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GC-mass characterization of tomato ethyl acetate extract and its antibacterial and antioxidant properties

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ABSTRACT: Tomatoes possess valuable medicinal properties with high lycopene and flavonoid content, recognized for diverse pharmacological impacts. The study aimed to evaluate the antibacterial and antioxidant traits of the ethyl acetate tomato extract while identifying its main components. The antioxidant potential was determined through the 2,2-diphenyl-1-picrylhydrazyl radical assay, while the antibacterial activity was evaluated using the agar well diffusion method. Additionally, the components present in the extract were explored through GC-Mass spectroscopy. The final extraction ratio was calculated at 31.38±0.76%. Over 25 individual compounds were discerned in the tomato extract, encompassing myricetin 50.7%, n-hexadecanoic acid 19.9%, salicylic acid 13.77%, octenyl succinic acid 1.58%, vanillic acid 1.41%, dimethyl benzene 1.02%, Iso-quercitrin 1.02%, Hexadecanol 0.85%, Nomane-a-tomatidine 0.77%, a-tocopherol 0.76%, Homoserine 0.76%, and other compounds in smaller quantities. The extract exhibited a broad spectrum of antibacterial activity against the tested bacterial strains (*S. aureus* and *P. aeruginosa*). Notably, *S. aureus* displayed higher susceptibility to the tomato diethyl acetate extract concentrations in the culture media than *P. aeruginosa*. The ethyl acetate tomato extract showcased distinct 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity. The results indicate that the tomato extract possesses significant antibacterial and antioxidant qualities, showing promise as a valuable source of natural compounds for new drug development.
Keywords: natural compounds; bacterial strains; free radical; Gas Chromatography - Mass Spectrometry.

Caracterização por espectroscopia de massa (GC) do extrato de acetato de etila de tomate e suas propriedades antibacterianas e antioxidantes

RESUMO: Os tomates possuem propriedades medicinais valiosas com alto teor de licopeno e flavonóides, reconhecidos por diversos impactos farmacológicos. O estudo teve como objetivo avaliar as características antibacterianas e antioxidantes do extrato de tomate com acetato de etila e identificar seus principais componentes. O potencial antioxidante foi determinado através do ensaio do radical 2,2-difenil-1-picrilhidrazila, enquanto a atividade antibacteriana foi avaliada pelo método de difusão em placas de ágar. Adicionalmente, os componentes presentes no extrato foram explorados através de espectroscopia GC-Mass. A taxa de extração final foi de 31,38±0,76%. Mais de 25 compostos individuais foram discernidos no extrato de tomate, abrangendo miricetina 50,7%, ácido n-hexadecanóico 19,9%, ácido salicílico 13,77%, ácido octenil succínico 1,58%, ácido vanílico 1,41%, dimetil benzeno 1,02%, iso-quercitrina 1,02%, Hexadecanol 0,85%, Nomane-a-tomatidina 0,77%, a-tocoferol 0,76%, Homoserina 0,76% e outros compostos em quantidades menores. O extrato exibiu amplo espectro de atividade antibacteriana contra as cepas bacterianas testadas (*S. aureus* e *P. aeruginosa*). Notavelmente, *S. aureus* apresentou maior suscetibilidade às concentrações de extrato de acetato de dietila de tomate no meio de cultura do que *P. aeruginosa*. O extrato de tomate com acetato de etila apresentou atividade distinta de eliminação de radicais livres 2,2-difenil-1-picrilhidrazil. Os resultados indicam que o extrato de tomate possui qualidades antibacterianas e antioxidantes significativas, mostrando-se promissor como uma fonte valiosa de compostos naturais para o desenvolvimento de novos medicamentos.
Palavras-chave: compostos naturais; cepas bacterianas; radicais livres; Cromatografia Gasosa - Espectrometria de Massa.

1. INTRODUCTION

On 4.85 million hectares, the tomato (*Solanum lycopersicum* L.) produces over 182.3 million tons of tomato fruits annually, making it the second most significant fruit or vegetable crop after potatoes (*Solanum tuberosum* L.) (QUINET et al., 2005). This one is quite significant of all the vegetable plants in the world.

Western South America originated, while Central America is where domestication is supposed to have taken place (DARWIN et al., 2003). Due to their critical role in

human nutrition, tomatoes have undergone extensive breeding to increase fruit quality, production, and tolerance to biotic and abiotic influences.

