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ESTIMATION OF THE PHYTOCHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF IRAQI *OCIMUM SANCTUM L.*EXTRACTS

SARAH S.M. AL-TEMIMI AND LAMIA A.M.AL-MASHHEDY*

Department of chemistry, College of Science, University of Babylon, Hilla, Iraq

ABSTRACT

This research focuses the phytochemical study of aqueous extracts (cold and hot) and ethanolic extract (70%) of *Ocimum Sanctum L*. that are collected from local markets in Iraq. The investigation of phytochemical constituents involved the qualitative and quantitative studies. The results for qualitative analysis explained that the aqueous hot extract and ethanolic extract contains more amounts of active components such as flavonoids, saponins, tannins, alkaloids, terpenoids, glycosides and amino acids or primary and secondary amines rather than cold extract, also the quantitative analysis illustrated that the aqueous hot and ethanolic extracts contains more amounts of flavonoids, saponins, tannins and alkaloids because the aqueous hot and ethanolic extracts still have high yield from all active components and this results may be due to the role of heating in the hot extract and the type of solvent in the ethanolic extract of these active components that cannot be extracted by cold water. Biological activity was also analyzed for the aqueous and ethanolic extracts of *Ocimum Sanctum L*. by using four different bacterial strains (*Staphylococcus aures*, *Streptococcus pyogenes, Salmonella typhi* and *Klebsiella)*and using agar diffusion method. The results showed that ethanolic extract has the biggest zone of inhibition for *Staphylococcus aures*, *Streptococcus pyogenes* and *Klebsiella* while the aqueous cold and hot extracts given less results of inhibition zone.

KEYWORDS: ***Ocimum sanctum L.*, phytochemical constituents, flavonoids, Saponins, anthroquinones, alkaloids, phlobatanins, Zone of inhibition.**

***Corresponding author**

LAMIA A.M.AL-MASHHEDY
Department of chemistry, College of Science, University of Babylon, Hilla, Iraq

INTRODUCTION

The world is rich with natural and individual medicinal plants. Medicinal plants study are now getting more interest than ever because they have many advantages to society or to all mankind, especially in the line of medicine and pharmacological[1] .Over three-quarters of the world population depends basically on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or another, were utilized for medicinal purposes [2]. The medicinal values of plants lie in their phytochemicals, which makes specific physiological actions on the human body. Phytochemicals are compounds found in plants that are utilized as food and medicine top reserve against illness and to ensure human health. Phytochemicals have antioxidant or hormone –like impact which helps in fighting against many diseases including cancer, heart disease, diabetes and high blood pressure .[3]
Phyto constituents work with nutrients and fibers to form in corporated part of protection system against many diseases and stress conditions. Phytochemicals are essentially divided into two groups: primary and secondary constituents; according to their mission in plant metabolism. Primary constituents include common sugars, amino acid, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins and so on [4].The important advantages supposed for therapeutic uses of medicinal plants in many ailments are their integrity besides being economical, effective and their simple availability. There are more than 35,000 plant species being utilized in various human cultures around the world for medicinal purpose. [5] The medicinal plant used in this study was Ocimum sanctum L. that belongs to the family Labiatae and is known as Tulsi in Hindi , Holy Basil in English and Rehan in Arabic. It is known to have various pharmacological activities. Many studies have determined that basil extracts have effective antioxidant, anti-aging, anticancer , and antimicrobial properties.[6] Ocimum sanctum plant have been shown to exert hepatoprotective effect in the models ofexpecting hepatotoxicity like paracetamol and carbon tetrachloride induced liver damage. Ocimum sanctum utilized on common cold, cough, fever. It is also used as mosquito and insect expeller [7] .Ocimum sanctum plant contains vitamin C and A, and minerals like calcium, zinc and iron, as well as chlorophyll and many other phytonutrients. Also enhances the efficient digestion, absorption and use of nutrients from food and herbs [8].

