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Biochemical Studies on Bio Extracts as Antioxidant and Antibacterial Activity

Farid, M[1].; Hanaa, F. M. Ali[2]; Gehan, F. A. Massoud[3] and Sherein, S. Abdelgayed[4]

1-Researcher of Biochemistry and Medicinal Plants-Egypt

2- Professor of Agri. Biochemistry, Faculty of Agriculture, Cairo University-Egypt.

3- Researcher of Medicinal and Aromatic Plants, Horticulture institute, Agric. Res. Center-Egypt

4- Professor of Pathology, Faculty of Vet. Med. Cairo University-Egypt

ABSTRACT:

Biochemical Evaluated of some bio extracts as follows: Chemical composition and Phytochemical screening were determined in *Tilia Cordata* and *Vitex Agnus* leaves. The total polyphenols and flavonoids contents of *Tilia Cordata* leaves have the highest of total polyphenols and flavonoids contents, which were 126.00mgGAE/g and 15.88mgQE/g, followed by *Vitex Agnus* leaves, which were 119.77mgGAE/g and 13.41mgQE/g, respectively. Methanolic and aqueous extracts of plants leaves were antioxidant activity by used (FRAP, LPO, DPPH and ABTS) The methanolic extract of *Tilia Cordata* and *Vitex Agnus* leaves, have the highest of reducing power which were 1.649 and 1.018 at the concentrations of 80mg/ml, respectively. Also, by used (LPO, DPPH and ABTS), were the highest antioxidants activity for methanolic extract respectively. Moreover, the methanolic extract of *Tilia Cordata* leaves produced the highest growth inhibition (18 and 17mm) for against *Escherichia coli* and *Bacillus subtillis* at 4mg/ml, respectively. While, The methanolic extract of Vitex Agnus produced the medium percentage of growth inhibition (10.5, 9.75 and 10mm) for against *Escherichia coli, St.coccus aureus* and *Bacillus subtillis* at 4mg/ml, respectively. And compared with antibiotic. This study concluded that *Tilia Cordata* and *Vitex Agnus* leaves extract has an antioxidant and antibacterial activity.

Key words: Chemical composition, Phytochemical, Antioxidant and Antibacterial activity.

1. Introduction

Tilia cordata (family: Tiliaceae) have been used in folk medicine as anxiolytic. Phytochemical studies have demonstrated that, *Tilia* species contained hydrocarbons, esters, aliphatic acids, terpenoids, quercetin and kaempferol derivatives, phenolic compounds, condensed tannins and a coumarin scopoletin. *Tilia Americana* has several flavonoids such as rutin, hyperoside, quercitrin and tiliroside [1].

Natural antioxidants from plants such as flavonoids and other polyphenols are known to play an important role in the protection of cells from oxidative damage. Consequently, the antioxidant activity is connected to anticancer activities including pro apoptotic, DNA damaging and antiangiogenic effects. Among polyphenols, flavonoids are the principal secondary metabolites that characterize the genus *Tilia*. This plant is an hybrids between *Tilia tomentosa* (native to Europe and western Asia) and *Tilia americana.* They, an ethanol extract from the plant leaves presented a selective antiproliferative action on a lymphoma cell line named BW 5147 in relation to free radical scavenging activity, in addition, one of its main compounds, the flavonoid rutin, showed antioxidant peroxidase activity related to the inhibition of normal cells proliferation by hydrogen peroxide modulation. Additionally, hydrogen peroxide plays an important role in the inflection of cell proliferation, stimulating cell proliferation but also leading cells to apoptosis. They, it was shown that, BW 5147 cells possess low level of hydrogen peroxide and high level of superoxide anion in comparison with normal lymphocytes in response to a low level of superoxide dismutase activity [2].

The Genus *Vitex* belongs to the Verbenaceae family, which grows in tropical and subtropical regions. This genus is an important natural source of food and medicine around the world and in Iran as well. There are approximately 270 known species of the genus *Vitex* distributed in many parts of the world. *Vitex agnus-castus* is a well-known medicinal plant widely distributed in the Middle East and Europe. Traditionally, it has been used for treatment of several female disorders such as endometriosis, abnormal menstrual cycles, menopausal conditions, insufficient lactation and acne. Recent studies revealed that, the plant has wide pharmacological properties such as antibacterial, antihistaminic, anti-inflammatory and antioxidant activities **[**3].

Free radical damage is one of the most prominent reasons of devastating diseases that are accountable for killing millions of people in the world and this can manifest as heart attacks and cancers. Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes such as adaptation of food into energy in the body. Antioxidants are powerful free radical scavengers in the human body. Several researches on antioxidants in biological systems have confirmed their neutralizing effects on oxidative stress that predispose the human body to lethal diseases and thus, making keen interest in assessment of antioxidant potentials of consumable food compounds antioxidants comprise a number of chemical compounds [4].

Medicinal plants having various phytochemicals and bioactive components such as trace metal ions, vitamins, alkaloids, carotenoids, polyphenols, fats, carbohydrates, and proteins are involved in enhancing long-term health benefits. Antioxidant activity of plants have been observed, using FRAP, ABTS and DPPH. They quench, scavenge and suppress the formation of reactive oxygen species (ROS) and oppose their actions [5].