Research material and food both make extensive use of tomatoes. Compared to other model plants like rice and Arabidopsis, the tomato plant possesses distinct and captivating traits such as fleshy fruit, a sympodial stalk, and compound leaves (PARNELL et al., 2004). These features are essential in agronomy and aren't observable in other model plant systems. Thirteen recognized wild tomato species showcase diverse phenotypes that can be hybridized with cultivated tomatoes. These wild tomato varieties hold significant value in breeding programs, as they offer sought-after characteristics (Agarwal; Rao, 2000) and are instrumental in evolutionary research. As the tomato genome sequencing effort progresses, valuable data has been produced to support tomato research. Furthermore, the tomato is a member of the vast Solanaceae family, which includes many other economically significant plants, including petunias, potatoes, eggplant, peppers, and tobacco (BAI; LINDHOUT, 2007).

Tomatoes are a useful study material since you can quickly apply knowledge from studies on these plants to other plants. Owing to these characteristics, the tomato is used as a model organism for plants in the Solanaceae family, particularly those with fleshy fruits (BLUM et al., 2007). All four primary carotenoids, lutein, lycopene, and beta- and alpha-carotene, are present in tomato extract. In addition to their potential individual advantages, these carotenoids also work synergistically. That is, they cooperate to promote health (BUTELLI et al., 2008). Eating a diet strong in tomato-based products may help lower the risk of pancreatic cancer. Researchers discovered that males with the greatest and lowest intakes of lycopene, a pigment found mostly in tomatoes, had a 31% lower risk of pancreatic cancer (KIM et al., 2019).

The present work aimed to reveal the antibacterial and antioxidant properties of the ethyl acetate extract of local tomatoes *in vitro*.

2. MATERIALS AND METHODS

2.1. Sample collection

Fresh, bright red Tomato fruits (1 kg) were collected from local fruits and vegetable farms in Al-Diwaniya province, transported to the laboratory, and kept at -4° C. The fruits were thoroughly washed under a running stream of water to remove all dirt, dust, and foreign materials attached to their surface, and the seeds and skin were removed. The fruit is then chopped into small pieces with a stainless-steel knife, ground in a mill, and passed through a 200-micron stainless steel mesh sieve. To obtain tomato paste, the sieved material was placed in the oven at 45 °C and then stored at -20 °C until use.

2.2. Tomato extraction

According to the method described by Haroon (2014), Ethyl acetate solvent was used to extract active ingredients from the tomato paste. Accurately weighed samples of dried tomato paste powder (approx. 40 grams) were placed in a cellulose extraction thimble (Wisd-Korea) and covered with glass wool. The thimbles were placed in the Soxhlet extraction unit and extracted for 18 hours with 500 mL of diethyl acetate. The remaining solvent was then evaporated in a vacuum-rotating evaporator (IKA-Korea). An allowance was made for the sample that had been removed for extract determination. The dried concentrate containing the extracted lycopene was weighed.

2.3. GC-Mass analysis

Gas chromatography-mass spectrometry (GCMS) was employed using an automated pyrolysis technique on a Shimadzu GCMS-QP 2010 plus instrument with electron impact ionization (at 70 eV) to identify various compounds within the extract. In the GCMS analysis, 1µl of the sample was introduced in split mode into the instrument. The injector temperature was maintained at 300°C, the interface temperature at 300°C, and the mass spectrometry (MS) scan ranged from 35 to 450 atomic mass units (AMU). A capillary column, specifically an RTX-5MS type, measuring 60 meters in length with an internal diameter of 0.25 mm and a film thickness of 0.25µm, composed of 5% diphenyl and 95% methyl polysiloxane, was used for the analysis. The temperature program within the oven began at 50°C for the initial 6 minutes and then increased to 280°C for the subsequent 21 minutes. Helium was employed as the carrier gas at a flow rate of 1 ml min-1. The mass spectrometry settings involved an ion source temperature of 200°C, electron impact ionization at 70 eV, and a mass range (BM) between 40 and 600 m/z. The extract compounds were identified by comparing the mass spectra to the library data within the GC-Mass system.

