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Original Research Article

Investigating the Anti-Diabetic Effects of Turmeric (*Curcuma longa*) by the Examination of Its Bioactive Compounds in an Ethanolic Extract

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Abstract: Phytochemicals are chemical molecules found in plants, possess physiological activity, and provide other health benefits to humans besides those of macronutrients and micronutrients. The plant, Curcuma Longa, scientifically referred to as turmeric, is a strange plant that finds some applications as a spice, dye, cosmetic, and medicine. The purpose of this study was to determine the antidiabetic efficacy of Curcuma longa leaves and also to determine the bioactive chemicals that could be found in the plant. The bioactive chemicals were extracted in the Soxhlet technique using GC-MS. Based on the results of the GC-MS, the water-based extract of the Curcuma longa leaves contained eleven chemical components. These are: 2, 6- Octadienal, 3- 7 Dimethyl -(E, 0 -Terpinene, o-Cymene, Dodecane, Hexadecanoic acid, methyl ester, n- Hexadecanoic acid, 9,17- Octadecadienal, Curcumene, Epiglobulol, Caryophyll Analysis of Curcuma longa leaves extract by GC-MS revealed that it contained bioactive constituents that had potential significant medicinal effects. As per the kind of extract (Crude, Ethyl acetate, Ethanol, Water and acarbose (Standard) indicated (95.71±0.68), (41.98±0.39), (63.00±0.41), (68.04±0.49), (19.11±0.08) and (19.11±0.08) respectively against 1-amylase. Although, recorded (61.002.22), (45.172.36), (31.276.76), (49.002.46), and (12.261.72) respectively inhibitory-potency against Alpha- glucosidase activity. Thus, the presence of these bioactive substances can explain the medicinal properties of the plant and this may result in the production of new antidiabetic drugs.

Keywords: *Curcuma Longa*, Antidiabetic Activity, Bioactive Compounds.

INTRODUCTION

Turmeric is an annual crop that is widely cultivated in the tropical and subtropical region and is a rhizomatous perennial plant of the family Zingiberaceae. The genus Curcuma is the one that is most famed with its most popular species, Curcuma longa. However, some other species belonging to this genus are Curcuma aromatica, Curcuma zedoaria, Curcuma amada, and Curcuma rakatakanta among others. In its full grow, Turmeric can be about 1 meter in height. The sheaths, false stems, petioles and blades of the leaves are produced by the two rows of long, rectangular, leaves of this plant. Rhizomes are roughly divided and have very specific smell; they can be between 2.5 and 7 centimeters long and are about 2.5 centimeters in diameter [1, 2]. There are two types of rhizomes which are defined by their form. The major rhizomes are in the form of a pear, and they are termed as the bulbs. The flowers are contained in the spike-like structures and they are dull yellow in color, 10-15 cm in length. This natural spice is used as a colorant in curries, mustard, spaghetti, yoghurt, cheese, baked, and salads [3]. As a food coloring additive, it has the E100 label and as an ecological dye, it is called Natural Yellow. The turmeric consists of carbohydrates 69.4, water, 6.3, protein, 5.1 and minerals at 3.5. The multiple chemicals in the 5.8% essential oil are the curcumin, which is a key curcuminoid in turmeric rhizomes. The curcumin derivatives have numerous positive properties such as fighting against diabetes, infections, allergies, cancer, blood clotting and infertility [4-6]. This bioactive compound has low bioavailability because it is not soluble in water. In order to increase its bioavailability, several scientists have tried different methods of micro and nano emulsions encapsulation. The three protons that can ionize and are found in an aqueous solution of curcumin are one enolic proton whose pKa is approximately 8.5, and 2 phenolic protons that have a pKa of between 10 and 10.5. The most important chemical reactions involved in the biological activity of curcumin are hydrolysis, enzyme reactions, electron-donating reactions, which result in the oxidation of the substance, reversible and irreversible nucleophilic addition, and electron-donating reactions [7, 8]. In our

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study, we want to learn more about bioactive constituents in ethanolic extract of turmeric (Curcuma longa) and its role in treating diabetes.

