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Original Research Article

Antidiabetic Potential and Antibacterial Activities of Garlic (*Allium sativum* L.) against the Multidrug Resistance Bacteria

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Abstract: Diabetes mellitus DM is the most frequently found chronic disease among people. Scientists are now using traditional herbs as an addition to regular treatment in an attempt to manage blood sugar levels and minimize any negative effects. For a very long time, garlic was considered necessary for regulating both nutrition and health. At present, people from all over the globe are using garlic more and more. The purpose of this study is to see how garlic (*Allium sativum*) can treat diabetes and also fight multidrug resistance bacteria. Adding 100 g of garlic fleshed and 250 ml of distilled water to a mixing machine allowed the juice to be prepared. Once the slurry was all squeezed and filtered, the liquid was immediately frozen at a temperature of -10 °C before it was used. The standard method was followed to react *Allium sativum* extract with α-amylase and α-glucosidase, and starch solution to check the ability to inhibit α-amylase. at the same time, data was also obtained using infrared spectroscopy in a wave number range of 4000 cm−1 to 500 cm−1. The antidiabetic potential of garlic (*Allium sativum*) extract obtained from methanolic crude extract, ethyl acetate fraction, ethanol fraction, and acarbose (Standard) was shown to be (89.87±0.61, 34.86±0.25, 60.05±0.30, and 12.92±0.11), respectively, in inhibiting α-amylase. Inhibitory potency against α-glucosidase activity was found to be (71.62±0.55, 50.27±0.39, 39.38±0.26, and 19.00±0.17) accordingly. The anti-*Escherichia coli* activity was 30.07±0.50 for the metabolites of *Allium sativum*.

Keywords: Antibacterial, Antidiabetic, Garlic (*Allium sativum* L.), FTIR.

INTRODUCTION

The condition is caused when the body either has a hard time burning glucose from food or if the liver produces glucose in large amounts while the person is fasting. Most diseases in the diabetes group, such as type 2 diabetes, result in the cellular glucose level being higher than normal [1, 2]. Considering the reality that some current T2DM drugs create side effects, people are looking for more natural ingredients to either control or treat hyperglycemia. Being a macromolecular substance, polysaccharides take part in different functions inside the body and are responsible for various physiological outcomes, for example, hypoglycemia, hypolipemia, immune regulation and anti-cancer effects. Having polysaccharides seems to decrease MDA and raise SOD levels to help β cells in the pancreas resist oxidative injury. Besides, they sometimes support the glycogen synthesis process by modulating certain enzymes, for instance, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase-α (G6pc). Apart from that, polysaccharides [3-5], acting as prebiotics could improve diabetes by correcting bacterial imbalance in the gut and increasing intestinal protection. Since the leading structure of polysaccharides and their recognized antioxidant and probe properties are there, we believed it could also control and stop both hyperglycemia and diabetes mellitus and lead to complications [6]. At present, there are about 150 million diabetics around the globe, and this figure is expected to grow to 300 million or more by the year 2025. Some causes for the rise are a decrease in activity, unhealthy diet rich in energy, obesity, and living longer. Asia and Africa show the greatest chance for a significant rise in diabetes mellitus (DM) rates in the future. Many doctors have suggested using herbal medicines to treat diabetes [7, 8]. Most people see plant drugs as safer than synthetic

drugs and with fewer side effects. Garlic (*Allium sativum* L.) is an herb that comes from southern Europe and Central Asia. After realizing the benefits of the ingredients and their health effects, people began purchasing more garlic products used for medicines and health foods. It increases the value of garlic in business and leads to better usage of natural resources. On the other hand, research is mostly on garlic sulfur compounds, so there isn't much study on the polysaccharide, which comprises the larger part of garlic, resulting in a greatly limited potential for the garlic industry. The spicy seasoning garlic (*Allium sativum* L., Liliaceae) has been a popular ingredient for a very long time. Iranians have grown garlic everywhere in the country for its flavor and health benefits [9]. For many centuries, garlic was popular among cultures, but until recently there was not enough scientific information to support its healing and drug properties. In the past decade, many studies and animal experiments have confirmed that garlic is beneficial to health. Because of their benefits, garlic oil, garlic powder, and pills form categories of garlic preparations that people regularly take to manage their blood pressure and improve their lipid profile [10, 11]. A medication called glibenclamide is often administered to people with diabetes in order to help β-cells produce insulin and lower the level of glucose. GP showed scavenging ability towards hydroxyl radical and superoxide anion. The study further reveals that a higher amount of GP causes a higher antioxidant effect. The presence of GP in alcoholic liver fibrosis mice may help by lowering MDA and increasing GSH-P, GSH and SOD in the livers, which demonstrates its antioxidant effect. It further prevented the rise of ALT and AST to help recover from liver damage. GP was found to act as prebiotics by controlling the gut bacteria and the levels of immune-related cytokines in the case of dextran sulfate sodium-induced colitis. Due to the positive effects, garlic and its extracts help lower blood sugar levels and prevent problems related to diabetes [12]. Still, experimental evidence revealed that treating rats with garlic water extract did not decrease their blood sugar. It might be because of differences in the experiments' setups, kinds of products, how much was given, and which effective ingredients were tested. Accordingly, it is unknown if GP contributes to low blood sugar and the methods it might use to do so. This research was done to measure the ability of garlic extract to fight diabetes and the antibacterial effects of garlic on multidrug resistance bacteria.

