**Integration of Gas Chromatography Mass Spectrometry Analysis of Phytochemicals in *Ziziphus jujuba* Targeting Multidrug Resistant *Shigella* speciesComplemented by Molecular Docking Study.**

***Ahmed ALSHARKSI1, Adam MUSTAPHA2, Serhat SIREKBASAN 3 and Tuğba Gürkök-TAN 4***

 ***1*** Department of Biology, Faculty of Sciences, Cankiri Karatekin University, Çankırı, Turkey,ahmedalsherkas87@gmail.com

2*Department of Microbiology, Faculty of Life Sciences, University of Maiduguri, Borno State, Nigeria.*

**3** Department of Medical Laboratory Techniques, Eldivan Vocational School of Health Services, Cankiri Karatekin University, Çankırı, Turkey; serhats@karatekin.edu.tr

4 Department of Field Crops, Food and Agriculture Vocational School, Cankiri Karatekin University, Çankırı, ,Turkey,t.gurkok@karatekin.edu.tr

**Abstract**

Antibiotic resistance emerging at the rate that surpass the development of new class of antibiotic. This study was conducted to explore the antimicrobial activity of Ziziphus jujuba extract against multi-drug resistant Shigella species. In-vitro antibacterial activity was assayed using agar diffusion technique. Total biochemical profile of the extract was screen using Gas Chromatography Mass Spectroscopy (GC-MS) analysis. In silico molecular docking was employed to determine activities of the compounds against PDB ID: Ix7i and the binding energies identified the potency of the compounds. The results demonstrated significant inhibitory effects using methanol solvent their 7.00mm at 25ml/dl, 7.00mm at 50ml/dl, 71.00mm at 75ml/dl and 20.00mm at 100ml/dl respectively. The result obtain from on the docking results CID-537118 had the best binding of -7.89kcal/mol and was analyze to interact with 1 hydrogen bond each with Lys32, (distance = 2.84Å). Likewise, CID-12760132 possess the binding affinity of -7.31kcal/mol interacting 2 hydrogen bonds with Lys32 (distance = 3.02Å) and Ser10 with (distance = 2.56Å), CID-56634694 has a binding energy of -7.30kcal/mol which interact with RNA dependent RNA polymerase via 2 hydrogen bond with Lys32 (distance = 2.98Å) and Ser10 with (distance = 2,55Å), Compound with Pubchem I.D of CID-101771, has binding energy of -7.25 kcal/mol and was examined to interact with via 2 hydrogen bond with Tyr228(distance =2.47Å) and Lys32 (distance =2.89Å). Compound with Pubchem I.D of CID-537118, has binding energy of -7.29 kcal/mol and was examined to interact with via 1 hydrogen bond with Tyr228 (distance =2.88Å). Compound with Pubchem I.D of CID-985, has binding energy of -5.00 kcal/mol and was examined to interact with via 1 hydrogen bond with Lys32 (distance =2.84Å) and Lys32 (distance =2.89Å). The results underscore the potential of Ziziphus jujuba as a potential source of source of bioactive compound with antibacterial activity and could be considered as an alternative therapeutic strategies and candidate for drug development.

Keywords: Multidrug resistant, Ziziphus jujube, Shigella, Molecular docking, In-vitro evaluation.

1. **INTRODUCTION**

A major adversity for the mortality and morbidity amongst humans and animals are infectious diseases [1]. Antibiotics serve the main basis for the therapy of microbial infections, since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical order that this would lead to the ultimate eradication of infectious diseases [2]. However, this headway is challenged by one of the 21st global challenge; antibiotic resistance and decline in development of novel antimicrobial agent [2].

Medicinal plants have been rich sources of bioactive compounds and been employed in the treatment of many infection in history [3]. Previous studies revealed the application of plants as source of bioactive compounds with antibacterial activities and have been recognized as promising in the process of drug discovery [4-7]. In fact, the use of medicinal plant have been recognized by World Health Organization (WHO) as widely used in many parts of the world in the treatment of many diseases [4]. Mustapha *et* *al*., [5] investigated the antibacterial activity of *Lawsonia inermis* Linn against multidrug resistant *Klebsiella pneumoniae*, and, Adeniyi *et* *al*., [6] confirmed the biological activities of plants found in certain regions of Ghana. In another review, plants such as *Matricaria recutita* L. *Hypericum perforatum* L. *Equisetum arvense* L. have exhibited wide range of antibacterial activity against pathogens [7].