2.4. Antibacterial activity

The antibacterial effectiveness of three varying concentrations (50, 100, 150 mg/ml) of tomato ethyl acetate extracts, in comparison to the activity of two distinct antibiotics (Ciprofloxacin and amoxicillin), was assessed using the agar well diffusion method, following the approach outlined by reference Tepe et al. (2005), with minor adjustments. Staphylococcus aureus (3 isolates) and Pseudomonas aeruginosa (3 isolates), known pathogenic strains for both humans and animals, were utilized in the study. A volume of one hundred microliters of the inoculum (at 1 × 108 CFU/mL) for each microorganism was dispensed onto the Petri dish and then evenly spread on specific media. Subsequently, one hundred microliters of each concentration of the extracts were placed into the designated wells. The plates were left to incubate at 37 °C for 24 hours, and after overnight incubation, the diameter (in mm) of the resulting zone of inhibition was measured.

2.4. Antioxidant activity of plant extracts

2.4.1. DPPH radical scavenging assay

Spectrophotometric analysis was utilized to evaluate the capability of scavenging radicals associated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) molecules, with slight modifications to the approach outlined by Tepe et al. (2005). In this method, each extract received an addition of 50 µL at varying concentrations, combined with 5 mL of a 0.004% methanolic solution of DPPH. Following a 30-minute incubation period at room temperature, the absorbance of the samples was measured at 517 nm using methanol as a blank. All measurements were performed in triplicate. The calculation of the percentage inhibition of free radical scavenging activity was determined using the formula:

$$\text{Inhibition (\%)} = [(\text{Abs.}_{\text{control}} - \text{Abs.}_{\text{sample}})/(\text{Abs.}_{\text{control}})]\times 100 \quad (01)$$

In this context, $Abs._{control}$ signifies the light absorption of the control reaction, encompassing all elements except for the compound under investigation, whereas Abs. The sample pertains to the light absorption of the compound being tested with all other components. Determination of the extract concentration achieving a 50% inhibition (referred to as IC_{50}) was derived from a graph that correlates the percentage of inhibition with varying concentrations of the extract. To construct the calibration curve (Barros et al., 2003), ascorbic acid at final concentrations ranging from 10 to 100 µg/mL served as the benchmark standard for the antioxidant drug.

2.5. Statistical analysis

The experiments were carried out three times, and the findings were displayed as average values accompanied by their standard errors. Statistical analysis using one-way ANOVA was performed using SPSS Statistics software (Version 31, United States) with a significance level set at $p < 0.05$.

3. RESULTS

3.1. Extraction yield

The final yield percentage of the tomato ethyl acetate extraction (TEE) process (99%) for three patches was 31.38±0.76%, and the characteristics of the final product of tomato extract after complete dryness were tiny powder and dark red in color Figure 1.

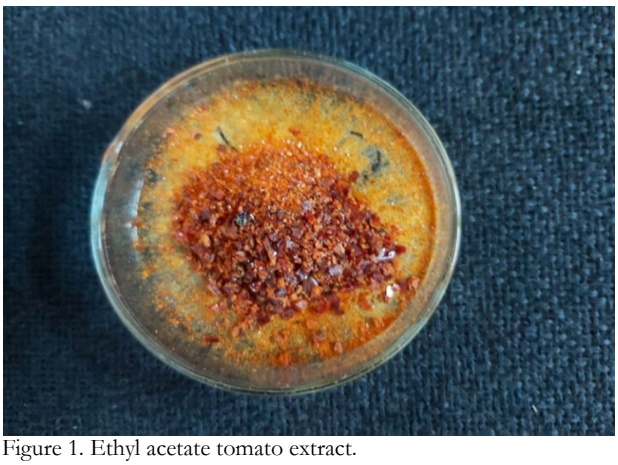

Figure 1. Ethyl acetate tomato extract.
Figura 1. Extrato de tomate com acetato de etila.

3.2. Composition of tomato extract

The chemical composition of Tomato ethyl acetate extract was detected by GC-Mass analysis. In the GC-Mass chromatogram, more than 29 individual compounds were identified in the extract after comparing the mass spectra with the NIST library, as shown in Table 1 and Figure 1. From the result of the chromatogram, it was observed that tomato extract contains myricetin 50.7%, n-hexadecanoic acid 19.9, salicylic acid 13.77, octenyl succinic acid 1.58, vanillic acid 1.41, dimethyl benzene 1.02%, Iso-quercitrin 1.02%, Hexadecanol 0.85%, Nomane-a-tomatidine 0.77%, a-tocopherol 0.76%, Homoserine 0.76% and other less quantity compounds.

Table 1. GC-Mass compounds of the Tomato extract.
Tabela 1. Compostos GC-Mass do extrato de tomate.