MATERIALS AND METHODS

Preparation of Garlic Extract

The garlic bulbs used were fresh ones (*Allium sativum* Linn) and were taken from Nader Markets in Hillah City, Babylon, Iraq. They were peeled, washed, and cut into small pieces. 250 ml of distilled water and 100 g of garlic were put into a mixing machine and crushed to get the juice. Following the removal of cow milk, the slurry was pressed and filtered with a fine cloth, and the filtrate was frozen right away at -10 °C for future use.

Determination of Antibacterial Activity

By using the disc diffusion method, antibacterial activity was checked for Garlic's methanol, ethyl acetate, and ethanol fractions. It took place using a 18-hour formation at 37°C in an amount of 10 ml of Mueller Hinton Agar. Suspending the tests followed the standard of the 0.5 McFarland turbidity scale which is the same as approximately 1.5×108 CFU/ml in sterile saline solution. It was purified by forcing it through a membrane filter with a size of 0.45 µm. Under sterile conditions, the empty sterilized discs (6 mm) were rubbed with 50 µl of each extract and put on the agar surface. On each petri plate, there was a sterile disc filled with diluent instead of bacteria to serve as vehicle control. Discs of AM-Amikacin and Bacteracin were applied as the reference control. Laboratory parafilm wrapped every Petri plate tightly to make sure that none of the test samples could evaporate due to air moving in or out of the plate [13-15]. The extract were kept at room temperature for 30 minutes to let mixing occur and were later placed at 37°C for 18 hours. To measure the zone of inhibition, vernier caliper was used after the incubation period finished.

In Vitro Antidiabetic Activity

Amylase Inhibitory Effect

The *Allium sativum* extract reacted with α-amylase enzyme and starch solution to learn how much α-amylase is inhibited by following the same protocol but with some alterations. In a solution with 150µL from each, 300µL of the sample and the same volume of sodium phosphate buffer (0.02 M, pH=6.9, enzyme α-amylase at 240 U/mL) were kept at 37°C for 20 min. After that, 300 µL of 1% starch solution in 0.02 M sodium phosphate buffer was poured into the mixture. The mix of substances was heated at 37 °C for 20 minutes. Then, one milliliter of dinitrosalicylic acid (DNS) was put in the reaction, and it was incubated at 37 °C for 20 minutes. Then, the mixture was kept in a boiling water bath for 15 minutes before being diluted with 2 mL of distilled water and the absorbance was checked at 560 nm on the spectrophotometer. Acarbose was selected to show that the experiment was performed correctly. Percent inhibition was used to show the results and was calculated as shown below:

$$\textbf{Inhibition (\%)} = (Ac - Acb) - (As - As) / Ac - Acb \times 100$$

Ac shows the absorbance measurement for the control (enzyme with buffer); Acb refers to the absorbance measurement for the control, blank (buffer with no enzyme);

As is the value of absorbance for the sample with both enzyme and inhibitor; and Asb shows the absorbance for the blank sample (no enzyme). Testing shown that mefloquine has an IC50 value of 63 µM, which means that 63 µM of mefloquine inhibits half of the enzyme's activity.

α-Glucosidase Inhibitory Assay

The extent to which the extract and fractions blocked the activity of α-glucosidase was found through analysis. Alternatively, the analysis was carried out as in the regular way with very few minor changes. The serum for testing was placed in a pre-cooled 96 well plate and set at 37 degrees centigrade for 15 minutes. Among other ingredients, the mixture had twenty liters of the first ten samples and fractions at 0.500 mg/mL, ten liters of purified alpha-glucosidase at one unit/mL, and fifty liters of the 6.8 phosphate buffer solution, at a concentration of 100 mM. It was during the pre incubation stage that the temperature was set at 37 degree centigrade. The next step was to incubate the mixture for twenty more minutes at thirty-seven degrees centigrade after that, twenty liters of P-NPG at five millimolar concentration were added. When 50 litres of 0.1 M sodium carbonate was added, the reaction was stopped. For comparison, all three tests were done about three times to get read findings. At the same time, a control experiment was done in which the chemical being studied was left out of the procedure. Each of the tests was done three times in order to find the assessments that are as precise as can be. The results were expressed as the percentage of α-glucosidase inhibition by using this expression.