MATERIALS AND METHODS

Collection of Vegetation and Derivation of Raw Materials

We purchased the Curcuma longa leaves (turmeric), which were fresh in the Iraqi market of Hilla. The authenticity of the leaves was confirmed by one of the plant taxonomists in the College of Science in the University of Babylon. The leaves were sprayed by running water to remove any dust or dirt. The plant extract was prepared using Soxhlet. The extraction was allowed after 30 minutes in an oven at temperatures of 105-110 degrees Celsius after which they were transferred to 500 ml of clean boiling flasks. The process now proceeded to allowing it to cool in a desiccator. A one hundred grams portion of the material was taken and moved to a Soxhlet thimble.

Gas Chromatography and Mass Spectrometry

The bioactive chemicals extracted in Curcuma longa were tested through gas chromatography-mass spectrometry with a chromatography system of Agilent Technologies made of GC-7890A/MS-5975C model and using an HP-5MS column measuring length, diameter and film thickness of 30 m, 250 µm and 0.25 µm, respectively. The GC-MS spectroscopic detection was necessary in the use of high-energy electrons (70 eV). The carrier gas was helium gas of 99.995% purity passing through the rate of 1 mL/min. We began with a temperature of 50 to 150 C, and heated it by 3C/min then maintained it approximately 10 minutes. The temperature was then added slowly to 300 o C with an increment of 10 o C/ minute. One microliter of the 1 percent dilutes extracts were injected using a splitless mode. The percentage of the chemical compounds in the extracts was calculated depending on the area of the peaks on the chromatogram.

Thermogravimetric Spectroscopy

The mixture was mortar-ground until thoroughly mixed, and 50 mg of Curcuma longa ethanolic extract and 950mg of KBr IR grade added. A Cloiset Scientific FTIR spectrophotometer ABB MB3000 was used to analyze all the samples with a germanium beam splitter and DTGS detector. It was scanned with the built-in Horizon MB FTIR software at a resolution of 4 cmG1 and 32 FTIR spectra were scanned in the 4000-650 cmG1 mid-infrared region. The HATR attachment was placed in a way that the samples were touching it well. All the spectra were rationed using a background of air spectra. After each scan a fresh reference air backdrop spectrum was taken. These spectra were measured twice at every data point in the form of absorbance.

Alpha- Amylase Inhibition Test

The traditional method of the a-amylase inhibitory activity of the extract and fractions was slightly altered. Initially, 20 mL of 2 units per milliliters 20 mL of 2 international units of 20 mL of 2 units per milliliters of 2 international units of 2 units per milliliters of 2 units per milliliters. Then followed 200 mL of extract and fractions at the various concentrations of 0.5 mL milliliters each. Finally, 500 mL of 6.8 phosphate buffer that contained 100 mM phosphate was added. The mixture was then subdivided into 96 wells and allowed to incubate at 37 o C in 20 minutes. The preincubation phase was set at 37 o C. Thirty minutes later, the above mixture was put back into an incubator of 37 degrees Celsius. This was followed by addition of 20 liters of 1% soluble starch in 100 mM phosphate buffer at pH of 6.8 in the substrate. Then the 100 liters of DNS color reagent were put into the liquid and it was cooked under constant pressure after 10 minutes. The aim of the measurement was to find out the final mixture absorbance in quantitative terms. The levels of control employed were 0.1 to 0.5 mg/ml acarbose that are regarded as normal. We synthesized a material which had grown without any experimental procedures (extracts and fractions) at once as a point of reference. As an illustration, three copies were made of each experiment. The findings were given out as a percentage of inhibition which is the product of the formula.

The percentage of inhibition could be determined by applying the following formula:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}) / \text{Abs}_{\text{control}} \times 100$$

The α-Glucosidase Inhibiting Assay

According to the outcomes of the analysis, we are aware that the extract and fractions inhibit α-glucosidase. The analysis was otherwise done as per the traditional method with minor adjustments here and there. The reaction mixture also included a total of fifty liters of 6.8 phosphate buffer solution, which had a concentration of 100 mM, ten liters of pure alpha-glucosidase, twenty liters of the nine different extracts and first nine fractions and a concentration of 0.500mg/ml. After the liters of P-NPG substrate were added to a concentration of five millimolar, twenty liters were used. This reaction was stopped by adding 50 liters of a solution containing 0.1 M sodium carbonate solution. In this test, the amount of the newly released nitrophenol was measured at 405 nm with a multiplate reader to determine its absorption. Acarbose 0.5 mg/mL was used as a standard, and the sample used was reviewed. These three tests were approximately repeated three times in order to compare the results. Also, another experiment was conducted simultaneously but without the chemical under investigation. The given studies were repeated thrice to give the best results. The inhibitory activity of the protein on the α-glucosidase was expressed as a percentage by the following equation:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

A control where [A] control is the absorbance of the control and [A] extract is the fractions. The IC50 values calculated on the basis of the visual representations represented the amounts of fractions that are used to inhibit enzyme activity to 50 percent.

