PHYTOCHEMICAL SCREENING OF GARLIC (*ALLIUM SATIVUM* L.) BULBS AND BALANITES (*BALANITES AEGYPTIACA* DEL.) FRUIT AND THEIR INHIBITION EFFECTS ON *ESCHERICHIA COLI*

Mohammad Hazaimeh
Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah-11952, Saudi Arabia
Corresponding author: md.khalaf@mu.edu.sa

ABSTRACT: *Resistance of some microbes to drugs is the subject of recent research that directed the effort towards natural products to solve these problems. The objective of this work was to run phytochemical screening for garlic (*Allium sativum*) bulbs and Balanites (*Balanites aegyptiaca*) fruit and to test their inhibition effects on Escherichia coli (E. coli). Samples of Balanites fruits and garlic bulbs were brought from the local market whereas, a pure and identified strain of E. coli sample was brought from the Central Medical Laboratory and was cultured on Eosin Methylene Blue (EMB) Agar. The phytochemical screening of both products was run following the standard methods. Polar extracts of both B. aegyptiaca fruits and A. sativum bulbs were prepared to test their inhibition activities on E. coli stain through the agar well diffusion method for 72 hours. The GC-MS data revealed that Balanites fruit contained saponins, tannins, glycosides, flavonoids and terpinoids, whereas garlic bulbs contained glycosides, sterols, flavonoids, resins, and terpinoids. Garlic bulbs at the concentrations of 127.78 and 25.56 mg/ml inhibited completely the formation of E. coli colony, while the concentration of 5.11 mg/ml showed an average inhibition zone of 19.7 mm which was almost about that of the positive control cityzol (average of 19.3 mm). Balanites fruits at the concentrations of 241.71 mg/ml caused a mean inhibition zone of 18.67 mm on E. coli colony after 72 hours, which was statistically similar to that of the positive control cityzol It was clear that garlic bulbs at the tested concentrations have a potential inhibitory effect better than that of Balanites fruit on E. coli local strains. There was a clear positive correlation between concentrations and zones of inhibition.*

Keywords: Microbial Resistance, Inhibition Activities, Phytochemical, Garlic Bulbs, Balanites Fruit

1. INTRODUCTION

Escherichia coli is a rod-shaped, coliform, and Gram-negative bacterium that is commonly found in the large intestine of humans [1]. Most *E. coli* strains are harmless, but some serotypes are sometimes responsible for food contamination [2]. The harmless strains can benefit their hosts by producing vitamin K [3], and preventing colonization with some pathogenic bacteria [4]. *E. coli* mostly gets to man through contaminated food or water, and it can easily transmit between humans [5]. When infected with *E. coli*, the symptoms involved fatigue, vomiting, severe abdominal cramps, watery or bloody diarrhea, and fever [6].

E. coli constitute about 0.1% of gut microbiota [7]. *E. coli* is able to survive for many days and grow outside a host [8], this makes them a potential bio-indicator to test for fecal contamination [9]. Plants as a source of natural compounds play a vital role in the maintenance of human health since. The natural compounds were used by humans as a traditional medicine to treat different diseases and infections [15]. Garlic (*Allium sativum* L.), family Liliaceae is the most commonly used plant in folk medicine. It has a pungent odor resulting from sulfur-containing compounds (e.g., allicin). Many cultures recognized garlic for the prevention and treatment of some diseases such as cholesterol metabolism, cardiovascular disease, atherosclerosis, and cancer [14]. Recent research focused on heart, cancer, and infectious diseases in addition to antioxidant activity. Garlic found to have antihypertensive, antioxidant, and antimicrobial action [10].

The desert date: *Balanites aegyptiaca* Del., family Zygophyllaceae, is a spiny shrub or tree commonly distributed in drylands of Africa and South Asia. Chemically, it contains some organic compounds, e.g., protein, lipid, carbohydrate, alkaloid, saponins, flavonoids, and organic acids, in addition to balanitoside, balanitin-3, balanitin-6 and -7, balagyptin, and diosgenin glycosides. Traditionally, it is commonly used against various ailments i.e. jaundice, intestinal worm, dysentery, diarrhea, and stomach aches [11]. The present work aimed to run phytochemical screening for garlic bulbs and Balanites fruit to identify the main classes of the organic compounds before testing the inhibition effect of the polar extracts of these products on *E. coli* strain using the agar well diffusion method.

2. MATERIALS AND METHODS

Samples

Samples of *Balanites aegyptiaca* fruits and *Alium sativum* bulbs were brought from the local market areas of Wad Medani city, Gezira State, Sudan. The pure and identified strain of *E. coli* sample was brought from the Central Medical Laboratory, University of Gezira.

Culture of *E. coli*

E. coli was cultured on a selective medium: Eosin Methylene Blue (EMB) Agar at 39:1000 (g:ml water) in sterilized plastic Petri-dishes (each containing 10-15 ml of EMB medium).