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

A control is indicated by the absorbance figure of the sample before its components were extracted, while A extract is for the figure representing the absorbance of extracted fractions. From the plotted data, IC50 indicates the number of fractions that cause the enzyme's activity to decrease to 50%.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of *Allium Sativum* L

FTIR data were processed in the lab with the help of software on computers before we could capture the FTIR spectra of the GLVs. The purpose was to measure the FTIR spectra of both kinds of the GLVs. To be prepared for FTIR analysis, enough laboratory crushed leaf samples were converted into pellets using KBr, and at the same time an even and thin layer was produced with a physical press on the studied mixture. At the same period, experiments were done to gather valid data by looking at information from 4000 cm−1 to 500 cm−1 in wave numbers. For these tests, three tests were conducted on the samples using KBr pellets that hadn't been processed to act as a control.

Statistical Analysis

All the experimental data were shown as mean±SEM (for n=5) and were analyzed statistically by one-way ANOVA using GRAPHPAD Prism software (version 8), with Student's t-test applied to find significant differences between groups. All of the significance tests required p-values of 0.05 for a difference to be spotted.

RESULTS AND DISCUSSION

The antidiabetic potential of garlic (*Allium sativum*) extract obtained from methanolic crude extract, ethyl acetate fraction, ethanol fraction, and acarbose (Standard) was shown to be (89.87±0.61, 34.86±0.25, 60.05±0.30, and 12.92±0.11), respectively, in inhibiting α-amylase. Inhibitory potency against α-glucosidase activity was found to be (71.62±0.55, 50.27±0.39, 39.38±0.26, and 19.00±0.17) accordingly. In diabetes mellitus, a metabolic problem develops over the long run, disturbing the workings of several body systems, because not enough insulin is produced or it is not effective. The lack of insulin causes the blood sugar to rise and this damages several body parts, especially the blood vessels and nerves, according to [4, 16-18]. Type 1 diabetes is the name given to hyperglycemia that occurs when insulin production drops, whereas the term type 2 diabetes describes hyperglycemia that arises when insulin is not used properly. Handling type 2 diabetes is not easy, mostly because of: insulin resistance, too much insulin, problems with secretion, and poor glucose uptake and metabolism related to insulin. Patients with this type of diabetes are given metformin, glibenclamide, α-glucosidase and α-amylase inhibitors. It has been widely known that garlic is believed to lower blood sugar. Alloxan is commonly applied to induce diabetes in different animals by harming and destroying the β-cells in the pancreas [19, 20]. It was found from our results that when mice are diabetic due to alloxan, their body weight goes down because fats and proteins are being catabolized. Several experiments have shown that garlic has strong abilities to lower blood glucose. As far as we know, the first report describing how allicin from garlic helps diabetic mice came out as early as b. More studies have shown that amino acids with sulfur in garlic send healthy effects to the blood sugar, lower the sugar in the body, and increase the liver conversion of sugar into glycogen in mice and rabbits with diabetes. Keeping insulin –SH active is another way for hypoglycemic to avoid harmful oxidation. Sulfhydryl group-induced inactivation of insulin is a usual occurrence [21, 22]. Garlic may join with cysteine glutathoine, albumins, and other important compounds, which lead to increased serum insulin rates.

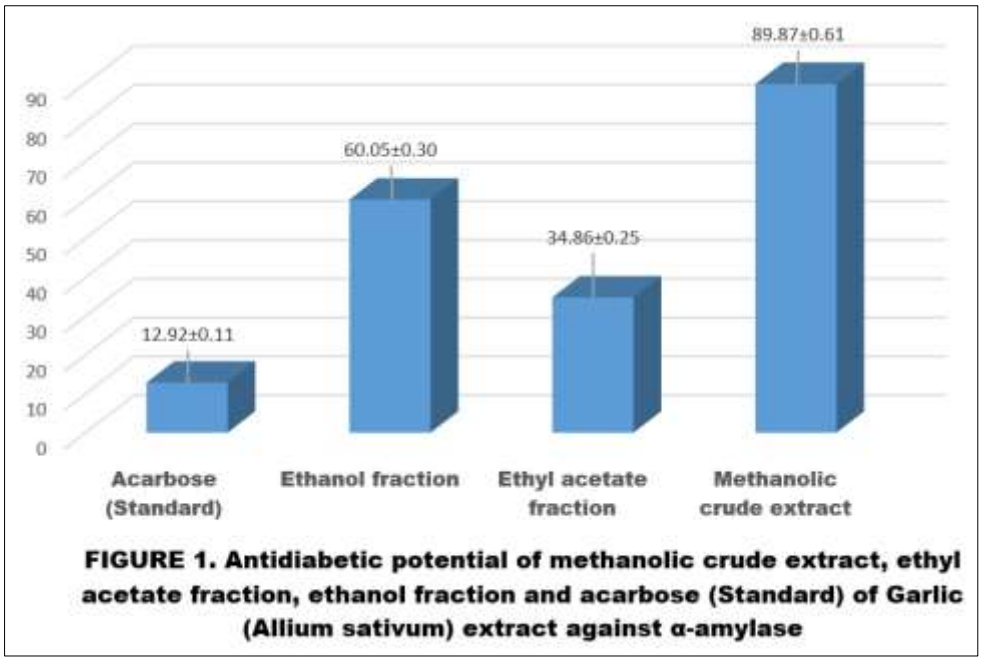

FIGURE 1. Antidiabetic potential of methanolic crude extract, ethyl acetate fraction, ethanol fraction and acarbose (Standard) of Garlic (Allium sativum) extract against α-amylase