Compounds	RT	Area (%)
Acetic acid	2.035	0.35
Dimethylbenzene	4.163	1.02
Methylcyclopentanone	4.479	0.30
Vanillic acid	6.029	1.41
Butanoic acid	7.214	0.23
Protocatechuic acid	8.057	0.22
P-coumaric acid	9.327	0.42
1-6 octadiene	10.01	0.17
Oxiranemethanol	10.16	0.45
6-octadanol ,3,7-dimethyl	10.29	0.34
Homoserine	10.73	0.76
Naringenin	13.19	0.24
N-hydroxybenzoic acid	13.54	0.55
Salicylic acid	14.52	13.77
1,5-hepadiene, 2,6 dimethyl	15.11	0.47
Octenylsuccinic anhydride	16.17	1.58
Veridiflorol	17.31	0.35
n-hexadecanoic acid	19.46	19.9
15- heptadecenal	21.19	0.72
Propanoic acid	23.11	0.29
Heptadecane	23.73	0.31
2-bromotetradecene	24.25	0.33
Nonane-a-tomatidine	25.81	0.77
Cyclopropane carboxylic acid	27.44	0.46
P-hydroxy-benzoic acid	28.11	0.36
a-tocopherol	29.36	0.76
Myricetin	30.56	50.7
Hexadecanol	31.07	0.85
Iso-quercitrin	31.99	1.02

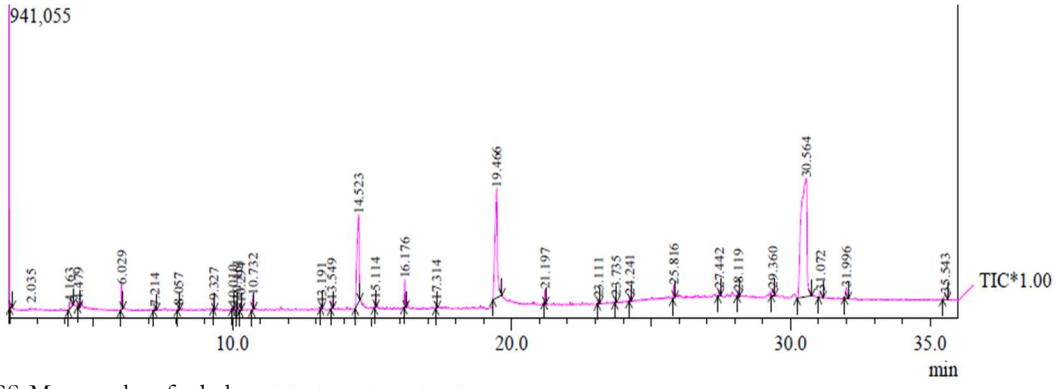

Figure 2. GS-Mass peaks of ethyl acetate tomato extract.
Figura 2. Picos de massa GS do extrato de tomate com acetato de etila.

3.3. Antibacterial screening

The sensitivity of both *S. aureus* and *P. aeruginosa* to the different concentrations (50, 100, 200 mg/mL) of tomato ethyl acetate extract and compared with two standard antibiotics (ciprofloxacin, amoxicillin) were tested in the culture media by agar well diffusion method. The results listed in Table 2 and Figure 3 showed a wide spectrum of antibacterial activity against both mentioned pathogenic bacteria. According to the extract concentration, the 150 mg/mL showed better antibacterial action against both bacteria as compared with other ethyl acetate extract concentrations with an average zone of inhibition of 18.14±0.98, 16.38±1.28 mm against *S. aureus* and *P. aeruginosa* respectively. At the same time, the 50 mg/mL showed less activity with average inhibition zones 11.08 ± 1.02 and 10.09 ± 0.76 mm against S. aureus and *P. aeruginosa*, respectively. In general, S. aureus was recorded as more sensitive to the tomato diethyl acetate extract concentrations as compared with the sensitivity of *P. aeruginosa* in culture media.

Table 2. Antibacterial activity of the tomato extract.