Scientific classification of the plant

- **Kingdom:** Plantae
- **Order:** Lamiales
- **Family:** Lamiaceae
- **Genus:** *Ocimum*
- **Species:** *sanctum*
- **Binomial name:** *Ocimum sanctum* L. [9]

MATERIALS AND METHODS

Collection of plant samples

The plant materials were brought from local markets in Iraq , the plant were washed in tap water and dried then used to make the aqueous and ethanolic extracts that analyzed.

Preparation of the extracts

The extraction performed by weighting 450g of the plant in 1.5 L of ethanol (70% v/v)and using blender to make the mixture .The mixture was heated by using the extraction apparatus for five hours, then filtered by filter paper and evaporated at 40°C up to one third of initial volume. Remaining solvent was completely evaporated at 40°C, using a hot air oven .The yield of ethanolic extract was(1.89%) In the same way , part of the plant was weighted and mixed with water only but the extraction was at 60 ºC to make hot extract for the purpose of comparison of the phytochemical constituents with that of ethanolic extract, the yield of hot extract was (1.13%) and of the cold extract was(1.04%) [10].

Phytochemical analysis

Chemical tests were conducted on the aqueous and ethanolic extracts powdered that form of the plant sample using standard methods.

Qualitative analysis of Phytochemical constituents

1 Test for Flavonoids

A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration [11].

2 Test for Saponins

A weight of 0.5 g of crude powder was shaken with distilled water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of Saponins. [12]

3 Test for Tannins

A weight of 0.5 g of the crude powder was stirred with 10 ml of distilled water. This was filtered and 0.1% ferric chloride reagent was added to the filtrate, a blue-black colouration was taken as evidence for the presence of tannin.[12]

4 Test for Anthroquinones

A weight of 0.5 g of crude powder was shaken with 10 ml of benzene and was filtered 0.5 ml of 10 % ammonia solution was added to the filtrate and the mixture was shaken well and the presence of the violet color in the layer phase indicated the presence of the anthroquinones. [12]

5 Test for Alkaloids

A weight of 0.5 g of crude powder was defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5 ml of aqueous hydrochloric acid on a boiling water bath. The resulting mixture was centrifuged for 10 min at 3000 rpm. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1 ml with Dragendroff's reagent and turbidity was observed.[12]

6 Test for Phlobatanins

An aqueous and ethanolic extracts of the plant sample were boiled with 1% aqueous hydrochloric acid to observe the deposition of red precipitate.[11]

7 Test for Terpenoids

5 ml of aqueous and ethanolic extracts of the plant sample were mixed with 2 ml of chloroform in a test tube. 3ml of concentrated sulphuric acid was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed if terpenoids constituent was present.[13]

8 Test for amino acids or primary and secondary amines

1ml of each extracts was boiled for few minutes in a boiling water bath with 1% of freshly prepared ninhydrin solution. The development of blue-violet colour was indicative of the presence of amino acids or primary and secondary amines.[14]

9 Test for glycosides

1ml of concentrated sulphuric acid was put in a test tube, 5 ml of aqueous and ethanolic extracts from the plant sample were mixed with 2ml of glacial acetic acid containing 1 drop of ferric chloride . The above mixture was carefully added to 1ml of concentrated sulphuric acid so that the concentrated sulphuric acid was underneath the mixture. If glycoside is present in the sample, a brown ring will appear indicating the presence of the glycoside constituent.[15]

Quantitative analysis of Phytochemical constituents

1 Flavonoids determination

10 g of the plant crude powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whitman filter paper .The filtrate was later transferred into a crucible and evaporated into dryness and weighed to a constant weight.[16]

2 Alkaloids Determination

0.15gm of the extracts were taken in a test tube and 5ml of 20% acetic acid in ethanol was added and covered to stand for 4 hrs. This was centrifuged and the supernatant was

concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighted.[3]

3 Tannin Determination

0.1gm of the extracts were weighed and 10ml of distilled water was added and shaken for 1 hr. in a mechanical shaker. This was centrifuged and 1ml of the filtrate was pipetted out into a tube and mixed with 200µl of 0.1 M ferric chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. The absorbance was measured in a colorimeter at 670nm wavelength, within 10 min. A blank sample was prepared with the reagent and distilled water and the absorbance taken at same wavelength.[3]