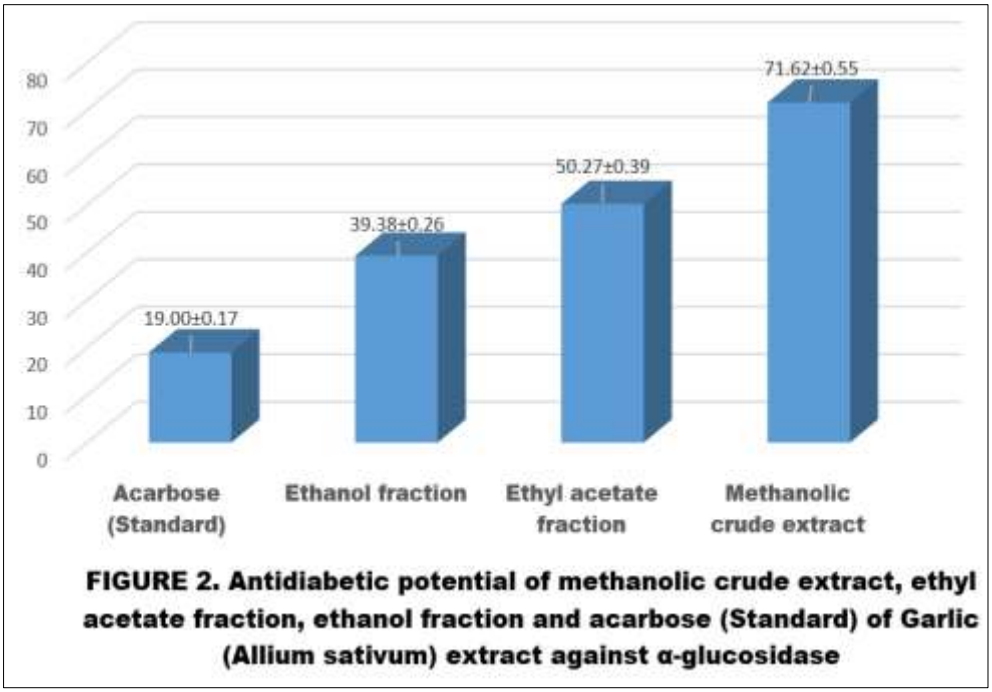

FIGURE 2. Antidiabetic potential of methanolic crude extract, ethyl acetate fraction, ethanol fraction and acarbose (Standard) of Garlic (Allium sativum) extract against α-glucosidase

Comparing the antimicrobial activity of the methanol, ethyl acetate, and ethanol fractions of garlic (*Allium sativum* L.) with those of AM-Amikacin and Bacteracin revealed: 28.01±0.45, 15.83±0.25, 24.00±0.39, 30.07±0.50 and 35.00±0.58 respectively in *Escherichia coli* FIGURE 3. While recorded 20.31±0.29, 11.09±0.20, 25.43±0.51, 28.07±0.46 and 33.00±0.53 respectively for *Klebsiella pneumoniae* FIGURE 4. Record 22.03±0.30, 15.92±0.25, 19.09±0.29, 26.92±0.50 and 31.89±0.51 respectively for *Staphylococcus aureus* FIGURE 5. While 18.00±0.29, 12.95±0.22, 29.00±0.49, 31.97±0.52 and 35.00±0.59 respectively for *Streptococcus pyogenes* FIGURE 6. Significant efficacy against *Escherichia coli* was demonstrated by the metabolites of *Allium sativum* (30.07±0.50). *Allium sativum* L., known as garlic, is known to fight bacteria, mainly because its oil-soluble compounds like allicin, ajoenes, and allyl sulfides have such powers. These compounds are able to prevent bacteria and their infections by killing them, preventing the accumulation of biofilms, fighting toxins, and reducing the signals that allow bacteria to thrive in groups. Scientific studies reveal that garlic extracts are effective at tackling both Gram-positive and Gram-negative bacteria and some acid-fast bacteria as well as Helicobacter pylori. For a long time, garlic has been regarded as the top plant that helps in treating bacterial infections. Since garlic has a high potential for antimicrobial drugs, this study looks at the antibacterial power of *Allium sativum* extracts and their effects along with some antibiotics on drug-sensitive and multidrug-resistant isolates of emerging hospital pathogens. In

the in vitro tests, the whole *Allium sativum* extract was seen to stop the growth of many different types of bacteria, including those that are resistant to several drugs, either by killing them or keeping them alive. At present, both humans and animals get treated with antibiotics in all parts of the world. Using antibiotics in so many ways has apparently led to the appearance of bacteria that are no longer cured by antibiotics, which is very dangerous for humans [23, 24]. Several researchers have observed this occurrence in both types of bacteria: Gram-positive and Gram-negative. This is an important social challenge, since interacting with the dense traffic leads to weakening of the human immune system.