Preparation of the polar extracts

Polar extracts of both *B. aegyptiaca* fruits and *A. sativum* bulbs were prepared by soaking a certain weight of each product in a certain volume of acetone for 24 hours, then filtered and poured into a clean Petri-dish and let it to dry by evaporation of acetone solvent under room temperature. The dry product of each extract was then weighed and mixed with a certain volume of water to prepare five different concentrations of each product.

The phytochemical screening

The phytochemical screening on the dry powders of both products for the presence of the main classes (saponins, tannins, glycosides, sterol and steroids, flavonoids, alkaloids, resins, and terpinoids) was run following the methods of [12].

Inhibition activity tests

Inhibition activities of *B. aegyptiaca* fruits and *A. sativum* bulbs were run through the agar well diffusion method. *E. coli* samples were streaked on the Petri-dishes, and immediately, on the center of each Petri-dish, a small disc paper (4 mm diameter) was impregnated for 5 minutes in one of the prepared concentrations placed, then incubated for 72 hours at 37^{o}C.

Cityzol 500 (metronidazole; produced by Cityphar Pharmaceutical Industry, Sudan, production date: June 2021) at a concentration of 20 mg/ml (positive control) and negative control were also set the same way. The formation of *E. coli* colonies at the negative control Petri-dishes considers the sign to calculate the inhibition zones formed in the rest of the Petri-dishes. The distance (in mm) between the disc paper in the middle of each Petri-dish and the nearest colony was taken as an inhibition zone for each concentration tested. All tests were triplicate.

Statistical analysis

The simple descriptive statistics and the least significant differences (LSD) among the tested concentrations and negative and positive control were used. Results were expressed as mean ± standard deviations (SD). Data with different letters reflected data with different levels of significance.

3. Results and Discussion

Phytochemical screening

The sample of Balanites fruit mesocarp revealed the presence of saponins, tannins, glycosides, flavonoids, and traces of terpenoids (Table, 1). These results agreed with the work of [11], who detect the presence of protein, lipid, carbohydrate, alkaloids, saponins, flavonoids, organic acid (rutinoside and rhamnogalactoside, diosgenin, and glucopyranosyl) and soluble tannins, except the presence of alkaloids in this study. Garlic bulbs show the presence of glycosides, sterols, flavonoids, resins, and terpenoids (Table, 1). This result was confirmed with the work of [12], except for the absence of saponins in this study, and this may be due to differences in time, location, and even garlic variety.

Table (1) The presence (+) of the main phytochemicals on Balanites and Garlic products

Phytochemicals	Balanites fruit mesocarp	Garlic bulbs
Saponins	+	-
Tannins	+	-
Glycosides	+	+
Sterols	-	+
Flavonoids	+	+
Alkaloids	-	-
Resins	-	+
Terpenoids	+	+

Inhibition activity of garlic bulb

Garlic bulbs at the concentrations of 127.78 (Figure, 1) and 25.56 mg/ml inhibited completely (25 mm) the formation of *E. coli* colony in all three Petri-dishes after 72 hours, while the concentration of 5.11 mg/ml showed an average inhibition zone of 19.7 mm which was almost about that of the positive control cityzol (average of 19.3 mm). The concentrations of 1.02 and 0.20 mg/ml showed considerable inhibition zones (10.0 and 7.0 mm, respectively; Table, 2). The inhibition activity revealed by garlic bulbs may be due mainly to its chemical composition in addition to its distinguish volatiles (terpenoids). A similar conclusion was suggested by [13] who stated that: natural product extracts that contain flavonoids and terpenoids were found to be active on various *E. coli* virulence traits.

Table (2) Inhibition zone (mm) of *E. coli* caused by Garlic bulb aqueous extract after 72 hrs

Conc (mg/ml)	Rep-1	Rep-2	Rep-3	Mean±SD
127.78	25	25	25	25 ± 0^{a}
25.56	25	25	25	25 ± 0^{a}
5.11	22	20	17	19.7 ± 1.5^{b}
1.02	12	10	8	10 ± 1.2^{c}
0.20	10	7	4	7 ± 1.7^{d}
Control	0	0	0	0 ± 0^{e}
Cityzol 500 (20 mg/ml)	20	20	18	19.3 ± 0.7^{b}

Inhibition activity of Balanites fruit

Balanites fruits at the concentrations of 241.71 mg/ml caused a mean inhibition zone of 18.67 mm in *E. coli* colony after 72 hours, which was statistically similar to that of the positive control cityzol (average of 19.3 mm). The concentrations of 48.34 and 9.67 mg/ml showed considerably high inhibition zones (12.33 and 10.67 mm, respectively). Relatively low inhibition zones were caused by the concentrations of 1.93 and 0.39 mg/ml (8.67 and 5.3 mm, respectively, Table, 3). The inhibition activity revealed by Balanites fruits may refer mainly to their chemical composition. [11] found that aqueous extract of *B. aegyptiaca* has an antibacterial effect against *Salmonella typhi*. Also, the activity of this plant extract was comparable with those of antibiotics commonly used for treating typhoid fever. This plant possesses saponins,