Statistical Analysis

To calibrate the PLS multivariate in the correlation of the real and predicted values of curcumin, we referred to the Horizon program that is supplied with the FTIR spectrophotometer. To check the calibration model, the leave-one-out cross-validation analysis was used. We considered the values of RMSEC and R-2 as a measure of the validity of the calibration model. Subsequently, the validation or prediction samples were computed on the basis of predictive capabilities of PLS calibration model.

RESULTS AND DISCUSSION

Given that most pharmaceutical industry is based on medicinal plants in terms of the derivation of pharmaceutical chemicals, biotechnology researchers take special interest in medicinal plants. Many of the chemicals that are used in contemporary medicine, including biochemicals, aromas, food colors, and flavors, are of plant origin [9, 10]. Most of the herbal medications and their derivatives are based on plant secondary metabolites or crude plant extracts. Such extracts have a complex compound of phytochemicals. The GC-MS analysis of the aqueous extract of the leaf of Curcuma longa showed the appearance of eleven chemical compounds. These are: 2, 6- Octadienal, 3-7 Dimethyl (E, 0 -Terpinene, o-Cymene, Dodecane, Hexadecanoic acid, methyl ester, n- Hexadecanoic acid, 9,17- Octadecadienal, 0 -Curcumene, Epiglobulol, Caryophyllene oxide, 6-Phenylundecane, 4-Phenylundecane, 0 -Muurol Curcumin reduces mitochondrial oxidative stress through an improvement on the activities of glutathione, catalase, and superoxide dismutase. Curcumin possesses three redox sites that on oxidation and hydrogenation give phenoxy radicals and stabilize the compound against the enol structure. The in vivo experiment has investigated the protective properties of curcumin on the heart and liver against the harmful effects of doxorubicin (DOX), which is a popular drug used to treat tumor diseases. Gas chromatography mass spectrometry (GC-MS) is one of the useful techniques in accurate determination of bioactive chemicals. It has been established that Curcuma longa leaves have valuable bioactive constituents [11-13]. In the GC-MS analysis of the methanolic extract of Curcuma longa leaves, a large number of bioactive components were observed. Identification of bioactive chemicals and essential oils in the rhizome of Curcuma longa using the GC-MS. Curcuma longa rhizomes have bioactive components whose research has attracted a lot of interest. Little is known of chromatographic analysis of Curcuma longa leaf extract. The reason why this research was conducted was to isolate bioactive chemicals in Curcuma longa leaves and exploit their therapeutic potential in developing novel antimicrobials [14, 15], that will combat infectious diseases.

Table 1: The volatile parts of the Turmeric (Curcuma longa) were analyzed by GC-MS

No.	Compounds	Retention times	Molecular Formula	No.	Compounds	Retention times	Molecular Formula
1.	2,6- Octadienal, Dimethyl-(E	7.6	C10H16O	8.	α-Curcumene	23.2	$C_{15}H_{22}$
2.	α-Terpinene	9.7	-	9.	Epiglobulol	25.2	$C_{15}H_{26}O$
3.	o-Cymene	9.9	$C_{10}H_{14}$	10.	Caryophyllene oxide	25.8	$C_{15}H_{24}O$
4.	Dodecane	15.5	$C_{12}H_{26}$	11.	6-Phenylundecane	26.7	$C_{17}H_{28}$
5.	Hexadecanoic acid, methyl ester	16.9	$C_{17}H_{37}O$	12.	4-Phenylundecane	27.0	$C_{17}H_{28}$
6.	n- Hexadecanoic acid	17.6	$C_{17}H_{34}O_2$	13.	α-Muurolol	27.4	$C_{15}H_{26}O$
7.	9,17- Octadecadienal	19.7	$C_{19}H_{36}$	14.	3-Phenylundecane	27.5	$C_{17}H_{28}$