Tilia cordata

Vitex agnus

2. Materials and Methods

PLANT MATERIALS

Leaves samples of *Tilia cordata* and *Vitex agnus* were kindly obtained from Agricultural Research Center, Giza, Egypt. Samples from two species were air dried in the shade and ground into a fine powder.

The powdered air dried leaves which were divided into dry part and two extracts. First extract: Powdered air dried leaves (1Kg) from each leaves samples were extracted by soaking at room temperature for six hours with methanol (10L), then the methanolic extracts were concentrated to nearly dryness under reduced pressure using the rotary evaporator at 45°C to achieve the crude methanol extract which kept for further investigation [6].

The yields of extracts were 21.8% and 18.7%, of Tilia cordata and Vitex aqgnus leaves, respectively. Second extract: Powdered air dried leaves (1Kg) of dried samples were extracted with distilled water by boiling at temperature from 80 to 100°C in reflux for 3h to achieve an initial extract. The extract was filtered after cooling to room temperature. Finally, the extract was lyophilized and preserved at −20°C until further use [7]. The yield of aqueous extract was11.39 and 9.2%, of Tilia cordata and Vitex agnus leaves, respectively. All tests were conducted in were obtained from Science Academy of Experimental Researches, Mansoura, Egypt.

Chemical composition of investigated leaves:

Determination of moisture content: Moisture content was determined according to the method described by [8]. A known weight of air dried leaves (2g) was dried at 105°C in an air drying oven to a constant weight. Percentage of moisture content was calculated.

Determination of ash content: Ash content was determined according to [8], as follow: Exactly 2g of air dried leaves were placed in a silica crucible and ignited at 600ºC in a muffle furnace till a constant weight, the percentage of ash content was calculated.

Determination of crude fiber content: Crude fiber is a mixed material and defined as the sum of lignin and polysaccharide contents which not digested by dilute acid and alkali. Crude fiber was estimated according to the method described by [8], A known weight of the air dried leaves (2g) was mixed with 0.5g asbestos, then 200ml of sulphuric acid (1.25%v/v H_2SO_4), were added. The mixture was boiled under reflex for 30 minutes, followed by filtration through Gooch crucible. The residue was boiled again with aqueous sodium hydroxide solution (200ml, 1.25%w/v NaOH) for 30 minutes, then filtration was repeated in the same manner. Finally the residue was washed with hot water followed by diethyl ether and dried at 110ºC to a constant weight. The content of Gooch crucible was then ignited in the muffle furnace at 600ºC to a constant weight. Fiber content was calculated by subtraction of ash content from the weight of digested sample. Percentage of crude fiber content was then calculated.

Determination of crude protein content: Crude protein content were determined by the official Kjeldahl method described in [8], as follow: A known weight of air dried leaves (0.5g) was digested with 8ml of concentrated sulphuric acid in Kjeldahl flask in the presence of (2.14g) digestion mixture [1kg potassium sulphate and 60g of mercuric oxide (red)]. After digestion, the solution was treated with 10ml of 40% sodium hydroxide solution. The liberated NH3 was received into 10ml of 1% boric acid in the presence of 2 drops of Tachero indicator (1.25g methyl red+0.32g methylene blue in one litre of 90% ethanol). The received ammonia was titrated with 0.01N sulphuric acid. The percentage of total nitrogen was estimated and the crude protein content was calculated by using 6.25 as a factor of protein [9].
Determination of crude lipid: Crude lipid of air dried leaves were determined according to [8], A known weight of air dried leaves (2g) was extracted in Soxhlet apparatus using n-hexane as a solvent for 24 hours. Then the solvent was removed and the percentage of crude lipid was calculated.

Determination of soluble carbohydrate content: The soluble carbohydrate contents were determined with a slightly modified phenol–sulphuric acid method according to [10]. The colour reaction was initiated by mixing 50µml of crude polysaccharide solution with 150µml of concentrated sulphuric acid, followed immediately with 30µml of 5% phenol, and the reaction mixture was kept at 90°C for 5 min. After cooling to room temperature, the absorbance of the mixture was measured at 490 nm, using a

Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The total carbohydrate content was calculated with D-glucose as a standard material.

Determination of reducing sugar: The reducing sugar was determined by the modified method of [11]. Briefly, 0.5 ml of 1% 3, 5-dinitrosalicylic acid (DNS) was added to an aliquot of sample (20–500 µml), and the volume adjusted to 5 ml with distilled water. After shaking, the mixture was heated in boiling water for 5 min and cooled to room temperature; 2.5 ml of distilled water were added to the mixture. The absorbance was measured at 540 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The total reducing sugar was calculated with D-glucose as a standard reducing sugar.

Calculation of non-reducing sugars: Insoluble sugars were calculated according to the following equation:

1- Non-reducing % = Total sugars % - Reducing sugars %

Preliminary phytochemical tests of crude methanolic and aqueous extracts of the investigated leaves:

Preliminary phytochemical tests were carried out on the crude methanolic and aqueous extracts by boiling for 3 hours, extract to detect the presence of: terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrates and/or glycosides, phenolic glycosides and resins.

Detection of terpenes, were detected according to the method adopted by [12]. A small amount of crude aqueous plant extract was dissolved in chloroform, then few drops of concentrated sulfuric acid were added carefully on the wall of test tube to form two separated layers, the resulted yellow ring changed to orange then red indicating the presence of terpenes.