*Ziziphus jujube* Mill, is a plant in a member of [Rhamnaceae](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/rhamnaceae) , and it is described as a medium-sized with seeds and fruit and has been widely used as medicine and food globally [8]. The plant is indigenious to many parts of the world and has been used for medicinal and nutritional purposes [9-11]. *Z. jujuba* is a home to many phytochemicals including amino acids, alkaloids, calcium, [cardiac glycoside](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/cardiac-glycoside), [flavonoids](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/flavonoid), lipids, phosphate, potassium, protein, [saponins](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/saponin), sugar, [tannin](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/tannin), and [terpenoids](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/terpenoid) [12-14]. There is concern for the increase trends of antibiotic resistance by *Shigella* spps to important lines of treatments such as third-generation cephalosporins (TGC), azithromycin and fluoroquinolones, [15, 16]

The discovery and development of new drugs design is a time-consuming and costly process, there is need for two phased search of potential bioactive compounds [17]. The aim of this study is to identify the chemical composition of *Zizhipus mauritiana* and investigated the antibacterial activity of Z. *mauritiana* against multi-drug resistant *Shigella spp* using in-vitro and in-silico studies.

1. **Materials and Methods**

**Bacterial Isolates**

*Shigella* isolates were obtained from the Department of Microbiology, Faculty of Life Sciences, University of Maiduguri, Nigeria. Standard Microbiological and biochemical methods were used to phenotypically identify and characterize the *Shigella* isolates as described by Cheesbrough [18].

**Phytochemical Plants Extraction**

Fresh leaves of *Ziziphus jujube* M. were collected within Maiduguri Metropolis, Nigeria and taken to the Department of Botany, University of Maiduguri, Nigeria, and processed for preliminary phytochemical screening. Three solvents were used Methanol, Chroloform and Distilled water according to Gul et al., [19].

**GC-MS Analysis**

Gas Chromatography Mass Spectroscopy was used according to Idris et al. [20] Identification of chemical compounds relied on retention time from Gas Chromatography, and mass spectra were matched with the National Institute of Standards and Technology database.

**Antibacterial activity of *Ziziphus jujube***

The agar well diffusion method was employed to determine the antibacterial activities of *Ziziphus jujuba* M. extracts Mueller–Hinton agar (MHA) plates using 25, 50, 75 and 100 mg/mL concentrations. These plates were incubated at 37 ◦C for 24 h, followed by the measurement of the zones of inhibition in millimeters using a Vernier caliper. Each antibiotic underwent testing in triplicates over four days, and the averages were calculated (mean ± standard deviation).

**Preparation of Crystal of Ix7i**

 In this study, the Ix7icrystal structure complex, involving GDP and 9PC ligands (PDB ID: Ix7i), was sourced from the Protein Data Bank (PDB) as per the work by Berman *et al*., 2000. Following acquisition, the associated ligands were excised, the structure underwent a comprehensive cleanup process. Finally, the protein structure was optimized, and its energy was computed utilizing a specific program integrated into SwissPDViewer [21].

**Molecular docking**

This study selected compounds with specific physicochemical properties for docking investigations. AutoDock 4.2, an extension of the Python Molecular Viewer, was employed for these docking studies. In this process, the torsion bonds and side chains of the ligands were allowed to rotate freely, while Ix7i remained rigid. The binding energy of the protein–ligand complex was calculated using the formula by Hariono et al. [23].

 **Pharmacokinetic Analysis**

Following the docking studies, compounds exhibiting favorable binding energies were subjected to a secondary screening. This screening focused on evaluating their pharmacokinetic properties, encompassing absorption, distribution, metabolism, and excretion (ADME), to ensure their viability as potential drug candidates. Pharmacokinetic Screening: Tools Used include the AdmetSAR tool and ADME/TOX program. Toxicity Assessment: Additionally, the toxicity of each identified compound was assessed to ascertain their safety profile Tools Used include the DataWarrior tool ,AdmetSAR tool and ADME/TOX program (<http://lmmd.ecust.edu.cn/admetsar3/>).

1. RESULTS AND DISCUSSION

In the current study, the results of the phytochemical analysis were presented in table 1. Flavonoid, saponins and volatile oil were not detected and cardial glycoside only found in distilled water as solvent. The presence of some secondary metabolites could attribute to the antibacterial activity of the plant.