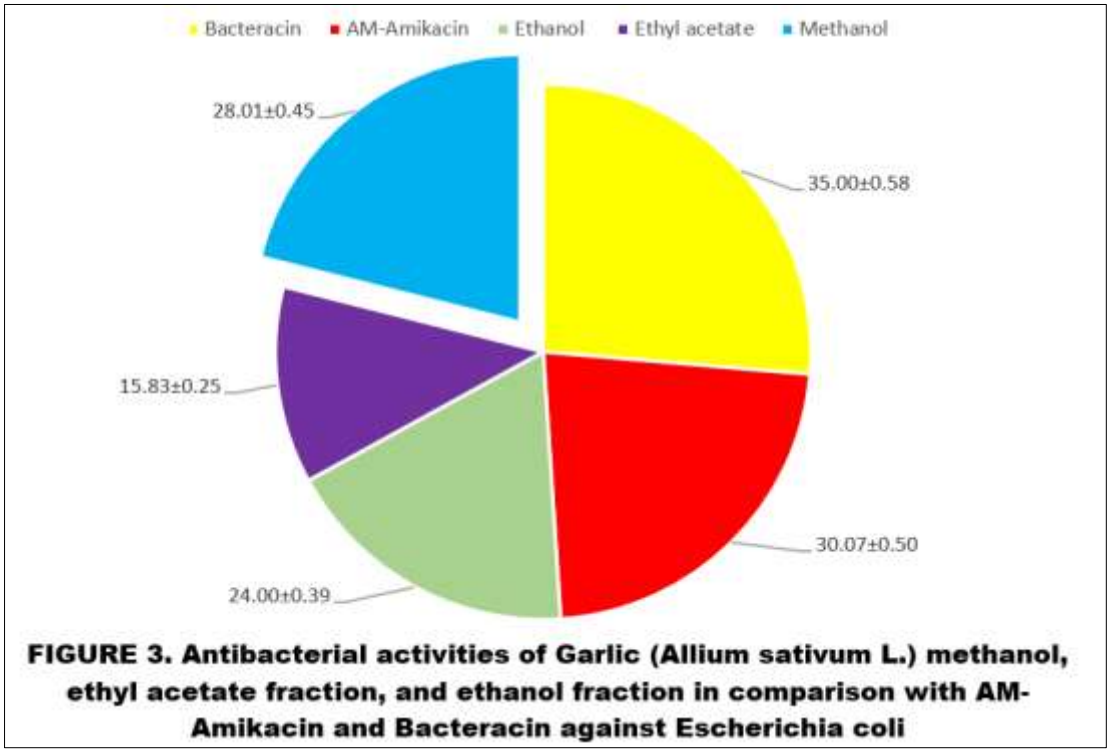

FIGURE 3. Antibacterial activities of Garlic (Allium sativum L.) methanol, ethyl acetate fraction, and ethanol fraction in comparison with AM-Amikacin and Bacteracin against Escherichia coli

