using an UV-visible spectronic 21D. Simultaneously, the calibration curve was established using ursolic acid. Terpenoid concentration was stated in milligrams of Ursolic Acid Equivalents per gram of dried samples (mg UAE/g dry sample) using the equation attained from the calibration curve.

Total Tannins Content:

With distilled water, the volume of each extract or standard was increased to 1 mL. 0.5 mL "Folin's phenol reagent" was added to the reaction mixture, followed by 5 mL of 35 percent $NaCO_3$, which was held at ambient condition for 5 minutes. The resulted blue color was read at 640 nm with the aid of a spectronic 21D. According to [24], tannin concentrations were determined using a gallic acid standard curve and the findings were reported as gallic acid equivalent (mg/g).

Total Phenolics Content:

The total phenolic content (TPC) of each extract was estimated using the Folin-Ciocalteu method [25, 26]. A hundred ml of each diluted extract (5 *mg*/ml) was mixed with 0.75 mL of Folin–Ciocalteu reagent (Folin-Ciocalteu reagent: distilled water. 1:9 w/w). After allowing the mixture to cool for 5 minutes, 2 mL $NaCO_3$ solution (75 g/L inH_2O) was thoroughly mixed. The absorption spectrum was estimated by the aid of spectronic 21D at 725 nm against blank after 90 minutes of incubation at ambient conditions. TPC was calculated as mg of Gallic Acid Equivalents (GAE)/100 g of dry mass.

Total Flavonoids Content:

Each extract's total flavonoid content (TFC) was attained using the techniques developed by [27]. Half a millilitre of sample extract (5 g/L) was combined with 1.5 ml of CH_3OH, then 0.1 ml of 1:10 "aluminium chloride", 0.1 ml of "potassium acetate", and 2.8 ml of deionized H_2O were added. For half an hour, the solution was kept at 22°C. A spectronic 21D was used to measure the absorption spectrum at 415 nm. "Quercetin Equivalents (QE)" per gram of extract (mg QE/g extract) were used to calculate the findings. Quercetin was used to create a standard curve in diferrent concentrations ranging from 12.5 to 100 mg/ L.

Antioxidant activity determination:

Estimation of antioxidant activity with "2,2'-diphenyl-1-picrylhydrazyl (DPPH)" radical scavenging Method:

The scavenging ability towards free radical of all the extracts from different plant samples was evaluated by (DPPH) according to [28] with a few adjustments utilising the stable "DPPH radical", which has peak absorbance of 515 nm. 2.4 mg "DPPH" was added in hundered mililitres of "CH_3OH" to make a radical solution. Test leave extracts (1.50 ml) were allowed to 2.85 ml of the "DPPH" reagent. The mix was strongly agitated and kept at ambient condition for half an hour in the dark. The absorption of the reaction mix and the "DPPH radical" (blank) were estimated spectrophotometrically at 515 nm. All the determinations were done in triplicate. If the absorption value of reaction mixture decreases indicates a rise of free radical scavenging activity. The following calculation to determine the proportion of "DPPH" scavenging were proposed by [29].

"DPPH Scavenged (%) = $((A_B–A_A)/A_B) \times 100$"

Given that, A_B is absorption of the control reaction at t = 0 min; A_A is absorption of the antioxidant at t = 30 min. The standard curve was plotted using the ratio "DPPH" cleared against the standard antioxidant concentration (Trolox). The IC_{50} (the microgram of extract to scavenge 50% of the radicals). From the calibration, the concentration of extract that provides 50% inhibition (IC_{50}) was determined. The lower IC_{50} value, the more significant antioxidant activity achieved.

"ABTS [2,2'-Azinobis(3-Ethylbenzothiazoline-6-Sulphonic Acid)]" Assay for Free Radical Scavenging Activity:

The procedure for the "ABTS" test was performed according to the method described by **[30]** with some alterations 7.4 mmole "$ABTS^{\cdot+}$ reagent (stock 1) and 2.6 mmole $K_2S_2O_8$ reagent were used as stock solutions (stock 2). The reagent mixture was made by combining equal amounts of stock 1 and 2 reagents and letting them to interact at ambient temperature in the dark for 12 hours. by the aid the spectrophotometer, this solution was mixed by 1mL "$ABTS^{\cdot+}$" reagent with sixty mililitres of CH_3OH to produce an OD of 1.1±02 units at 734 nm. For each test, a new "$ABTS^{\cdot+}$" solution was produced. For two hours in the dark, all test extracts (1.50 mL) were kept to interact with 2.85 mL of the "$ABTS^{\cdot+}$" solution. Using a spectrophotometrically, the optical density (OD) was then measured at 734nm. The standard curve was constructed by plotting the OD versus µM Trolox (25 and 600). The outcomes are shown in µM Trolox Equivalents (TE)/g fresh mass. Extra low concentrations of "ABTS" was performed if the the values did not fall within the straight line of the standard curve.