**TABLE 1.** Phytochemical Analysis of *using* different *Ziziphus jujuba* solvent

|  |  |  |
| --- | --- | --- |
| **S/No.** | **Phytochemical Constituents** |  |
| **Methanol** | **Chloroform** |  |
| **1** | **Flavonoid** | - | - |  |
| **2** | **Saponins** | - | - |  |
| **3** | **Alkaloid** | + | + |  |
| **4** | **Volatile Oil test**  | - | - |  |
| **5** | **Steroid** | + | - |  |
| **6** | **Tannin** | + | + |  |
| **7** | **Cardial glycoside** | - | - |  |

(-) indicate the absence, while (+) indicate the presence

The results of the GC-MS analysis revealed the different constituents of the phytochemicals, including their compound names, chemical formula, peak value and retention time. The compounds identified from GC-MS analysis revealed different molecular weight (MW) ranging from 142 to 524 in the 39 compounds detected.

**Antibacterial activity of *Ziziphus jujube***

The result of the antibacterial activity of the extracts of *Z. jujube* against *Shigella* ranging from 7.00-20.00mm for Methanol and 7.00-11.00 mm for Chloroform.

**Pharmacokinetic analysis and Docking score**

A molecular docking analysis was carried out on the twelve compounds to evaluate their binding energies with the *Shigella* protein (**Ix7i**). The molecular docking revealed free binding energies ranging from −0.00 kcal/mol to +8.07 kcal/mol.

*Ziziphus jujuba* a susceptibility testing was conducted in the research aiming to identify chemical compounds in, with potential drug activity against multidrug-resistant *Shigella*. Fresh leaves were collected, air-dried, and extracted using methanol and ethanol. The methanol extract exhibited a significant inhibition zone of 7.00mm at 25mg/dl. 7.00mm at 50mg/dl, 17.00mm at 75mg/dl and 20.00mm at 100 mg/dl concentrations. While in choloform solvent the extract showed inhibition zone at 25 mg/dl, 50 mg/dl, 75mg/dl and100mg/dl, and 7.00mm, 8.00mm, 10.00mm and 11.00mm respectively. This reveals the potential antibacterial effect of the plants against the bacterial isolates and could be attributed to the phytochemicals embedded.

The totals of 39 compounds were obtained from GC-MS analysis of *Ziziphus jujuba*, compounds were further filtered based on their physiochemical properties according to **Christopher A. Lipinski‘s rule of five or Pfizer’s rule of five (Molecular weight (≤500), Number of HBA (≤10), Number of HBD (≤5), MolLogP (≤5))**  to evaluate Drug likeness or determine the chemical and physical properties of pharmacological agent**.** This led to the selection of all the 39 compounds. These Molecules were assayed in docking studies using the **AutoDock 4.2 tool** to calculate the binding free of each protein-ligand complex. Based on the docking results CID\_537118 had the best binding of -7.89kcal/mol and was analyze to interact with RNA dependent RNA polymerase via 1 hydrogen bond each with Lys32, (distance = 2.84Å). Compound with Pubchem I.D of CID\_537118, has binding energy of -7.29 kcal/mol and was examined to interact with via 1 hydrogen bond with Tyr228 (distance=2.88Å). Compound with Pubchem I.D of CID\_985, has binding energy of -5.00 kcal/mol and was examined to interact with via 1 hydrogen bond with Lys32 (distance=2.84Å) and Lys32 (distance=2.89Å). Compound with Pubchem I.D of CID\_550119, has binding energy of -5.45 kcal/mol and was examined to interact with via 1 hydrogen bond with Tyr228(distance=2.98Å). Moreover, other compounds interact with RNA dependent RNA polymerase via the weak hydrophobic bond, these include; CID\_537083, CID\_537671, CID\_5364533, CID\_13760785, CID\_543346, CID\_554143, CID\_23618376, CID\_319211683, CID\_3449717, CID\_5284421, CID\_5319737, CID\_11748436, CID-5364509, CID-5366244, CID-9601436, CID-554143, CID-24585, CID-5367644, CID-5367644, CID-13760785, CID-5283646, CID-554143, CID-535324, CID-249903130, CID-5364495, CID-550072, CID-249914677, CID-6420608, CID-550119, CID-523023, CID-445070, and CID-638072.\_which possessed the binding energy of **-3.67**kcal/mol, **-3.67** kcal/mol,  **-4.25** kcal/mol,  **-5.88** kcal/mol, **-6.02** kcal/mol, **-4.82** kcal/mol, **-4.04** kcal/mol, -3.96 kcal/mol, **-0.00** kcal/mol, **-5.00** kcal/mol**-7.89** kcal/mol, **-4.63** kcal/mol, **-4.34** kcal/mol, **-4.12** kcal/mol, **-4.62** kcal/mol, **-3.72** kcal/mol, **-4.18** kcal/mol, **-4.89** kcal/mol, **-0.00** kcal/mol, **-4.74** kcal/mol, **-0.30** kcal/mol, **-4.38** kcal/mol, **-6.02** kcal/mol, **-3.59** kcal/mol, **-3.80** kcal/mol, **-7.43** kcal/mol, **+5.82** kcal/mol, **-3.46** kcal/mol, **+8.07** kcal/mol, **+8.07** kcal/mol, **+5.62** kcal/mol, **-5.04** kcal/mol, **-4.74** kcal/mol, **-4.55** kcal/mol, **-4.63** kcal/mol, respectively.