Tomato extract conc. (mg/mL)	Type of bacteria	
	S. aureus	*P. aeruginosa*
50	11.08±1.02	10.09±0.76
100	13.22±1.14	13.01±1.12
150	18.14±0.98	16.38±1.28
Ciprofloxacin	21.25±2.08	20.12±1.64
Amoxicillin	19.34±1.26	16.56±1.05
LSD(P<0.05)	1.78	

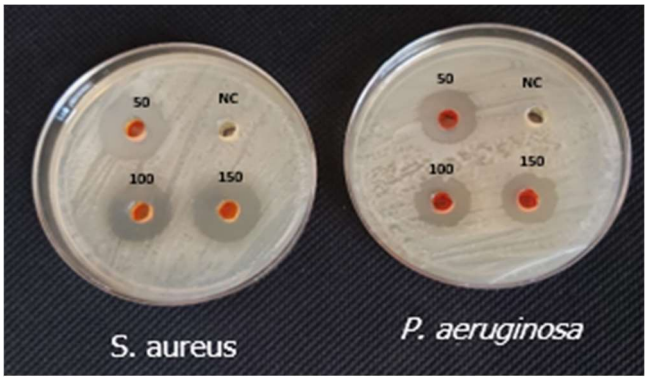

Figure 3. Antibacterial activity of concentrations of ethyl acetate tomato extract.
Figura 3. Atividade antibacteriana de concentrações de extrato de tomate com acetato de etila.

3.4. Antioxidant screening

As shown in Table 3 and Figure 3, the DPPH radical scavenging activity at 517 nm was used to measure the in vitro antioxidant (DPPH) activity of the 10 graded concentrations of ethyl acetate tomato extract about the ascorbic acid activity. Tomato extract dissolved in ethyl acetate had the greatest ability to scavenge the DPPH radical, with an IC50 value of 46.22 µg/mL, compared to 23.84 µg/mL for the standard drug (ascorbic acid). On the other hand, the results also showed the scavenging activity of the extract had a proportional relationship with the extracted figure's used concentration (DARWIN et al., 2003).

Table 3. Percentage of PDDH inhibition of tomato extract as compared with standard drug (ascorbic acid).
Tabela 3. Porcentagem de inibição de PDDH do extrato de tomate em comparação com o medicamento padrão (ácido ascórbico).

Concentration (µg/mL)	Tomato extract	Ascorbic acid
10	18.2±0.78	30.76±1.06
20	26.38±1.22	43.2±1.24
30	36.12±1.08	56.24±3.07
40	44.16±3.26	66.28±2.35
50	57.89±3.01	78.6±2.18
60	64.68±2.78	80.22±2.85
70	72.55±4.12	85.5±3.54
80	78.42±2.28	90.8±2.14
90	84.54±3.05	92.3±3.02
100	88.48±2.74	96.45±2.96
LSD(P<0.05)	3.18	
IC_{50} value	46.22 (µg/mL)	23.84 (µg/mL)

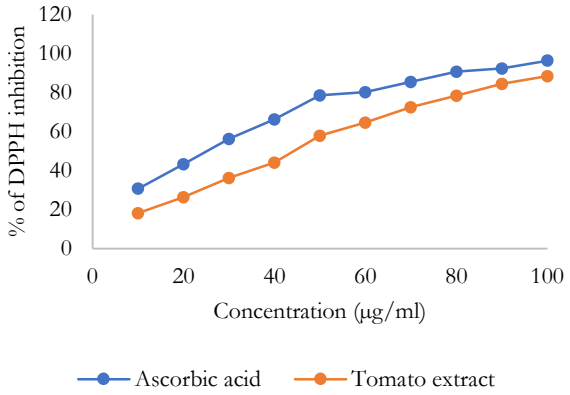

Figure 3. Percentage of PDDH inhibition of tomato extract and ascorbic acid.
Figura 3. Porcentagem de inibição de PDDH de extrato de tomate e ácido ascórbico.

4. DISCUSSION

Finding naturally occurring plant chemicals with both antioxidation and antibacterial properties becomes the ideal target in food additive development (DOMÍNGUEZ et al., 2020). Tomatoes extracts are rich in a variety of active phytochemicals and nutrients with different biological and healthy beneficial medical activities (EBRAHIMABADI et al., 2010). Tomatoes not only contain lycopene and vitamin C but also additional antioxidants, including beta-carotene and phenolic substances like ferulic acid, hydroxycinnamic acid, chlorogenic acid, and flavonoids (KOOHSARI et al., 2021).