Ferric Reducing Antioxidant Power "FRAP" Assay:

The "FRAP" assay was carried out according to procedure of [31]. 0.01M "TPTZ (2, 4, 6-tripyridyl-s-triazine)", 0.30M

"acetate buffer (3.1 g $C_2H_3NaO_2$ - $3H_2O$ and 16mL $C_2H_4O_2$)", pH 3.6 solution in 0.04M HCl, and 0.02M "$FeCl_3$-$6H_2O$" solution were among the stock solutions. After combining two and half mililitres "TPTZ reagent", twenty-five mililitres of acetate buffer and 2.5 mL "$FeCl_3$-$6H_2O$" reagent, the new working reagent was heated to 37 ^oC before use. For half an hour in the dark, all leaf extracts (1.50 mL) were prepared by reacting with 2.85 mililitres of the FRAP reagent. At 593 nm, measurements of the resultant colors "ferrous tripyridyltriazine complex" were obtained. The standard curve was constructed by using concentrations ranging from 25 and 0.80 mmole "Trolox". The attained outcomes were stated in mmole "Trolox Equivalents (TE/g)" fresh weight. Extra dilution was required on condition that the "FRAP reading" values were beyond the extent of the standard curve line.

Statistical analysis:

All experiments and analytical data were achieved in triplicate and stated as mean ± standard deviation. When the treatment was found to be significant, it was shown by a significant "F-test" ($p<0.05$). The differences among the individual means were determined using **[32]** and were to be significant when ($p<0.05$), for mean ± standard deviation. Correlation coefficient values for "ABTS" were calculated from dose response curve.

RESULTS AND DISCUSSION

Qualitative Analysis of Phytochemicals

Plant material contains various phytoconstituents from many chemical classes, including flavonoids, anthocyanins, phenols, saponins, terpenoids, tannins, emodins, glycosides, steroids, and fatty acids. This phytoconstituent individual has been used, as anticarcinogenic, antioxidant, antidiabetic, antimicrobial, antimutagenic, and anti-inflammatory [33].

Preliminary Phytochemical Screening of *Cymbopogon citratus*

The results of phytochemical constituents of *Cymbopogon citratus* extract **(**Table 1**)** exhibited the presence of terpenoids, steroids and phenols in hexane, 80% ethanol and ethyl acetate extracts in high amounts, respectively. Glycosides and flavonoids in 80% ethanol extract; steroids, saponins and phenols in hexane extract as well as terpenoids and tannins in ethyl acetate extract were found in moderate amounts, respectively, whereas tannins, anthocyanin and phenols in 80%ethanol extract; fatty acids and glycosides in hexane extract and saponins, glycosides, flavonoids and emodins in ethyl acetate extract were found in low amounts. Table (1) also showed that saponins, fatty acids and emodins in 80%ethanol extract; tannins, anthocyanins and flavonoids and emodins in hexane extract and anthocyanins and fatty acids in ethyl acetate extract were absent.

The phytochemical constituents were examined from both stem and leaves extracts obtained from *C. citrates*, The results indicated diverse constituents such as tannins, phenol, flavonoids and cardiac glycosides but the absence of alkaloid and saponins [34]. These chemicals have been discovered to have a spectrum of biological actions, which may aid in the prevention of a variety of human illnesses. Epidemiological studies reported that flavonoids have a cure for coronary heart disease, whereas saponins exhibit antibiotics properties and treat hypercholed- terolemia [35].

Table (1): Qualitative analysis of phytochemical Screening tests of *Cymbopogon citratus* 80%ethanol, hexane and ethyl acetate extracts**.**

Chemical Constituents	*Cymbopogon citratus*		
	80%Ethanol	**Hexane**	**Ethyl acetate**
Terpenoids	+	+++	++
Steroids	+++	++	+
Tannins	+	-	++
Saponins	-	++	+
Anthocyanins	+	-	-
Fatty Acids	-	+	-
Glycosides	++	+	+
Phenols	+	++	+++
Flavonoids	++	-	+
Emodins	-	-	+

(+++), (++), (+) and (-) indicate to high, moderate, low and absent concetration, respectively.