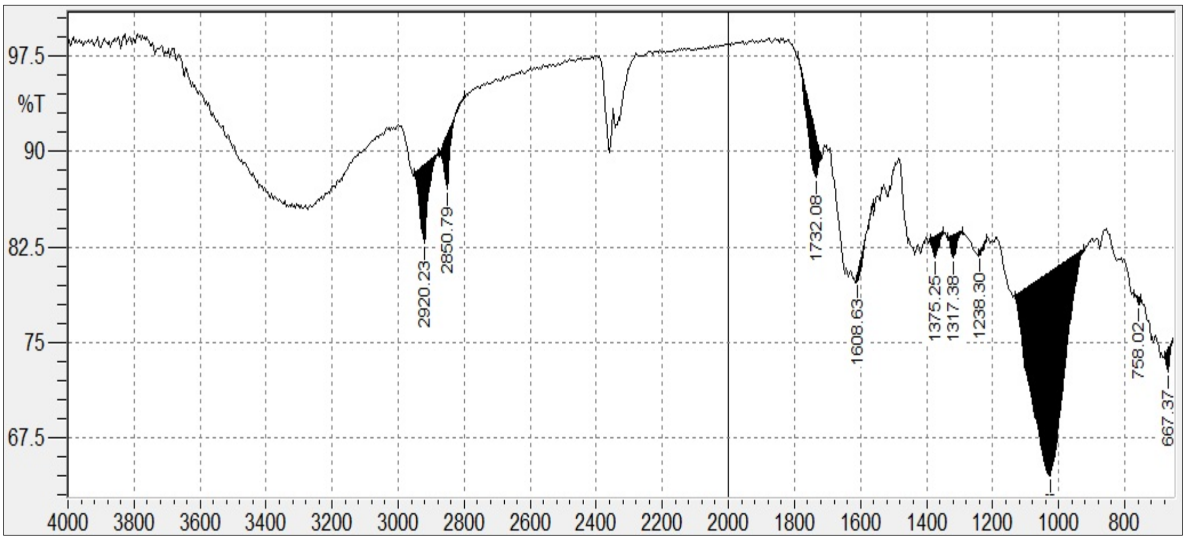

Figure 1: Infrared spectroscopic profile solid analysis of Turmeric (Curcuma longa) using Fourier-transform Wavenumber (cm^{-1})

Table 2. FT-IR peak values of solid analysis of Turmeric (*Curcuma longa*).

No.	Peak (Wave number cm^{-1})	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.3	72.695	1.895	678.94	651.94	3.527	0.113	Strong	=C–H	Bending	Alkenes	650-1000
2.	758.0	77.926	0.633	771.53	752.24	2.045	0.030	Strong	=C–H	Bending	Alkenes	650-1000
3.	1026.1	64.543	16.005	1132.21	925.83	29.401	9.952	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1238.6	81.859	0.339	1242.16	1219.01	1.948	0.031	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1317.2	81.685	1.786	1334.74	1292.31	3.501	0.183	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1375.2	81.635	1.701	1388.75	1350.17	3.215	0.181	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1608.5	79.778	0.565	1612.49	1562.34	4.152	0.079	Bending	N-H	Stretch	Amide	1550-1640
8.	1732.1	87.925	3.027	1788.01	1720.50	2.601	0.583	Strong	C=O	Stretch	Aldehyde	1720-1740
9.	2850.4	87.077	4.293	2875.86	2800.64	3.025	0.369	Strong	C-H	Stretch	Alkane	2850-3000
10.	2920.9	82.847	6.132	2951.09	2877.79	4.516	0.849	Strong	C-H	Stretch	Alkane	2850-3000