 Based on the docking analysis, 7 out of 39 compounds had a good binding energies with the protein, these compounds were further subjected to pharmacokinetic analysis to scrutinize the pharmacokinetic properties of individual compound (via **absorption, distribution, metabolism, excretion, and toxicity).** The properties such as **Human Intestinal absorption (HIA), Cytochrome P450 (CYP450 2D6) inhibition, and Blood-Brain Barrier (BBB)** were determined using **ADMETSAR 2.0 tool**.

The toxicity parameters such as Mutagenicity, Tumorigenicity, Reproducibility, and Irritability was accessed using the DataWarrior tool, and two compounds (CID- 3449717and CID-5364495) has **high mutagenicity**, one compound CID-609887 the others are none mutagenic. These compound CID-985 has high tumorigenic while two compounds with CID-5283646, CID-249903130 has low tumorigenic while the others are none tumorgenic.in the analysis, six compounds has high reproducibility (CID-101771, CID-537118, CID-319211683, CID-537118, CID-56634694, CID-5364495, CID-249914677, and CID-6420608) and the remaining compounds are non-reproducible. And finally, seven compounds has high irritability (CID- 537083, CID-543346, CID-537118, CID-985, CID-537118, CID-3449717, and CID-5364495) and CID-5364495 has low irritability, the rest of the compounds are none irritable.

1. **Conclusion**

The extracts of Z. jujube showed better inhibitory effect t of the *Shigelle* isolates due to the presence of the secondary metabolites in the plant. A total of 39 compounds with good affinities against RNA dependent RNA polymerase were selected and screened for pharmacokinetic properties. Seven compounds with desirable pharmacokinetic properties were selected. Therefore, these results could be considered as suitable prospective inhibitors of multidrug *Shigella* species.

**References**

1. The Lancet: Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050 2024; 404: 1199–226
2. de Kraker, M.E.A., Stewardson, A.J., & Harbarth S. (2016).Will 10 million people die a year due to antimicrobial resistance by 2050? *PLoS Med*, 13: e1002184
3. Isaac, A., Samuel, A.O., Woasiedem, T., Francis, A.A., & Lawrence,S.S. (2023).Applications of molecular docking in natural products-based drug discovery. <https://doi.org/10.1016/j.sciaf.2023.e01593>
4. Breijyeh, Z., & Karaman, R. (2024). Antibacterial activity of medicinal plants and their role in wound healing. *Futur J Pharm Sci* **10**, 68. <https://doi.org/10.1186/s43094-024-00634-0>
5. Mustapha, A., AlSharksi, A.N., Eze, U.A., Samaila, R.K., Ukwah, B.N., Anyiam, A.F., Samarasinghe, S., & Ibrahim, M.A. (2024). Phytochemical Composition, In Silico Molecular Docking Analysis and Antibacterial Activity of *Lawsonia inermis* Linn Leaves Extracts against Extended Spectrum Beta-Lactamases-Producing Strains of *Klebsiella pneumoniae*. *BioMed*, 4, 277–292. https:// doi.org/10.3390/biomed4030022
6. Adeniyi, A., Asase, A., Ekpe, P.K., Asitoakor, B.K., Adu-gyamfi, A., & Avekor, P.Y. (2018). Ethnobotanical study of medicinal plants from Ghana ; confirmation of ethnobotanical uses, and review of biological and toxicological studies on medicinal plants used in Apra Hills Sacred Grove, *J. Herb. Med*. 14 76–87, doi:10.1016/j.hermed.2018.02.001
7. Bittner,F.S., Rendeková, K., Mučaji, P., Nagy, M., & Slobodníková, L. (2012).Antibacterial Activity of Medicinal Plants and Their Constituents in the Context of Skin and Wound Infections, Considering European Legislation and Folk Medicine-A Review. *International Journal of Molecular Science.*22(19):10746. doi: 10.3390/ijms221910746.
8. Priya, A., Talever, S., Devender, P., & Himansu, C. (2023). An updated review of Ziziphus jujube: Major focus on its phytochemicals and pharmacological properties. Pharmacological Research - *Modern Chinese Medicine,* 8, 100297, <https://doi.org/10.1016/j.prmcm.2023.100297>.
9. Pareek S. Nutritional composition of jujube fruit Emirates J. Food Agric., 25 (6) (2013), pp. 463-470, [10.9755/ejfa.v25i6.15552](https://doi.org/10.9755/ejfa.v25i6.15552)
10. Lu, Y., Bao, T., Mo, J., Ni, J., & Chen, W. (2021). Research advances in bioactive components and health benefits of jujube (*Ziziphus jujuba* Mill.) fruit. *J Zhejiang Univ Sci B*. 15;22(6):431-449. doi: 10.1631/jzus.B2000594.
11. Gao, Q.H., Wu, C.S., & Wang, M., (2013). The jujube (Ziziphus jujuba Mill.) fruit: a review of current knowledge of fruit composition and health benefits. *J Agric Food Chem*, 61(14): 3351-3363. 10.1021/jf4007032
12. Asma, H.S.,  Moza, T.H.G.,  Hossain, M.A. (2016). Comparative evaluation of total phenols, flavonoids content, and antioxidant potential of leaf and fruit extracts of Omani Ziziphus jujuba L Pac. *Sci. Rev. A Nat. Sci. Eng.,* 18 (1) 78-83
13. Shams,N., Najafabadi, M.A., Sahari, M.B.,  Hamidi, Z.E. (2017). Effects of concentration method and storage time on some bioactive compounds and color of jujube (Ziziphus jujuba var. vulgaris) concentrate. *J. Food Sci. Technol*., 54 (9) (2017, August), pp. 2947-2955, [10.1007/s13197-017-2733-2](https://doi.org/10.1007/s13197-017-2733-2)
14. Miklavčič, A.V., ,  Baruca, A.A.,  Hladnik, M.,  Ota, A.,  Skrt, M.,  & Butinar, B*.* (2019). An integrated characterization of jujube (Ziziphus jujuba Mill.) grown in the north adriatic region