4 Saponin Determination

20 g of each extract was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated as percentage.[17]

Biological activity

Preparation of the media

The agar diffusion method was adopted to estimate the antibacterial activity of the prepared extracts. The media was prepared by dissolved 28g of nutrient agar in 1L distilled water in a conical flask and then put in the autoclave at 121°C and 1atm for 15-20 minutes. Then the conical flask was put out and cold to 45°C and distributed into petri dishes In order to leave solidifies and in each of these petri dishes, 5mm in diameter, were cut using the cork borer to make holes. These holes were filled with 0.1ml of the aqueous and ethanolic extracts at three different concentrations 50,100 and 200mg/ml for each type of the extracts then left for 24hr at 37°C. After incubation the antibacterial activity of the extracts were determined by measuring the diameter of zone of inhibition (ZI) exhibited by the extracts [18].

Bacterial strains

The antibacterial activity was evaluated against four microorganisms, Gram positive *Staphylococcus aures*, *Streptococcus pyogenes*; Gram negative *Salmonella typhi and Klebsiella*. The bacterial culture were grown and maintained on nutrient broth medium at 37°C for 24hr .

Antibiotics

Six different types of antibiotics (Gentamicin, Ampicillin, Tetracycline, Erythromycin, Cefixime and Norfloxacin)were used to evaluate and control the pattern of antibiotic sensitivity of the different target strains, The activity of antibiotics were determined by measuring the diameter of zone of inhibition (ZI) on different types of bacteria.

RESULTS AND DISCUSSION

In the preparation of extracts as shown before, we found that the yield of aqueous cold and hot extracts were(1.04%) and(1.13%) respectively, while the yield of ethanolic extract was (1.89%). These results may be due to the effect of heating and nature of solvent which were used in the extraction and may be effective in the presence of several active chemical species in ethanolic extract more than presence in aqueous cold and hot extracts. Phytochemical analysis is very useful in the evaluation of some active biological components of medicinal plants. *Ocimum Sanctum L*. extracts showed positive results for all constituents analyzed,

except for phlobatanins and anthroquinones (Table 1).

Table 1
The qualitative analysis of phytochemicals screening of Ocimum sanctum L. extracts.

Phytochemical constituents	Cold extract	Hot extract	Ethanolic extract
Alkaloid	+ve	++ve	++ve
Flavonoid	+ve	++ve	++ve
Saponin	+ve	++ve	++ve
Tannin	+ve	+ve	+ve
Terpenoid	+ve	++ve	++ve
Glycosides	+ve	++ve	++ve
Amino acids and primary or secondary amines	+ve	++ve	++ve
Anthroquinons	-ve	-ve	-ve
Phlobatannin	-ve	-ve	-ve

Phytochemical analysis conducted on the plant extracts detected the presence of constituents which are known to show medicinal as well as physiological activities. Ocimum Sanctum extracts contain different phytochemicals with biological activity that can be of beneficial therapeutic indicator .For example, Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and terpenoids show the analgesic properties .[19] Alkaloids which are one of the largest groups of phytochemicals that observed in *Ocimum sanctum L*. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria.[5] The presence of terpenoids acts as strengthen for the skin, by increasing the concentration of antioxidants in wounds, and restored inflamed tissues by increasing blood supply[1]. Also the presence of tannins are used for urinary tract infection and diarrhea, while saponins are used for diabetes treatment .[20] All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects, including anti-inflammatory, antitumor activities. [21] The presence of phytochemicals likes flavonoids work as protectors against a wide variety of environmental stresses and work as antioxidants, antimicrobials, photoreceptors, visual attractors, and for light screening. Flavonoids show biological activities, including antiallergenic, antiviral and anti-inflammatory actions. However, most interest has been focused on the antioxidant activity of flavonoids, because of their ability to reduce free radical formation and scavenge free radicals [22]. The results of quantitative analysis of *Ocimum Sanctum L*. extracts (Table 2) shown the presence of saponin, flavonoids, alkaloids and tannin in different quantities in all extracts. These results may be due to the increasing of active component solubility with temperature, and the efficiency of extraction depends on the nature of the solvent.