Phytochemical constituents of *L. inermis*

The results of phytochemical constituents of *L. inermis* extract **(**Table 2**)** exhibited the presence of steroids, tannins in 80%ethanol extract; terpenoids in hexane extract and phenols in ethyl acetate extract were found in high amounts. Terpenoids, anthocyanins and phenols in 80%ethanol extract; steroids and tannins in hexane extract and glycosides and flavonoids in ethyl acetate extract were found in moderate amounts, whereas glycosides, flavonoids and emodins in 80%ethanol extract; anthocyanins, phenols and emodins in hexane extract and terpenoids, steroids, tannins and emodins in ethyl acetate extract were found in low amounts.

Table (2) also revealed that saponins and fatty acids were absent in all extracts; glycosides and flavonoids in hexane extract as well as anthocyanins in ethyl acetate extract were absent.

These results disagree with [36]**,** who discovered saponins, alkaloids, glycosides, tannins, flavonoids, and resins in *L. inermis* by phytochemical screening.

Phytochemical screening of different extracts of leaves of *Lawsonia inermis* were studied using petroleum ether, ethyl acetate, ethanol and water, All extracts contain flavonoids "(Quercetin equivalent 16.2-85.6 g/Kg)", antocyanins "(Cyanidin equivalent 0.75-5.48 mg/Kg)", tannins "(Catechin equivalent 31.3-477.9 g/Kg)" and polyphenols "(Gallic acid equivalent 71.7-129.6 g/Kg)" [37]. The ethanolic extracts exhibited the strongest free radical scavenging activity, with an IC_{50} of 6.90.1 mg/L, while the petroleum ether extract had "antimalarial activity of 27 mg/L." Henna extracts containing "ethyl acetate extract 27 mg/L" and "petroleum extract 22 mg/L" were found to have anticancer activity against "human breast cancer cells (MCF7)"[38].

Table (2): Qualitative analysis of phytochemical constituents of Lawsonia inermis leaves 80% ethanol, hexane and ethyl acetate extracts.

Chemical Constituents	*Lawsonia inermis*		
	80%**Ethanol**	**Hexane**	**ethyl acetate**
Terpenoids	++	+++	+
Steroids	+++	++	+
Tannins	+++	++	+
Saponins	-	-	-
Anthocyanins	++	+	-
Fatty Acids	-	-	-
Glycosides	+	-	++
Phenols	++	+	+++
Flavonoids	+	-	++
Emodins	+	+	+

*(+++), (++), (+) and (-) refer to high, moderate, low and negligible concentration, respectively***.**

Phytochemical Constituents of *H. sabdariffa.*

The results of the phytochemical constituents of *H. sabdriffa* extract (Table 3) exhibited phenols and flavonoids in 80% ethanol extract; terpenoids in hexane extract and emodins in ethyl acetate extract in high amounts.

Terpenoids, anthocyanins in 80% ethanol extract; glycosides and emodins in hexane extract and steroids, tannins, phenols and flavonoids in ethyl acetate extract were found in moderate amounts, whereas steroids, tannins, glycosides and emodins in 80%ethanol extract; saponins, phenols and flavonoids in hexane extract and terpenoids, anthocyanins and glycosides in low amount. Data in (table 3) also showed that saponins and fatty acids in 80%ethanol extract; steroid, tannins, anthocyanins and fatty acids in hexane extract and saponins and fatty acids in ethyl acetate extract were absent.

The preliminary phytochemical analysis *in H. sabdariffa* were studied and stated that "many herbal bioactive constituents found in the extract such as glycosides, , saponines tannins, alkaloids, flavonoids and phenols and their quantities were 13%, 0.96% ,17.0%, 2.14%, 20.08%, and 1.1%, respectively[39].

Table (3): Phytochemical Screening tests of *H. sabdariffa* 80% ethanol, hexane and ethyl acetate extracts.