The bioactive chemicals in plants are widely used in both healthcare and the manufacturing industry, and in personal care. The discovery of functional groups can help to understand better bioactive chemicals present in plant extracts like those with antioxidant, antibacterial and anti-inflammatory properties. The FTIR spectroscopy can be used to identify organic and inorganic compounds and thus identify secondary metabolites that contribute to the pharmacological properties of plants. These metabolites are alkaloids, flavonoids, terpenes and phenols. The hydroxyl (O-H) bands of phenolic compounds are strong unlike the carbonyl (C=O) stretching bands of aldehydes, ketones, and esters. The fourier transform infrared spectra of plants can teach researchers more about the chemical composition of the plants and which functional groups make them have this biological activity. Possible antidiabetic properties of Turmeric (Curcuma longa) through the inhibition of α-amylase and alpha-glucosidase activity using Curcuma longa fractions Inherent inhibitory effects of Curcuma longa fractions on α-amylase and α-glucosidase activity: These values indicate that the Curcuma longa fractions had significant anti- amylase and anti-glucosidase activities as shown in Figures As noted, the enzymatic inhibitor test of Curcuma longa fractions showed that the inhibition activities depended on the dose in addition to the fraction. The highest dose that was being studied was the most inhibited and the least inhibited was the lowest dose. Recorded (95.71±0.68), (41.98±0.39), (63.00±0.41), (68.04±0.49) and (19.11±0.08) respectively in inhibitory potency against α-amylase in accordance with the type of extract (Crude, Ethyl acetate, Ethanol, Water fraction and acarbose (Standard)) were used. In the process of recorded (61.00+0.40), (45.17+0.39), (31.27+0.29), (49.00+0.46) and (12.26+0.06) respectively inhibitory potency against 8- glucosidase activity. Curcumin also has antiinflammatory and free radical properties besides being a hydrogen donor. The two most significant constituents of the plant, cinnamonaldehyde (Cin) and trans-cinnamaldehyde (Cin) which are the major components of the essential oil of Cinnamomum zeylanicum, give the oil its aroma as well as numerous biological activities.

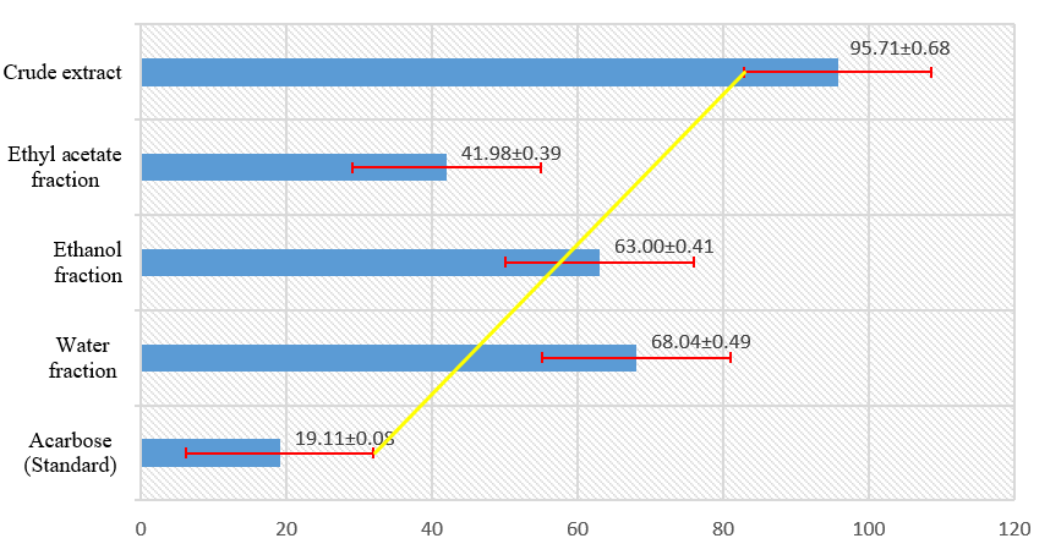

Figure 2. Inhibitory Potential antidiabetic effects of Turmeric (*Curcuma longa*) by α-amylase inhibition.