Table 2
The quantitative analysis of phytochemical screening of Ocimum Sanctum L. Extracts.

Phytochemical constituents	Cold extract	Hot extract	Ethanolic extract
Alkaloid	34%	43.3%	43.6%
Flavonoid	7.5%	16.7%	16.9 %
Saponin	0.39%	8.7%	8.71%
Tannin	0.833%	1.376%	1.377%

In this study ,the dose of extracts (50, 100, 200) mg/ml of *Ocimum Sanctum L.* were tested for antibacterial activity against four types of bacteria Gram positive *Staphylococcus aures*, *Streptococcus pyogenes*; Gram negative *Salmonella typhi* and *Klebsiella* by using agar

diffusion method.The results were recorded by measuring the zone of inhibition(mm) that showthe ethanolic extract has the biggest zone of inhibition at the concentration 200mg/ml against *Staphylococcus aures* ,*Streptococcus pyogenes* and *Klebsiella* and at(100)mg/ml against *Staphylococcus* only ,when the aqueous cold extract doesn't give any inhibition zone at(50,100)mg/ml against all types of bacteria under study while (200)mg/ml has an inhibition zone against Klebsiella only as well as the hot extract give an inhibition zone at(100)mg/ml against *Staphylococcus* and at(200)mg/ml against *Staphylococcus aures* ,*Streptococcus pyogenes* and *Klebsiella* as shown in Table (3)and Figure (1,2).

Table 3

The inhibition zone(mm) of Ocimum Sanctum L. extracts on some types of bacteria where (-)=no activity, STR, Streptococcus; STA, Staphylococcus; Kleb;, Klebsiella, Salm;, Salmonella.

Type of extracts	Concentration mg/ml	Diameter of inhibition zone (mm)			
		STA	STR	*Salm*	*Kleb*
Cold extract	50	-	-	-	-
	100	-	-	-	-
	200	-	-	-	12
Hot extract	50	-	-	-	-
	100	11	-	-	-
	200	12	12	-	5
Ethanolic extract	50	-	-	-	-
	100	10	-	-	-
	200	13	17.5	-	13
Antibiotics	Concentration mg/ml				
Ampicilin	10	-	8	-	-
Cefixime	10	-	-	30	-
Erythromycin	10	4	-	4	5
Gentamicin	10	16	17	18	13
Norfloxacin	10	15	15	20	20
Tetracycline	10	16	-	12	-

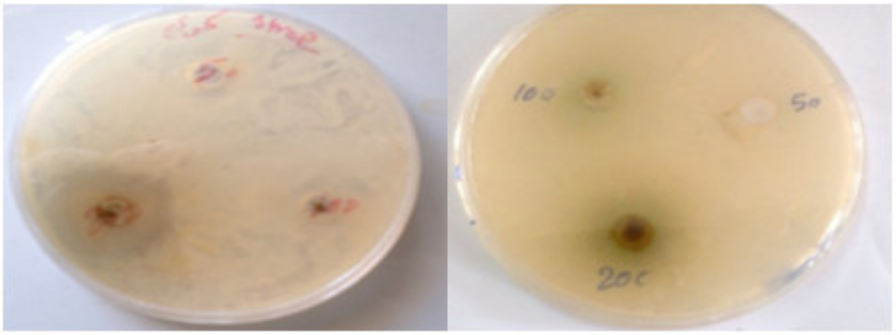

Figure 1
Antibacterial activity of ethanolic extract of Ocimum Sanctum L. on two types of bacteria Staphylococcus aures and Streptococcus pyogenes.