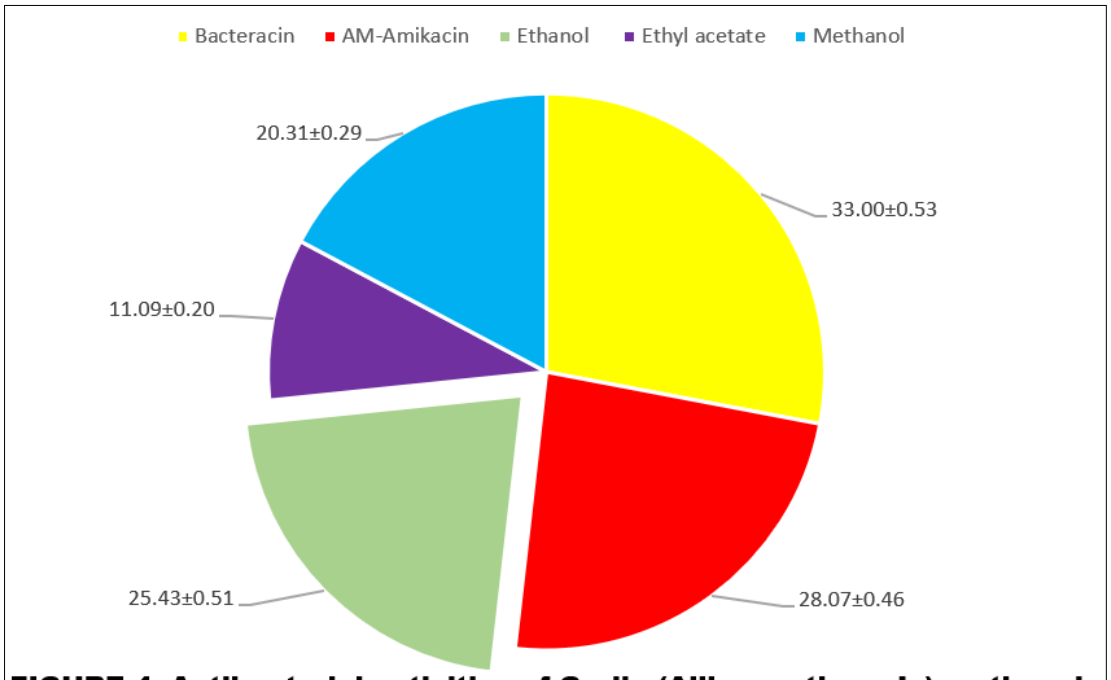

FIGURE 4. Antibacterial activities of Garlic (Allium sativum L.) methanol, ethyl acetate fraction, and ethanol fraction in comparison with AM-Amikacin and Bacteracin against Klebsiella pneumoniae

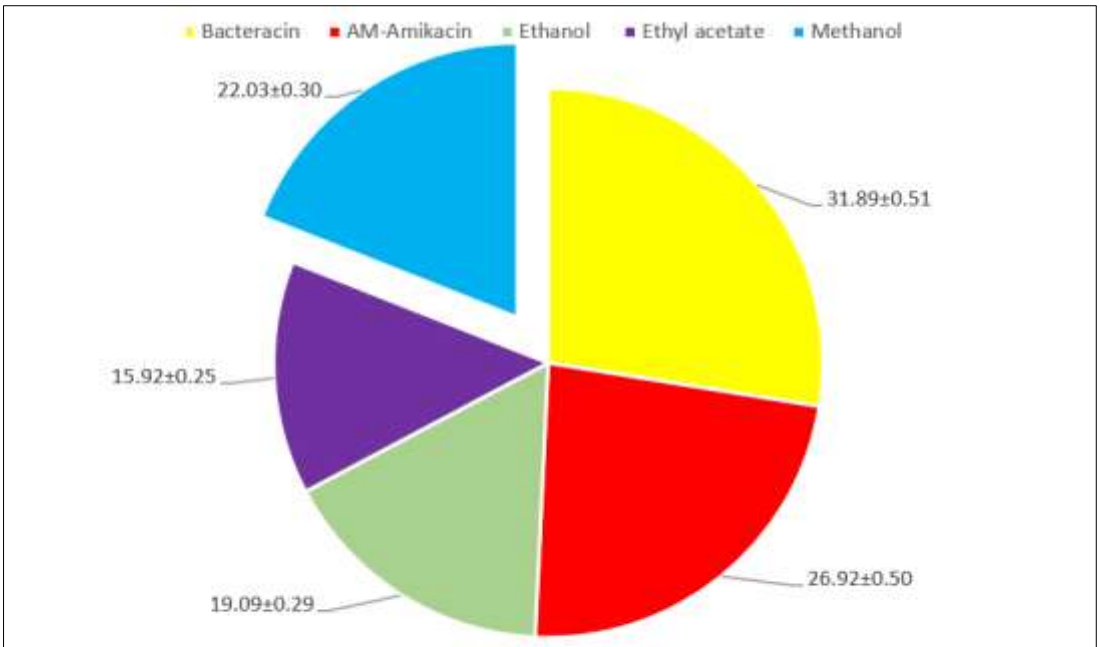

FIGURE 5. Antibacterial activities of Garlic (Allium sativum L.) methanol, ethyl acetate fraction, and ethanol fraction in comparison with AM-Amikacin and Bacteracin against Staphylococcus aureus