Detection of tannins, were detected by the method described by [12]. Few milliliters of distilled water were added to few milliliters of aqueous extract and filtrate, then, ferric chloride solution (5%) was added to the filtrate. The presence of tannins yellowish green color was obtained.

Detection of flavonoids, were detected according to [12]. A small amount of crude plant extract was macerated in hydrochloric acid (1%) overnight, then sodium hydroxide solution (10%) was added to the filtrate, the appearance of yellow color indicates the presence of flavonoids.

Detection of saponins, were detected according to [12]. The aqueous crude plant extract was vigorously shaken developing a voluminous froth which persisted for almost one hour indicate the presence of saponins.

Detection of carbohydrate and/or glycosides, in crude plant extract were detected using Molish's reagent according to [12]. Some drops of α-naphthol in ethyl alcohol were added to 1ml of crude boiling water extract, then 1ml of concentrated sulfuric acid was added carefully without shaking, a purple ring was appeared indicating the presence of carbohydrates and/or glycosides in crude plant extract.

Detection of alkaloids, were detected according to [12]. by adding 2ml of diluted hydrochloric acid to 1ml of plant extract. Then five drops of Wagner's reagent were added to 1ml of the solution and shaking after addition of each drop. After leaving for sometimes, the formed precipitate indicating the presence of alkaloids.

Detection of phenolic glycosides, were detected according to [12]. by the following technique: some drops of concentrated sulfuric acid were added to 1ml of plant extract, a red color was produced which disappear when water was added.

Detection of resins, were detected according to the methods described by [12]. The crude boiling water extract was boiled on water bath for 20 minutes and distilled water was added to extract, a white precipitate was formed in presence of resins.

Total phenolic contents:

Total phenolic contents of air dried leaves were determined by using Folin–Ciocalteu reagent method according to [13]. About 0.1g of air dried leaves was dissolved, separately in 1 ml distilled water. Aliquots of 0.1 ml from The solution was taken and mixed with exactly 2.8 ml of distilled water, 2.0 ml of (2% w/v) sodium carbonate and finally 0.1 ml of 50% (v/v) of Folin–Ciocalteu reagent was added. Mixture was incubated for 30 minutes at room temperature and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. For quantitatively determination a standard curve of Gallic acid (0-200mg/l) was prepared in the same manner. Total phenolic contents were expressed as milligram gallic acid equivalent (GAE)/g based on dry weight.

Total flavonoid contents:

Total flavonoid contents of air dried leaves were determined calorimetrically using aluminum chloride as described by [14]. About of 0.1g of air dried leaves were dissolved in 1ml of distilled water. Resulting solution (0.5 ml) was mixed with 1.5 ml of 95% ethyl alcohol, 0.1 ml of 10% aluminum chloride ($AlCl_3$), 0.1ml of 1M potassium acetate (CH_3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. Quercetin was chosen as a standard of flavonoids for making the standard curve (0–50mg/l). The concentrations of total flavonoids contents were expressed as milligram quercetin equivalent (QE)/g based on dry weight.

Determination of reducing power, (FRAP) radical scavenging activity:

Reducing power of methanolic leaves extracts was determined according to the method of [15]. Extract (0–100mg) from each sample in 0.20mol phosphate buffer, pH 6.6 (2.5ml) was added to 2.5ml potassium ferricyanide (10mg/ml), mixture was incubated at 50^{0}C for 20min. Trichloroacetic acid (TCA) (2.5ml, 100mg/ml), was added to the mixture then centrifuged at 650g for 10 minutes. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml ferric chloride solution (1mg/ml) was added and the absorbance of the resultant color was measured using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer at 700nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of lipid peroxidation (LPO) measurement of MDA-TBARS:

MDA analysis of plant extract was made conferring to the method [16]. For this purpose: 0.05 M TRIS-HCl pH 7.4/0.15 M KCl and 0.2% Tween 20 with a buffer solution containing 1 mM hydrogen peroxide FR and 3 ml were prepared daily. LPO for the measurement, 1 ml samples on after receipt of 0, 6% TBA solution and 2 ml distilled water was added and vortexes. Then, 90°C for 30 minutes and the reaction was allowed resulting pink color was extracted with 3 ml of n-butanol. Samples were centrifuged and the supernatant fraction obtained after centrifugation of the color density was measured in a Spekol 11 (Carl Zeiss-Jena) spectrophotometer at 532 nm.

Determination of (DPPH) radical scavenging activity:

The DPPH free radical scavenging activity of the plant leaves extracts at different concentrations were measured from bleaching of the purple colour of (2.2 Diphenyl -1-picryl hydrazyl) was based on the method of [17]. Exactly 0.1 ml solution of different concentration of the extract was added to 1.4 ml of

DPPH and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The percentage inhibition was calculated by using the following equation:

$$\text{Percentage inhibition (\%)} = \frac{(\text{A Blank} - \text{A Test})}{(\text{A Blank}) \times 100}$$

Were A= Absorbance

Determination of ABTS radical scavenging activity:

ABTS (2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of [18]. with slight modifications. 2ml of ABTS solution (1mg/1ml 0.1M phosphate buffer, pH 7.0) were added to 3ml of MnO_2 (25mg/ml in The phosphate buffer). Mixture was shaken and centrifuged for 10 minutes, clear supernatant was separated. Exactly 1mg of crude methanolic leaves was dissolved in a mixture solvent (1ml) of methanol and The phosphate buffer in the ratio of 1:1 (v\v). Resultant extract solution (20µl) was added to the ABTS solution mixture, as Thely described. Positive control sample was prepared exactly in the same manner but differ only in the addition of 20µl of 2mM ascorbic acid, instead of extract solution. Blank sample was prepared exactly in the same manner but differ only in the addition of 20µl of distilled water, instead of extract solution. Absorbance of the resulting greenish-blue solution was recorded at wave length 734nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The decrease in absorbance is expressed as a percentage of inhibition which was calculated from the following equation:

$$\text{\% Inhibition} = \frac{\text{A Blank} - \text{A Test}}{\text{A Blank}} \times 100$$

Crude methanolic and aqueous extracts of investigated leaves as antibacterial activity:

To study the effect of investigated leaves extracts, three cultures of bacteria namely: *Escherichia coli, Staphylococcus aureus* and *Bacillus subtillis* were used according to [19]. Briefly, nutrient agar media and 30 ml were dispensed in each Petri plate. Three wells of diameter 0.7 centimeter cut in each plate with the help of corn borer and then sealed with nutrient agar. In each prepared plate, extracts of the same concentration were poured in all wells using micro-pipette and three concentrations were made of each plant extract e.g. concentration of methanolic and aqueous extracts of Tilia cordata and *Vitex agnus* were (1, 2 and 4µg/ml), in separate nutrient agar plates. Then, plates were incubated at 37°C for 24 hrs. The diameter of inhibition zones were calculated as percentages. The mean diameter of the zone of inhibition during the 3 concentration was recorded as the final diameter (antibacterial power of the plant extract

compared with tested antibiotics (Penicillin) according to the standard table of the antibiogram test of the mentioned company. [20].

Statistical analysis: Statistical analyses of all experimental data were done using the statistical software package [21]. All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan's multiple range test at $P<0.05$ as the level of the significance [22].

3. Results and Discussion

Chemical composition of investigated leaves:

As showed in table (1) the percentages of moisture content for air dried investigated leaves were 10.88 and 9.14% for *Tilia cordata* and *Vitex agnus,* respectively. These results were in accordance with those obtained by [23]. who established that, (*Tilia cordata* Mill.) seed samples were drying and frozen in liquid nitrogen for 24 h at 11 different levels of seed moisture content (m.c.), ranging from 3.1% to 22.8% (fresh weight basis). While, the results of *Vitex agnus,* were higher than those reported by [24], who stated that, the effects of five different drying conditions on the physical, chemical and antioxidant properties of *Vitex agnus*, were evaluated. The results showed that, 30% of dried leaves with moisture content of less than 7% could be produced from fresh leaves.

Furthermore, ash, crude fiber, crude lipids, crude protein, total sugars, reducing sugar and non-reducing sugars were determined in Tilia cordata and Vitex agnus leaves. All results were calculated as (g/100g dry weight) and recorded in table (1).

Data presented in table (1), showed that, Tilia cordata leaves have the highest percentage values for moisture, protein, total sugars and non-reducing sugars, which were 10.78, 15.93, 36.85 and 22.80%, respectively. While, the average percentage value of crude fiber and lipid which were 28.89 and 2.98% for *Tilia cordata* leaves.

Data in table (1) showed that, *Vitex agnus* leaves have the highest percentage values for ash, crude fiber, lipid and reducing sugars which were 4.77, 33.79, 4.17 and 18.03%, respectively. While, the medium percentage value of protein which was 12.55% for *Tilia cordata* leaves.

Table (1): Chemical composition of investigated leaves

Plant leaves extract	Moisture %	Ash %	Crude fiber %	Protein %	Lipid %	Total sugars %	Reducing sugars %	Non reducing sugars %
Tilia cordata	10.78	4.65	28.89	15.93	2.98	36.85	14.05	22.80
Vitex agnus	9.74	4.77	33.79	12.55	4.17	35.08	18.03	17.05

The chemical composition for *Tilia cordata*, ranged from 2 to 9%, of lipid and protein content was 7%, respectively [25]. The chemical composition of *Vitex doniana*, were, 16.66, 11.50, 8.24, 0.58, 34.62 and 28.40% dw, of moisture, ash, crude protein, crude fiber, crude fat and carbohydrate available, respectively [26].

Preliminary phytochemical screening of crude aqueous and methanolic leaves extracts:

Table (2) represented the phytochemical constituents of crude methanolic and aqueous extracts of Tilia cordata and Vitex agnus leaves. The crude methanolic and aqueous extracts of investigated leaves were rich in terpenes, tannins, flavonoids, alkaloids, carbohydrate and /or glycosides and phenolic glycosides within the acceptable confines. But, methanolic and aqueous extract were poor in resins of Tilia cordata and Vitex agnus leaves. The methanolic extract was higher in focus than aqueous extract, where it is more polarity according to [27].