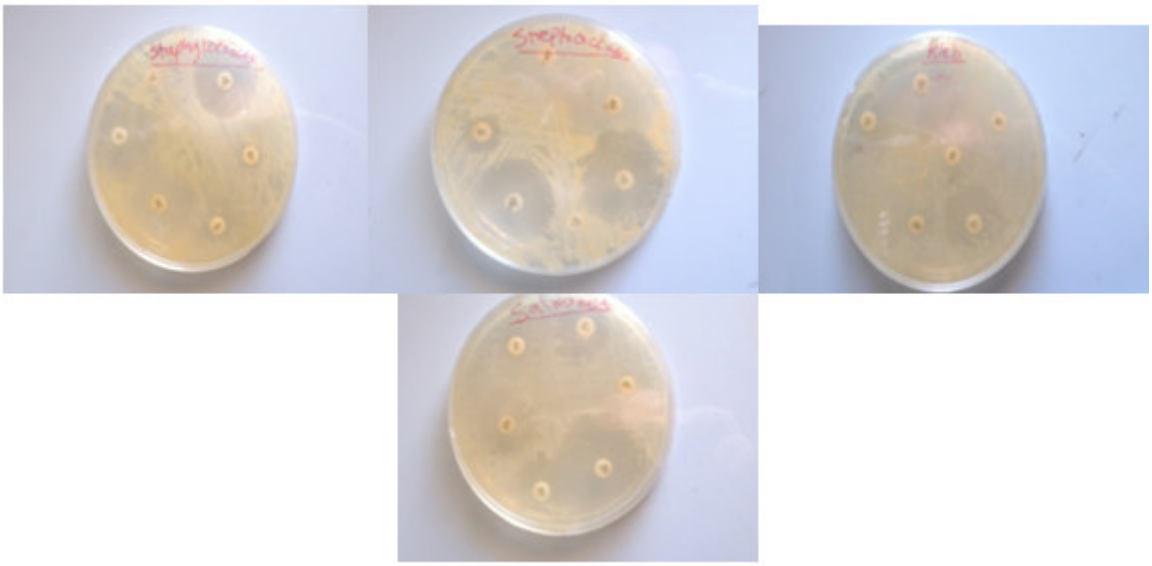

Figure 2
Antibacterial activity of antibiotics against four types of bacteria (Staphylococcus aures, Streptococcus pyogenes ,Salmonella typhi and Klebsiella).

These results enhance the qualitative and quantitative study that shows the ethanolic extract 70% better than aqueous extracts(cold and hot), moreover the biological activity indicated the concentration (200 mg/ml) of the ethanolic extract (70%) given the best results of inhibition zone against *staphylococcus ,streptococcus* and *Klebsiella* compared with other concentrations in this experiment, this result means increasing the concentration of extract leads to increase the zone of inhibition .[23] It appears that overall the microorganisms were not sensitive to the water extracts compared with the ethanolic extract as determined by agar diffusion methods. The reasons for this could be that all of the identified components from plants active against microorganisms, aromatic or saturated organic compounds, are most often obtained through initial ethanol extraction.[24] Antibacterial activity of different extracts of Ocimum sanctum L. and according to the results given in Table 3 which illustrated that alcoholic extracts shown higher zones of inhibition compared with antibiotic disk therefore, the results of this study suggest that Ocimum Sanctum L. organic extracts could be a source of natural antimicrobial agents that can be used as antimicrobial agents in designing and developing new drugs and also utilized for food or pharmaceutical industries to control pathogenic bacteria.[25] It indicates that the leaves of Ocimum species may possess

compounds with antimicrobial properties which are effective against infectious diseases. The extracts of this plant were shown to have bioactive compounds like anti-oxidative, anti-mutagenic and hypoglycemic activities.[26] The results of this study indicate that this plant extract could possibly use as antibiotics .The antimicrobial activity of *Ocimum Sanctum* is due to the presence of secondary metabolites that serve as a defense mechanism against prediction by many microorganisms. Largest zones of inhibition were observed at 200mg/ml of all extracts. These results of inhibition depend largely on the extraction solvent and plant part. Organic extracts provided more potent antibacterial activity as compared to aqueous extracts. The polarity of secondary metabolites and antibacterial compounds make them more readily extracted by organic solvents because Secondary metabolites are more soluble in organic solvents than water, and using organic solvents does not affect their bioactivity against bacterial species suggesting that organic solvents are clearly better solvents of antimicrobial agents.[5]

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