Chemical Constituents	*H. sabdariffa*		
	80%**Ethanol**	**Hexane**	**Ethyl acetate**
Terpenoids	++	+++	+
Steroids	+	-	++
Tannins	+	-	++
Saponines	-	+	-
Anthocyanins	++	-	+
Fatty Acids	-	-	-
Glycosides	+	++	+
Phenols	+++	+	++
Flavonoids	+++	+	++
Emodins	+	++	+++

(+++), (++), (+) and (-) *indicate to high, moderate, low and negligible values, respectively***.**

Quantitative analysis of some Phytochemicals

The result of the terpenoids, steroids and tannins of three plant leaves in different solvents is shown in Table (4). 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* 80% ethanol extract had the highest tannins content (4573.28±214.47), followed by hexane extract (4150.69±389.08) and ethyl acetate extract (3973.37±231.7) mg/g (Table 4).

Quantitative analysis results of phytochemicals of *H. sabdariffa* were approximately similar to the results attained by[39].

Quantitative analysis of Total Phenolic and Flavonoid Content.

Ethyl acetate extracted the highest TPC and TFC in *L. inermis* (141.84 ± 6.7 and 29.56 ± 1.63) mg /100g, respectively (Table 5). 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 TFC (37.23± 2.05mg/100g) when compared to other extracts.

Table (4): Quantitative Determination of Phytochemical extracts of *C. citratus, L. inermis and H. sabdariffa* leaves.

Plant	Extract	Terpenoids (mg/g).	Steroids (mg/g).	Tannins (mg/g).
Cymbopogon citratus	80%Ethanol	6.70±0.52	54.87±7.31	23.36± 4.16
	Ethyl acetate	7.48± 0.37	46.51± 7.69	24.25± 3.77
	Hexane	5.75± 0.77	24.35± 5.87	1625.43± 51.53
Lawsonia inermis	80%Ethanol	12.35± 1.27	310.25± 4.44	4573.28± 214.47
	Ethyl acetate	13.34± 5.78	280.76± 8.87	3973.37± 231.70
	Hexane	10.58± 2.77	73.84±8.10	4150.69±389.08
Hibiscus sabdariffa	80%Ethanol	13.40± 1.33	112.82±11.75	1037.93± 129.14
	Ethyl acetate	13.88± 1.33	12.82±1.60	18.96± 1.59
	Hexane	11.28± 1.45	546.15± 13.32	1220.02± 65.45

Values are mean ± SD (standard deviation).

Table (5): Total phenolic and flavonoid content of *C. citratus, L. inermis and H. sabdariffa* leaves extract.

Plant	Extract	Total phenolic content (mg/100g)	Total Flavonoid content (mg/100g)
Cymbopogon citratus	80%Ethanol	14.01 ± 0.38	37.23± 2.05
	Ethyl acetate	66.8 ± 3.6	6.19 ±1.17
	Hexane	29.74 ± 1.13	0.00±0.00
Lawsonia inermis	80%Ethanol	114.96 ± 2.58	23.45±0.90
	Ethyl acetate	141.84 ± 6.7	29.56±1.63
	Hexane	34.84 ± 2.25	0.00±0.00
Hibiscus sabdariffa	80%Ethanol	83.91 ± 6.28	34.09±2.11
	Ethyl acetate	58.76 ± 3.13	12.33±0.91
	Hexane	32.41 ± 2.4	8.87±0.63

Values are mean ± SD (standard deviation).

These outcomes goes in line with the previous finding of [40] who reported that TPC of *H. sabdariffa* was to be "41.07 mg Gallic acid/g". The TPC and TFC were determined by [**39]** who reported that phenols, flavonoids, were recorded to be 1.1%, 20.08%, , respectively.

The results of phytochemicals of *H. sabdariffa* confirmed the presence of saponins, flavonoids, tannins, and alkaloids in the plant's stem, root, and leaves. The quantitative study of stem, root and leaves exhibited the best findings in which phenolics were more abundant in plant leaves. However, alkaloids were present in all parts of the plant[41].

DPPH free radical scavenging activity 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 (Table 6) showed for 80% ethanolic extracts IC_{50} of 68.78 ± 2.3 µg/mL in "DPPH radical" scavenging assay to be significantly higher than that of ethyl acetate and hexane extract (296.9± 10.8 and above 1000 mg/g, respectively**).**

Table (6): Antioxidant activity based on "DPPH radical" scavenging activity of extracts of *C. citratus, L. inermis* and *H. sabdariffa* leaves.