Table (2): Phytochemical tests (Qualitative)

Plant leaves	Extracts	Terpenes	Tannins	Flavonoids	Saponin	Glycosides	Alkaloids	ph. glycoside	Resins
Tilia cordata	Methanolic	++	++	++	++	++	-	++	+
	Aqueous	++	++	++	+	++	-	+	+
Vitex agnus	Methanolic	+	++	++	+	++	-	+	+
	Aqueous	+	++	++	+	++	-	+	+

- = negative results + = positive results ++ = Strongly positive results

Conditions are classified depending on the concentration of the active ingredient in the solution by Spectrophotometer

Our data about *Tilia cordata* leaves were in agreement with those reported by [28], they found that, the methanolic extract of the *Tilia* flower, flavonoid glycosides are present, while quercetin is barely or not

detectable. [29], established that *Tilia* as a source of bioactive flavonoid glycosides and highlight it as an appropriate option for further pharmacognostical studies. [30], reported that, *T. cordata* extract showed the presence of coumarins, triterpenes, flavonoids, tannins, saponins, and carbohydrates.

The results of aqueous and methanolic extracts of *Vitex agnus* leaves were agreed with those suggested by [3], who found that, phytochemical analysis of the of *Vitex agnus-castus* mainly 1, 8-cineole, (E)- β-farnesene, sabinene, α-pinene, α-terpinyl acetate, β-caryophyllene and bicyclogermacrene. Phytochemical screening showed presence of alkaloids, iridoid glycosides, tannins, carbohydrates and flavonoids in methanol and water extracts of *V. agnus-castus* [31]. Phytochemical screening of *Vitex agnus* leaves extract revealed the presence of terpenoids, steroids, flavonoids and carbohydrates [32]. The *V. doniana* contained alkaloid, tannins, saponins, carbohydrates and proteins at varying levels [33].

Total polyphenols and total flavonoids content of investigated leaves:

Total polyphenols include several classes of phenolic compounds that are secondary plant metabolites and primary part of human and animal diets. Flavonoids are large group of the phenolic compounds consisting mainly of flavonols, flavanols and anthocyanins. Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damaging by lipid peroxides and scavenging or neutralizing free radicals [34].

It has been reported that, free radical scavenging and antioxidant activity of several medicinal plants are accountable for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular diseases [35], Also, it was found that, high total phenols content increase the antioxidant activity and there is a linear association between phenolic content and antioxidant activity in fig leaves extract [36].

Table (3) showed the total polyphenols (mgGAE/g) and total flavonoids (mgQE/g) contents of *Tilia cordata* and *Vitex agnus* leaves. Data in table (3) illustrated that, *Tilia cordata* and *Vitex agnus* leaves contained average values of total flavonoids which were 15.88 and 13.41mgQE/g, respectively. Moreover, *Tilia cordata* and *Vitex agnus* leaves have the highest concentration of total polyphenols, which were126.00 and 119.77 mgGAE/g, consecutively.

Table (3): Total polyphenols and total flavonoids content

Plant leaves	Total polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)
Tilia cordata	126.00	15.88
Vitex agnus	119.77	13.41

The total phenolic and total flavonoids content of alcoholic extract which were 25.98 and 5.22 mg/g dry plant. While, the total phenolic and total flavonoids content were 7.23 and 2.07 mg/g dry plant in aqueous extract of Tilia flos [37].

The findings were lower than the results of *Tilia cordata* leaves for total polyphenols contained was 126.00mgGAE/g, while were highest for total flavonoids contained, was 15.88mgQE/g, recorded by [38], who found that, the total polyphenols and total flavonoids contents in Tilia cordata leaves which were 28.74mgGAE/100g and 22.01mgCE/100g, respectively.

The results were in the same line with those reported by [39], who stated that, total phenolic contents of Vitex agnus leaves was123.9mgGAE/g. While the total phenolic contents of Vitex agnus fruits, was 114.59mgGAE/g.
The highest total polyphenols content were found in inflorescences and leaves of each plant (up to 6.65%). The highest total flavonoid content in V. agni-casti inflorescences was (7.59%)[40].

Reducing power of leave extracts:

Efficiency of methanolic and aqueous leave extracts to reduce Fe+++ to Fe++ was determined according to the method described by [15], Optical density of reaction mixture was measured at wave length 700nm using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The obtained data are presented in table (4), the absorbance showed the reducing power for different concentrations of crude methanolic and aqueous extracts of Tilia cordata and Vitex agnus leaves. Data expressed as absorbance at 700nm for producing color as a result for using three concentrations (20, 40 and 80 mg/ml) for each sample. From table (4), some points could be inferred: The reducing power capacity increased with increasing the methanolic and aqueous extracts concentrations for all samples.

Tilia cordata leaves have the highest percentage of reducing power which was ranged from 0.972 to 1.649 for methanolic extract at the concentrations of 20 and 80mg/ml, respectively. While, they have the reducing power of aqueous extract which was reached from 0.641 to 1.114 at the concentrations of 20 and 80 mg/ml, consecutively.

Vitex agnus leaves have the average percentage of reducing power which was ranged from 0.729 to 1.018 for methanolic extract at concentrations of 20 and 80mg/ml, respectively. While, they have the reducing

power of aqueous extract which were ranged from 0.513 to 0.814 at the concentrations of 20 and 80 mg/ml, consecutively.

Tilia cordata leaves have the highest percentage of reducing power of methanolic and aqueous extracts which were 1.290 and 0.855 at concentrations of 40mg/ml, respectively. While, Vitex agnus leaves have the average percentage of reducing power of methanolic and aqueous extracts which were 0.901 and 0.687 at concentrations of 40mg/ml, respectively.

High levels of reducing power specified the presence of some compounds which could be considered electron donors and could react with free radicals to convert them into more stable products [41]. Different studies indicated that, the electron donation capacity which reflecting the reducing power of bioactive compounds was connected with high antioxidant activity [42].