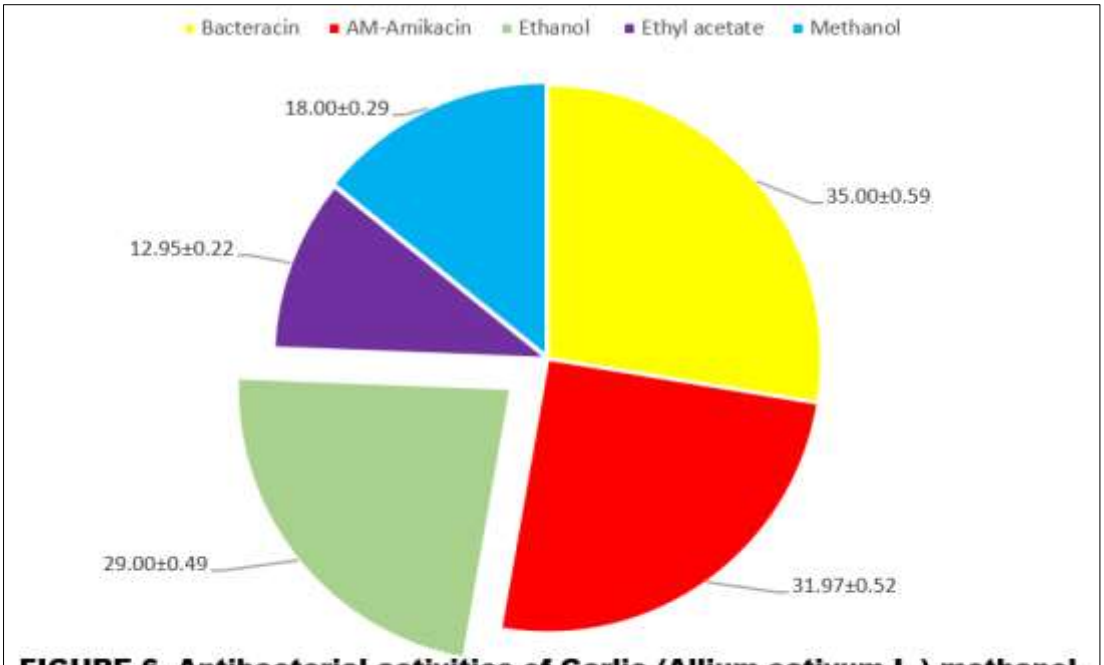

FIGURE 6. Antibacterial activities of Garlic (Allium sativum L.) methanol, ethyl acetate fraction, and ethanol fraction in comparison with AM-Amikacin and Bacteracin against Streptococcus pyogenes

Fourier-transform infrared spectroscopic profile solid analysis of Garlic (*Allium sativum* L.). Peak (Wave number cm-^{l}) 715.59, 1014.56, 1047.35, 1095.57, 1234.44, 1242.16, 1317.38, and 1597.06, with Functional group assignment Alkenes, alkyl halides and Aromatic FIGURE 7. Many researchers use molecular absorption spectroscopy to measure the number of compounds in different products and examine the composition of mixtures made in the lab. The downside of using these methods is that active components usually absorb in the UV region, and their overlapping spectra stop them from being analyzed together. The FTIR spectroscopy is able to assess plant composition and structure. Analysis can happen with both single compounds and blended mixtures using intact samples. It is more precise and selective to use IR spectrometry than to rely on colors. Besides, FT-IR spectroscopy allows you to rapidly identify and evaluate

microorganisms and monitor different biotechnological processes [25]. FTIR is not used to determine the exact amount of any metabolite; instead, it gives a quick view of the metabolic makeup of the tissue examined. FTIR helps scientists find out the structure of materials they do not know and observe how strongly the molecules absorb light in the chemical groups they contain. FT-IR analyzes the bonds inside chemical groups to detect specific vibrations and produces a spectrum that works like a unique signature for the sample. Monitoring IR spectra of plant material may make it feasible to spot minor changes in primary and secondary metabolites. At present, particularly in phytochemistry, tests using FTIR are applied to find out the precise structure of plant secondary metabolites. Still, FTIR is being investigated as a new technique to assess and find out which commercial products are part of the adulterant. FT-IR has been proven to be helpful in identifying the traits of bacteria, fungi, and plants. FT-IR is a popular technique for discovering the contents of a compound and showing how it is built, and is required by Pharmacopoeia in many countries for medicine identification.

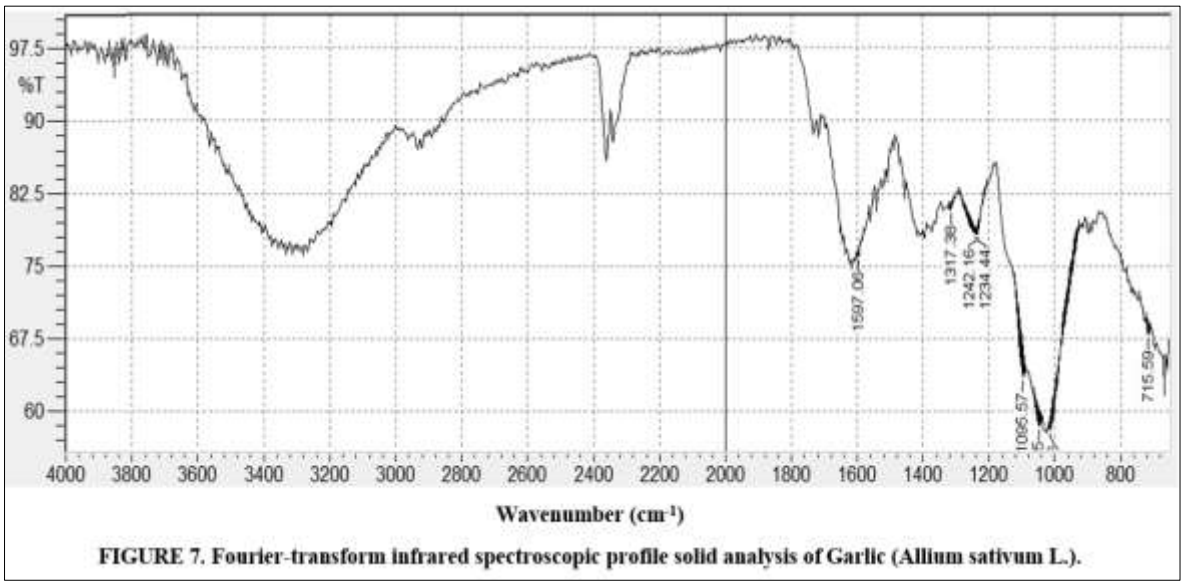

FIGURE 7. Fourier-transform infrared spectroscopic profile solid analysis of Garlic (Allium sativum L.).