Among the most popular analytical techniques for revealing primary, significant metabolites such as organic and amino acids, sugars, sugar alcohols, phosphorylation intermediates, and lipophilic compounds in gas chromatography-mass spectrometry (GC-MS), which is used to analyze various plant samples. Using this method to analyze samples of diverse plant extracts might assist in determining their composition and enhance their biological characteristics (SAUCEDA et al., 2017). GC-MS analysis of tomato ethyl acetate extract showed 29 constituents. The major components were myricetin 50.7%, n-hexadecanoic acid 19.9, salicylic acid 13.77, octenyl succinic acid 1.58,

vanillic acid 1.41, dimethyl benzene 1.02%, Iso-quercitrin constitutes 1.02% of it. These isolated compounds may significantly contribute to antibacterial and antioxidant activity, especially due to their content of phenolic compounds (CAVALIERE et al., 2018). Gas chromatography-mass spectrometry analysis of the tomato extract might support its biological characteristics and reveal its composition. It is highly challenging to identify every chemical by chromatography since natural products contain many active compounds and isomers and their diversity (MA et al., 2014).

The ethyl acetate extract from tomato showed antimicrobial activity against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (pseudomonas aeruginosa) in culture media. Previous works have shown that different tomato parts and extracts showed antibacterial activities against various microorganisms (CHORIANOPOULOS et al., 2004; VIEITEZ et al., 2018). A possible explanation for these results could be attributed to the variety of chemical components in tomato extract, which contains significantly higher amounts of flavonoid glycosides, which have been confirmed to be present by GC-Mass. The extract's many bioactive components exhibit antibacterial activity due to their capacity to permeate the bacterial membrane and impede the functioning characteristics of the cell (TAVEIRA et al., 2010). Phenolic acids are good possibilities for antibacterial compounds that work against infections and germs. Phenolic chemicals can cause microbial cell death by altering the permeability of the cell, damaging the cytoplasmic membrane, interfering with the cellular energy generating system (ATP), and disrupting the proton motive force (KAVITHA et al., 2017). The present work found that S. aureus is more susceptible than E. coli to the different tomato extract concentrations. The physical makeup of the cell wall distinguishes Gram-positive bacteria from Gram-negative bacteria. According to (DOMÍNGUEZ et al., 2020), Gram-negative bacteria appear less vulnerable to the effects of natural extracts because of the external cell membrane surrounding their cell wall, which contains a significant quantity of lipopolysaccharides. Products made from tomatoes are high in bioactive ingredients, including lycopene and ß-carotene, which have been demonstrated in several studies to have potent antioxidant and antibacterial properties. Therefore, these bioactive components are good candidates for creating innovative drugs due to their health-promoting qualities. Kavitha et al. (2021) mentioned that the effectiveness factor of the tomato is lycopene, which possesses antibacterial and antifungal properties.

The high phytochemical profile of tomato seeds makes them a potential natural source of antioxidants (COLLINS et al., 2022). They are a storehouse of bioactive carotenoids and phenolic compounds essential for regulating several physiological and metabolic processes in the body. Tomato parts contain phenolic compounds (flavonoids, phenolic acids), carotenoids (β-carotene, lycopene), and nucleosides (guanidine, inosine, adenosine) in particular (LI et al., 2020). In the hippocampus and cerebral cortex regions of the brain, the pre-treatment of rats with tomato pomace powder also reduced the lipid peroxidation product malondialdehyde and enhanced the activities of antioxidant enzymes glutathione peroxidase and superoxide dismutase (COLLINS et al., 2022). It has been found that tomatoes contain compounds known to be antioxidants, including carotenoids, phenols, flavonoids, condensed tannins, and ascorbic acid. Tomatoes include iron, potassium, lycopene, vitamin C, and folate as minerals and phytochemicals. Tomatoes are rich in flavonoids, hydroxycinnamic acid, chlorogenic acid, homo-vanillic acid, ferulic acid, and lycopene, a strong lipophilic antioxidant. Tomatoes are a helpful nutraceutical due to interactions between their various components and their lycopene content (ROSA-MARTÍNEZ et al., 2023).

5. CONCLUSIONS

According to GC-MS tests, 29 distinct components were found in the tomato extract. The major compounds were found to be myricetin 50.7%, n-hexadecenoic acid 19.9, salicylic acid 13.77, octenyl succinic acid 1.58, vanillic acid 1.41, dimethyl benzene 1.02%, Iso-quercitrin 1.02%. The tomato ethyl acetate extract showed high antioxidant activity with an IC50 value of 46.22 µg/mL. Compared with ciprofloxacin and amoxicillin, the tomato extract exhibited moderately antibacterial activity, which suggested that the tomato extract showed a broad-spectrum antibacterial activity.

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Data availability: The data used to verify the findings of this study can be obtained by contacting the corresponding author upon request

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