Table (4): Reducing power of crude methanolic and aqueous leaves extracts

Extract	Concentration	Optical density at 700nm	
	mg/ml	*Tilia cordata*	*Vitex agnus*
Methanolic	20	0.972	0.729
	40	1.290	0.901
	80	1.649	1.018
Aqueous	20	0.641	0.513
	40	0.855	0.687
	80	1.114	0.814

The results were in the same trend with those reported by [43], who found that, reducing power of essential oil, hexane, dichloromethane, ethyl acetate, methanol and water extracts of *V. agnus castus* at a concentrations (1.0mg/ml^{-1}), were 0.432, 0.185, 0.453, 0.287,0.524 and 0.751 absorbance at 700nm, consecutively.

The ethanolic extract of *Tilia cordata* inflorescences were analyzed using FRAP post-column assay possessed strong antioxidant activity which was 10.89 mg/g (dry weight) [44]. The present data agreed with those obtained by [24], who indicated that, FRAP of vacuum- and freeze-dried *Vitex* leaves were significantly higher than those of microwave-, oven- and low-temperature dried leaves, which was 0.989 absorbance at 593nm .

Lipid peroxidation (LPO) of leave extracts:

Lipid peroxidation (LPO) measurements by as standard Fenton Quercetin, Resvesterol reagents MDA-TBARS by the method of [16]. Optical density of reaction mixture was measured at wave length 532nm using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The obtained data are presented in table (5), the absorbance showed the lipid peroxidation for different concentrations of crude methanolic and aqueous extracts of *Tilia cordata* and *Vitex agnus* leaves. Data expressed as absorbance at 532nm for producing color as a result for using three concentrations (20, 40 and 80 mg/ml) for each sample. From table (5), some points could be inferred: The lipid peroxidation (LPO) capacity increased with increasing the methanolic and aqueous extracts concentrations for all samples.

Tilia cordata leaves have the highest lipid peroxidation which was reached from 1.210 to 0.589 for methanolic extract at the concentrations of 20 and 80 mg/ml, respectively. While, have the lipid peroxidation of aqueous extract was ranged from 1.391 to 0.712 at concentrations of 20 and 80 mg/ml, consecutively.

Vitex agnus leaves have the medium lipid peroxidation which was reached from 1.529 to 0.831 for methanolic extract at concentrations of 20 and 80 mg/ml, respectively. While, have the lipid peroxidation of aqueous extract was ranged from 1.613 to 0.988 at concentrations of 20 and 80 mg/ml, respectively.

Tilia cordata leaves have the highest percentage of lipid peroxidation for methanolic and aqueous extracts which were 0.990 and 1.111 at concentrations of 40mg/ml, respectively. While, *Vitex agnus* leaves have the medium percentage of lipid peroxidation for methanolic and aqueous extracts which were 1.055 and 1.217 at concentrations of 40mg/ml, consecutively.

ROS-mediated oxidation of membrane lipids results in the formation of lipid peroxidation of membrane (LPO) product as MDA (malondialdehyde) is generally considered to be degradation of polyunsaturated lipids. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells [45].

Table (5): Lipid peroxidation of crude methanolic and aqueous leaves extracts

Extract	Concentration	LPO at 532nm	
	mg/ml	*Tilia cordata*	*Vitex agnus*
Methanolic	20	1.210	1.529
	40	0.990	1.111
	80	0.589	0.831
Aqueous	20	1.391	1.613
	40	1.055	1.217
	80	0.712	0.988

In general, *T. platyphyllos* evidenced a higher antioxidant potential than *E. giganteum*, both as free radical scavenger and also as lipid peroxidation inhibitor. Not least interesting to highlight that this biological activity seems to be directly correlated with the relative abundance in phenolic compounds: for the plant extract with advanced antioxidant effects, i.e. *T. platyphyllos,* a high concentration of phenolic compounds [46].

The data of *Tilia cordata* leaves for lipid peroxidation which agreed with those obtained by [47], who found that *Tilia cordata* containing the higher polyphenol concentration had the lowest lipid peroxidation IC_{50} values, 15.8 µL/mg of microsomal protein.

Antioxidant activity of essential oil, hexane, dichloromethane, ethyl acetate, methanol and water extracts of *V. agnuscastus* at some concentrations (1.0 mg/ml^{-1}), were 86.17, 33.75, 64.51, 52.35, 53.14 and 94.07%, respectively [43]. Also, the main flavonoid constituents of the *V. agnus* extract contained, casticin, vitexin and orientin were assayed for antioxidant activity and showed that, only casticin possesses a marked lipid peroxidation inhibitory effect (IC_{50}=0.049mM) paralleled with that of the positive control ascorbic acid (IC_{50}=0.703mM) [48].

Determination of antioxidant activity using the 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

The antioxidant activity of both methanolic and aqueous extracts ready from the two studied plant species are reported in table (6). The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely adapted to measure the antioxidant activity [49]. The lower EC_{50} pointed to the higher antioxidant activity.

The antioxidant activity of the confirmed extracts was measured using DPPH radical scavenging activity. The antioxidants scavenging activities of DPPH are attributed to their hydrogen-donating abilities [50]. Vitamin C was used as the reference compound.