TABLE 1. Fourier-transform infrared spectroscopic profile solid analysis of Garlic (Allium sativum L.).

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.	715.59	67.897	1.285	723.31	709.08	2.223	0.062	Strong	=C–H	Bending	Alkenes	650-1000
2.	1014.56	58.136	1.133	1018.41	925.83	14.941	0.220	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1047.35	58.483	1.724	1064.71	1039.63	5.639	0.184	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1095.57	63.618	2.463	1122.57	1089.78	5.698	0.354	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1234.44	78.418	0.762	1238.30	1207.44	2.814	0.036	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1242.16	78.354	0.521	1286.52	1238.30	4.712	0.143	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1317.38	80.864	0.636	1321.24	1294.24	2.359	0.043	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1597.06	76.023	0.704	1602.85	1579.70	2.611	0.039	Medium	C=C	Stretch	Aromatic	1400-1600

Usually, insulin causes lipoprotein lipase to function, converts triglycerides to fatty acids, and allows more fat intake into the adipose tissue. Without enough insulin, lipolysis is not controlled and it increases, bringing about hyperlipidaemia. Insulin deficiency causes the level of free fatty acids in serum to increase since they are released from fats in larger quantities because triglyceride breakdown is favored over building up. Because gluconeogenesis and urea formation lead to more protein breakdown, there could be an increased release of tissue transaminases in the diabetic body. Increased transaminase activity is caused by liver cell damage, and this normally comes with higher levels of aspartate transaminase. The use of plant extracts in diabetes was shown to enhance the activities of serum transaminases: AST and ALT. Most often, an oral antidiabetic agent is given in the beginning, however, because of the chronic nature of the disease, patients later require additional treatments. A certain drug is chosen based on the individual's health and any other cardiovascular risk factors such as problems with cholesterol and high blood pressure. Since diabetes is spreading worldwide and bringing many problems, it's necessary to look for drugs that successfully control blood sugar and are safe and affordable. Many people today use natural products for health issues since they can get them without medical help,

they are less expensive, and also because they believe they are safer than artificial drugs. Several researchers have looked into different natural substances for possible help against diabetes. Many people have relied on garlic to offer protections against risks for heart diseases for long periods. Even though the hypoglycemic effects of different garlic preparations are known from studies in lab animals with diabetes, proof from human research is scarce and conflicts. Since there isn't enough research on hypoglycemia from garlic and the interest in natural remedies is increasing, we carried out a study to learn how garlic affects blood glucose levels in type 2 diabetic patients. In addition, because garlic stimulates antioxidants, it is seen as a possible solution for treating diabetes. Experts consider that the molecule onion S-allyl cysteine sulfoxide, a natural product found in garlic, shows the ability to reduce the effects of glycation [26, 27]. It was noted that garlic prevents insulin from entering the inactive form by sulphydryl groups. Diabetic dyslipidemia usually accompanies having diabetes and greatly increases a patient's chance of getting cardiovascular disease. Having both antidiabetic and antilipidemic agents is commonly recommended for diabetes patients to prevent them from heart disease complications. All the lipid parameters showed a sizable decrease in garlic group compared with placebo group. The lipid profile changes that were seen here are consistent with those from previous medical studies.

CONCLUSIONS

In conclusion, Data from the present study concluded a decrease in blood glucose levels when the extract of Allium sativum was given. The explored extract appears to have a good ability to block α-amylase. Thus, this research suggests that *A. sativum* aqueous extract can be looked at as a possible treatment for DM. Besides, garlic shows promise for future research on type 2 diabetes mellitus. *Allium sativum*'s metabolites were found to act powerfully against *Escherichia coli* (30.07±0.50). For this reason, garlic can offer a better choice to the antibiotics used nowadays. Moreover, researchers are searching for fresh drugs because some bacteria have become resistant to the antibiotics used previously. It seems that combining garlic extracts and currently available antibiotics in such a way that their actions work together would be an answer to the problem. Garlic, according to the research, could be used along with antibiotics to enhance the result of AM-Amikacin and Bacteracin in treatment. Clinical studies covering many aspects are expected to prove whether garlic works well when used alone or together with other methods for treating or preventing diabetes as well as cardiovascular risks.

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