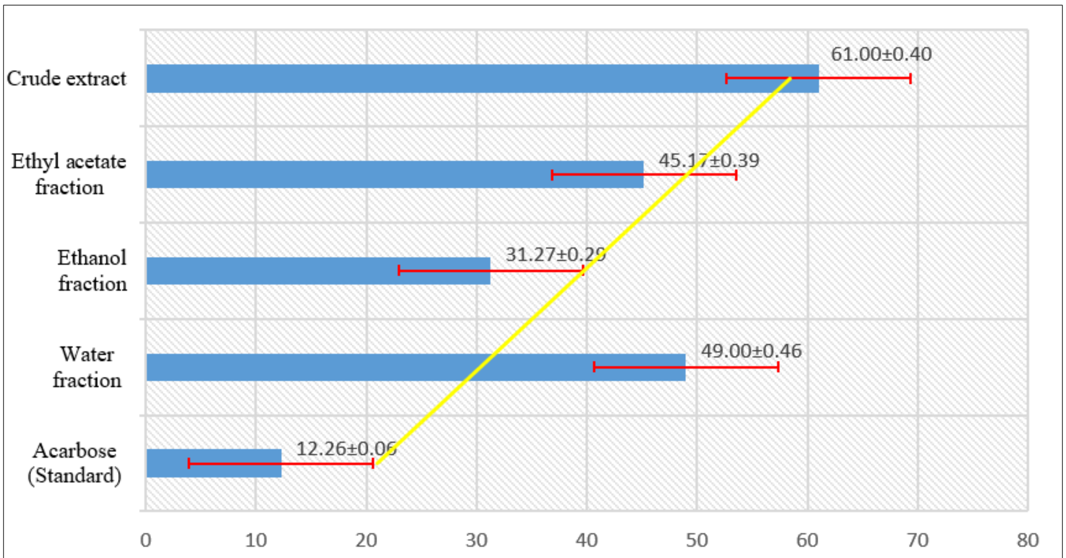

Figure 3. Inhibitory Potential antidiabetic effects of Turmeric (*Curcuma longa*) by α-glucosidase inhibition.

A persistent hyperglycemia is a symptom of diabetes mellitus, a metabolic disorder, where the body becomes unable or unable to use insulin to control the level of sugar in the blood. According to the American Diabetes Association, the different types of DM are as follows: (I) type 1 diabetes is the first variant that is characterized by the autoimmune of beta cells and complete deficiency of insulin, (II) type 2 diabetes is the second variant, which involves 90-95% of all cases of diabetes and is characterized by insulin resistance and consequent hyper-secretion of insulin in beta cells of Langerhans, and (III) gestational diabetes mellitus is the third type, which affects seven percent of pregn Sedentary lifestyle predisposes one to diabetes [16-18], particularly whereby one is overweight or obese. Diabetes is related to glucotoxicity, hyperglycemia, and oxidative stress. The processes combined increase the cellular production of ROS by the formation of AGEs and lipid peroxidation products. In the long run, diabetes may lead to failure, dysfunction, or damage in many organs. These are the eyes, kidneys and the blood vessels. Intestinal, hepatic, and large intestinal microbiota are the main locations of curcumin metabolism after having the product [19-22]. The metabolism process enzyme catalyzed has two steps. In the former, the four double bonds of the planar heptadiene- 3,5-dione of the molecule are cleaved in a heterocytic and hepatocytic reductase into dihydrocurcumin, tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin. Enzymes such as glucoronidases and sulphatransferases are capable of attaching hydroxyl groups to glucuronic acid and sulphate molecules respectively, and can thus be used to convert unmetabolised curcumin and the metabolites formed in the first step to glucuronidic and sulphate-O-conjugated metabolites in the second step. Various clinical trials on diseases and conditions have demonstrated good prospects of curcumin as an antioxidant, antidiabetic, antibacterial, antidepressant, and anticancer. Many studies have indicated that curcumin has antimicrobial, antiviral, antifungal, as well as antimalarial properties among others [23-25]. The emergence of antibiotic-resistant strains and the growing popularity among the population about the danger of modern drugs has raised the interest of the scientific community in therapeutic plants [26, 27].

CONCLUSION

These and numerous other health benefits of the perennial turmeric as a curcumin plant render it the most sought-after ingredient of food other than their color and taste. The phytochemical analysis of the Curcuma longa methanolic extract revealed the presence of some important phytochemicals like 2,6-Octadienal, Dimethyl-(E, α-Terpinene, o-Cymene, Dodecane, Hexadecanoic acid, n-Hexadecanoic acid, 9, 17-Octadecadienal, 9, 17-Alpha-Terpinene, Epiglobulol, Cary FTIR spectroscopy turned out to be an effective instrument in categorizing the plant samples under study in a number of functional groups. These phytoconstituents have important pharmacological activity including antidiabetic traits. Resistin, insulin, and leptin are some of the products of insulin resistance and thus curcumin is a good drug to treat diabetes. Further phytochemical research on sequential extraction using solvents of diverse polarities is very important.

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