From table (6), it is clear that, the scavenging effect (IC_{50}) of methanolic extracts for *Tilia cordata* leaves have the most effective of inhibition percentage (0.24), followed by aqueous extract which was (0.28), respectively. While, the scavenging effect (IC_{50}) of methanolic extracts for *Vitex agnus* leaves have the lowest effective of inhibition percentage (0.41), followed by aqueous extract which was (0.57), respectively.

Table (6): antioxidant capacity determined by ($DPPH^+$) radical scavenging

Plant leaves	Extracts	$DPPH^+$ IC_{50}
Tilia cordata	Methanolic	0. 24
	Aqueous	0. 28
Vitex agnus	Methanolic	0. 41
	Aqueous	0. 57

The antioxidant activity of water and ethanol *Tilia cordata* flower extracts using ($DPPH^+$), which were 63.0±3.8 and 36.7 ± 1.8 (mg/g), respectively [51]. They reported that, the antioxidant activity of each plant was evaluated *in vitro* using a standard model system, the DPPH assay of *Tilia cordata* leaves was 32.2% of methanolic extract [38].

The present data of *Vitex agnus* leaves for antioxidant capacity are agreed with those attained by [43], who found that, the antioxidant activity using ($DPPH^+$) of essential oil, hexane, dichloromethane, ethyl acetate, methanol and water extracts of *V. agnus castus* at a concentrations (0.2 mg/ml^{-1}), were 0.51, 0.51, 4.38, 4.60, 10.89 and 26.26%, respectively.

The data were parallel with that recorded by [39], who found that, IC_{50} values for *Vitex agnus* using ($DPPH^+$) assays were 0.449±0.001mg/ml and 0.612±0.004 mg/ml for leaf and fruit extracts, respectively.

Antioxidant capacity of leaves extracts determined by ($ABTS^+$) cation radical:

The capacity of *Tilia cordata* and *Vitex agnus* leaves methanolic and aqueous extracts to scavenge the ABTS radical were determined alone and compared with the reduction of ascorbic acid as a control sample which is known as a strong reducing agent.

From table (7), it could be seen that, all extracts showed different degrees of inhibition capacity, but their capacities were inferior than that of ascorbic acid which have the maximum inhibition (91.41%).

Also, from table (7), it is clear that, the methanolic extract of *Tilia cordata* leaves have the lowest absorbance value (0.062) with the highest value of inhibition percentage (81.79%) followed by aqueous extract which have (75.43%) at absorbance value (0.081) as inhibition capacity. While, the methanolic extract of *Vitex agnus* leaves have the highest absorbance value (0.092) with the lowest value of inhibition percentage (71.48%) followed by aqueous extract which have (67.19%) at absorbance value (0.089) as inhibition capacity.

Table (7): Antioxidant capacity determined by ($ABTS^{+}$) cation radical:-

Plant leaves	Methanolic extract		Aqueous extract	
	Absorbance	% Inhibition	Absorbance	% Inhibition
Tilia cordata	0.085	81.79	0.081	75.43
Vitex agnus	0.092	71.48	0.089	67.19
+ve Control (Ascorbic acid)	91.41			
-ve Control	0			

The antioxidant capacity (ABTS) values of *Vitex agnus-castus* leaves water, ethanol and n-hexane extracts, which were 2.50, 1.57 and 0.27(mM) trolox [52]. Similarly, The total antioxidant capacity using ($ABTS^{+}$), which were f48 and 62% of drying *Vitex agn*us leaves at 30ºC and freeze drying *Vitex agnus* leaves, respectively [24].

Determine of the radical scavenging activity of the *Tilia cordata* methanolic extracts determined with ABTS and DPPH assays and compared with Trolox, used as a standard molecule, IC_{50} value were 139.9 and 116.72µg/ml, respectively [30]. Likewise, they found that, antioxidant activity of *Tilia cordata* flower (80 % acetone and water) extraction, were 1020±88 and 97±11µmol-TE/g, respectively [53].

Effect of investigated leaves extracts as antibacterial agents:

The effect of various concentrations (1, 2 and 4µg/ml) of *Tilia cordata* and *Vitex agnus* leaves crude methanolic and aqueous extracts on the growth inhibition of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtillis*, compared with Penicillin as chemical antibiotic, are shown in table (8). It is clear that, growth inhibition percentage increased gradually with increasing the concentration of the extracts for all microbial strains under investigations.

From table (8), it could be observed that, methanolic extract of *Tilia cordata* leaves produced the highest growth inhibition (18 and 17mm) against *Escherichia coli* and *Bacillus subtillis* at concentration 4µg/ml, respectively. While, the percentages of growth inhibition for *Staphylococcus aureus* ranged from (8.25 to 15.5mm) in the same extract at concentration 1 and 4µg/ml, respectively. Moreover, the aqueous extract of *Tilia cordata* leaves produced the lowest growth inhibition ranged from (6.25 to 11mm) against *Staphylococcus aureus* at concentration 1 and 4µg/ml, respectively. While, the medium percentages of growth inhibition of *Escherichia coli* and *Bacillus subtillis*, were (14.5 and 14mm) in the same extract at concentration 4µg/ml, respectively.