*Food Technol. Biotechnol*., 57 (1) (2019), 17-28, [10.17113/ftb.57.01.19.5910](https://doi.org/10.17113/ftb.57.01.19.5910)

15. Taneja, N., Mewara, A. (2016). Shigellosis: epidemiology in India. Indian J Med Res. 2016;143(5):565. doi: 10.4103/0971-5916.187104

16. Li, Y.L., Tewari, D., Yealy, C.C., Fardig, D., & M’ikanatha, N.M. (2016). Surveillance for travel and domestically acquired multidrug-resistant human Shigella infections—Pennsylvania, 2006–2014. Health Secur*it*y *14*(3):143–151. doi: 10.1089/hs.2016.0026

17 El-Beltagi, H.S., Aziz, S.M.S., Aboshady, A.I., Ibrahim, M. A. R., Ibrahim, M. F. M., Alenezi, M. A., Darwish, D. B. E., Al-Qahtani, S. M., Al-Harbi, N. A., & Darwish, H. (2023). Isolation and Identification of Flavonoids from Black Cumin (Nigella sativa) by HPLC-MS and In Silico Molecular Interactions of Their Major Compounds with Fusarium oxysporum Trypsin-like Serine Protease. *Separations*. 10, 360.

18. Cheesbrough, M. (Ed.) Biochemical tests to identify bacteria. In Laboratory Practice in Tropical Countries; Cambridge Edition; Cambridge University Press: Cambridge, UK, 2002; pp. 36–70.

19. Gul, R., Jan, S.U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. *Science World Journal*, 5873648.

20. Idris, A.H., Haruna, Z., Iliyasu, M.Y., Sahal, M.R., Inusa, T., Salisu, A., Isma’il, S., Umar, R.D., Kabeer, Z.M.; Tahir, H. (2023). Antibacterial Activity of Lawsonia inermis Leaf Extracts against Multidrug-resistant *Pseudomonas aeruginosa* from Infected Wounds. *European Journal of Medicinal Plants*, 34,1–8

21. Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., & Ferrin, T.E. (2004). UCSF Chimera—A visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

22. Morris, G.M., Huey, R., Lindstorm, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., & Olson, A.J. AutoDock4 and AutoDockTools4: Automated Docking with Selective receptor flexibility. J. Comput. Chem. 1998, 30, 2785–2791.

23. Hariono, M., Abdullah, N., Damodaran, K.V., Kamarulzaman, E.E., Mohamed, N. Hassan, S.S. Shamsuddin, S., & Wahab, H.A. (2016). Potential new H1N1 neuraminidase inhibitors from ferulic acid and vanillin: Molecular modelling, synthesis and in vitro assay. Scientific Report, 6, 38692.