Plant	Extract	IC_{50} (µg/mL)
Cymbopogon citratus	80%Ethanol	68.78 ± 2.3
	Ethyl acetate	296.9 ± 10.8
	Hexane	above 1000
Lawsonia inermis	80%Ethanol	69.49 ± 3.1
	Ethyl acetate	92.96 ± 4.1
	Hexane	823.8 ± 30.2
Hibiscus sabdariffa	80%Ethanol	110.4 ± 7.35
	Ethyl acetate	308.0 ± 15.45
	Hexane	486.7 ± 21.4

Values are mean ± SD (standard deviation).

On the other , IC_{50} value of 80% ethanolic extracts 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 hexane leaf extract of *L. inermis* and *H. sabdariffa* extract had higher IC_{50} values (above 1000 and 823.8± 30.2 mg/ml), respectively, which indicated insufficient scavenging activity (Table 6).

The result of antioxidant activity of *C.citratus* was 26.03±1.60 µM Trolox/100 g dry weight in DPPH radical scavenging assay[42].

The antioxidant activity was determined using DPPH radical scavenging activity of extracts of *Hibiscus sabdariffa* leaves. They revealed that *Hibiscus sabdariffa* could be a potential source of antioxidant activity[40].

The ethanolic extracts of Thirteen Malaysian plants, among them *L. inermis* were better activity in free radical scavenging and inhibition of Lipid peroxidation assays than their corresponding aqueous extracts. They also found appositive correlation between ethanol extract, Phenolic concentration, and antioxidant activity[43].

Free radical scavenging: "**ABTS 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 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.

Table 7: Antioxidant activity "ABTS assay" extracts of *C. citratus, L. inermis and H. sabdariffa* leaves.

Plant	Extract	ABTS (µM TE/mg)
Cymbopogon citratus	80%Ethanol	719.93±79.09
	Ethyl acetate	266.65±14.7
	Hexane	30.13±3.8
Lawsonia inermis	80%Ethanol	719.33±4.5
	Ethyl acetate	799.63±11.5
	Hexane	69.5±6.3
Hibiscus sabdariffa	80%Ethanol	719.93±79.09
	Ethyl acetate	209.57±13.11
	Hexane	158.69±13.3

Values are mean ± SD (standard deviation).

The antioxidant activity of *L. inermis* was determined using DPPH activity . The data showed that "$CH_3COOC_2H_5$ extract had an IC_{50} of 8.6± 0.2 mg/L and 29.5± 0.8 mg/L in "ABTS" and "DPPH" assays, respectively. However, the IC_{50} of the exthanolic extract was 14.1± 0.5 mg/L and 14.1± 0.5 mg/L in "ABTS" and "DPPH" assays, respectively. Petroleum ether extract exhibited lower antioxidant of (738.7 ± 9.6 and 161.6 ± 2.3mg/L) in "ABTS" and "DPPH", respectively. Ethanol extract was the best extract and appears to be more potent in comparison to other extracts in both "ABTS" and in "DPPH" assays[38].

Estimation of "Ferric Reducing Antioxidant Power (FRAP)":

The ferric reducing antioxidant powers of all extracts of *L.inermis, H. sabdariffa* and *citrates* are shown in Table (8), 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 *citrates* (729.26 ± 52.02, 171.31 ± 17.28 and 135.44 ± 12.50, respectively, while hexane extract had the lowest reducing ability than the rest of the extracts under this study.

Table (8): Total Antioxidant activity; FRAP Assay extracts of *Cymbopogon citratus, Lawsonia inermis and Hibiscus sabdariffa* leaves.

Plant	Extract	FRAP Assay (µM TE /mg)
Cymbopogon citratus	80%Ethanol	630.22 ± 25.7
	Ethyl acetate	135.44 ± 12.5
	Hexane	18.91 ± 2.6
Lawsonia inermis	80%Ethanol	746.69 ± 54.49
	Ethyl acetate	729.26 ± 52.02
	Hexane	52.01 ±4.8
Hibiscus sabdariffa	80%Ethanol	546.70 ± 20.3
	Ethyl acetate	171.31±17.28
	Hexane	124.08±8.93

Values are mean ± SD (standard deviation).

The robust association was found among the concentration of anthocyanins and antioxidant activity based on "DPPH", "ABTS" and "FRAPS assays" of different *H. sabdariffa* extracts. The results also presented that *H. sabdariffa* extracts have possible use as a treasured source of anthocyanins with strong antioxidant ability[44].

Conclusion

The finding of the present work revealed that *C. citratus, L. inermis* and *H. sabdariffa* leaf extract showed significant antioxidative potential *in vitro*. Further study is necessary to evaluate the therapeutic properties of these plants under study.

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