From the obtained results outlined in table (8), it could be noticed that, aqueous extract of *Vitex agnus* leaves produced the average percentage of growth inhibition (13, 13.25 and 12.75mm) against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtillis* at concentration 4µg/ml, consecutively. While, the lowest percentages of growth inhibition of the same tested bacterial strains, which were (10.5, 9.75 and 10mm) in the same extract at concentration 4µg/ml, respectively. In table (8), it could be observed that, penicillin produced the highest growth inhibition, which were (24 and 22mm) against St. aureus and B. subtillis at concentration 4µg/ml, respectively. While, the percentages of lower growth inhibition of E. coli, ranged from (5.75 to 8.0mm) at concentration 1 and 4µg/ml, respectively. At compared with plant extracts.

Table (8): Effect of crude methanolic and aqueous extracts of investigated leaves as growth inhibition of the tested bacterial strains

Plant leaves	Extract	Concentration (mg/ml)	mm of growth inhibition for:		
			E. coli	*St. aureus*	*B. subtillis*
Tilia cordata	Methanolic	1	$9.75^{f}\pm0.11$	$8.25^{g}\pm0.18$	$9.5^{f}\pm0.12$
		2	$14.0^{b}\pm0.9$	$12.50^{c}\pm0.04$	$13.0^{c}\pm0.06$
		4	$18.0^{a}\pm0.02$	$15.5^{a}\pm0.09$	$17.0^{a}\pm0.02$
	Aqueous	1	$7.5^{h}\pm0.23$	$6.25^{i}\pm0.14$	$7.25^{g}\pm0.31$
		2	$11.5^{d}\pm0.08$	$10.5^{e}\pm0.09$	$10.5^{e}\pm0.08$
		4	$14.5^{b}\pm0.09$	$11.0^{d}\pm0.09$	$14.0^{b}\pm0.06$
Vitex agnus	Methanolic	1	$8.0^{g}\pm0.33$	$7.25^{h}\pm0.52$	$7.0^{g}\pm0.11$
		2	$10.75^{e}\pm0.11$	$9.0^{f}\pm0.11$	$10.0^{e}\pm0.2$
		4	$13.0^{c}\pm0.07$	$13.25^{b}\pm0.9$	$12.75^{d}\pm0.4$
	Aqueous	1	$6.75^{i}\pm0.13$	$5.5^{j}\pm0.17$	$6.5^{h}\pm0.17$
		2	$8.5^{g}\pm0.13$	$7.25^{h}\pm0.13$	$7.50^{g}\pm0.07$
		4	$10.5^{e}\pm0.08$	$9.75^{f}\pm0.22$	$10.0^{e}\pm0.41$

Penicillin	1	5.75^{c}±0.03	14.25^{c}±0.52	17.0^{c}±0.11
	2	7.5^{b}±0.12	17.5^{b}±0.11	20.75^{b}±0.09
	4	8.0^{a}±0.01	22.5^{a}±0.9	24.5^{a}±0.14

The results of several authors were agreed with that obtained, for instance [54], found that, antibacterial activity for several extracts of *Tilia cordata* flowers. The most promising plants, according to the authors, are those which exerted their effects to several of the test organisms, especially toward the gram-negative *Escherichia coli*. Moreover, the antimicrobial test of *T. cordata* leaf oil showed a significant activity against Gram-positive bacteria e.g. *S. aureus, S. lutea* and *B. cereus*, which were 17, 20 and 12mm, at a concentrations 10mg/ml, consecutively. [55].

The effect of cold aqueous extract of linden (*T. cordata*) on the growth of bacterial pathogenesis races (*Escherichia coli* and *Staphylococcus aureus*), ranged from 8 to 10 and 11 to 14 mm, at a concentration 5 and 10mg/ml, respectively [56]. While, the effect of cold alcoholic extract of linden (*T. cordata*) on the growth of bacterial pathogenesis races (*Escherichia coli* and *Staphylococcus aureus*), ranged from 1 to 3 and 2.1 to 4.1mm, at a concentration 5 and 10mg/ml, respectively.

The *Vitex* demonstrated antimicrobial activity against *Staphylococcus aureus, Streptococcus faecalis, Salmonella species* and *Escherichia coli* (10 to 20%), *Candida albicans, C. tropicalis, C. pseudotropicalis,* and *C. krusei* (10 to 40%), [57]. The extract of *Vitex agnus* seeds was reported to possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavonoids and glycosides content [58]. The antimicrobial activity of *Vitex doniana* methanol extract, against E. *coli, S. aureus, B. subtilis* and *S. typhi,* which were 7.63, 4.55, 0.00 and 10.41mm, at concentration 25mg/ml, respectively. Additionally, the higher antimicrobial activity of *Vitex doniana* acetone extract, against E. *coli, S. aureus, B. subtilis* and *S. typhi,* were 11.95, 7.36, 2.62 and 13.41mm, in the same concentration, consecutively [33].

4. Conclusion:

Chemical composition of the two investigated plants showed the highest content of moisture, ash, fiber, protein, lipid and total sugars. Medicinal plant extract containing high percentage of active compounds such as polyphenols and flavonoids of *Tilia cordata* and *Vitex agnus* leaves. The impact of natural extracts as antioxidant tested using (FRAB-DPPH-LPO-ABTS radical) showed high ability of these plants to scavenging the free radicals in laboratory. Moreover, methanolic extracts were the most effective bacterial

inhibitor followed by aqueous extract on *E. coli, St. aureus* and *B. subtillis* at compared with antibiotic (penicillin).

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