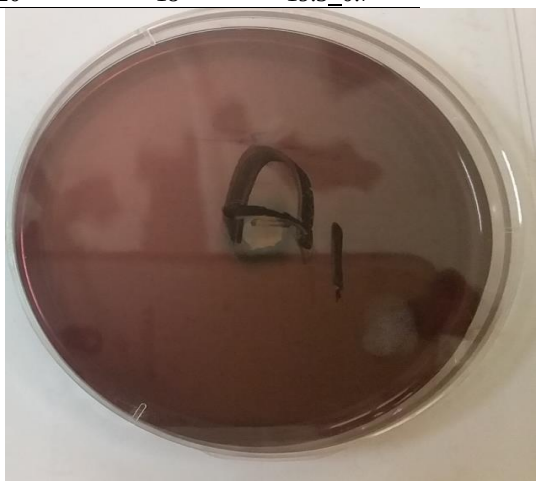

Figure (1) the Garlic bulb inhibition test on *E. coli* strain at conc. (127.78 mg/ml)

Table (3) Inhibition zone (mm) of *E. coli* caused by *B. eagyptiaca* fruit aqueous extract after 72 hours

Conc (mg/ml)	Rep-1	Rep-2	Rep-3	Mean±SD
241.71	18	18	20	18.67 ± 0.67^{a}
48.34	12	15	10	12.33 ± 1.45^{b}
9.67	10	12	10	10.67 ± 0.67^{b}
1.93	8	10	8	8.67 ± 0.67^{c}
0.39	8	5	3	5.3 ± 1.5^{d}
Control	0	0	0	0 ± 0^{e}
Cityzol 500 (20 mg/ml)	20	20	18	19.3 ± 0.7^{a}

tannins, phenols, and anthraquinones which were combined and responsible for its activity.

GC-MS chromatogram for the aqueous extracts

The main compounds that were identified using the GC-MS database from garlic bulb aqueous extract (Table, 4) were sucrose (19.02%), diallyldisulfid-S-oxid (17.52%; which is well known as allicin), acetaldehyde (8.62%), 2-Hydroxy-gamma-butyrolactone (7.13%), 2-Propanone (7.10%; the common acetone), propanoic acid (7.04%), trisulfide, allyl trisulfide (6.22%; which is well known as allitridin), acetic acid (6.21%), heptadecanoic acid (6.11%) and other traces (less than 2%).

Table (4) Details of the identified compounds from garlic bulbs aqueous extract

No	Compound name	Mol. formula	Mol. weight	Area %
1	Sucrose	$C_{12}H_{22}O_{11}$	342	19.02
2	Diallyldisulfid-S-oxid	$C_6H_{10}OS_2$	162	17.52
3	Acetaldehyde	C_2H_4O	44	8.62
4	2-Hydroxy-gamma-butyrolactone	C_4H_6O	120	7.13
5	2-Propanone	C_3H_6O	58	7.10
6	Propanoic acid	$C_3H_6O_2$	74	7.04
7	Trisulfide, allyl trisulfide	$C_6H_{10}S_3$	178	6.22
8	Acetic acid	$C_2H_4O_2$	60	6.21
9	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	6.11
10	4H-Pyran-4-one	$C_5H_4O_2$	96	4.07
11	Mononitromesitylene Benzene, 1,3,5-trimethyl-2-nitro	$C9H_{11}NO_2$	165	3.20
12	Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	254	3.12
13	Bisoflex Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	2.84
14	Other traces			>2.0

The main compounds that were identified using the GC-MS database from Balanites fruit aqueous extract (Table, 5) were the diosgenin 3-O-beta-D-glucopyranoside (24.13%) and 2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose (18.04%) which considered as a product of the hydrolysis of balanitin-6 and -7 (the steroidal glycosides). The database also detected -β-D-glucopyranosyl-(1→4) (9.09%), α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose (7.26%), 3β, 20S,22R,25R- 26-(β-D-glucopyranosyloxy) the protogracillin (steroid saponins; 7.22%), -22-methoxyfurost-5- en-3-yl β-D-xylopyranosyl-(1→3) (7.12%; the steroidal glycosides).

Table (5) Details of the identified compounds from Balanites fruit aqueous extract

No	Compound name	Mol. formula	Mol. weight	Area %
1	Diosgenin 3-O-beta-D-glucopyranoside	$C_{33}H_{52}O_8$	576	24.13
2	2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose	$C_{12}H_{22}O_{10}$	326	18.04
3	Balanitin	$C_{51}H_{82}O2_2$	1047	12.24
4	-β-D-glucopyranosyl-(1→4)	$C_{18}H_{32}O_{16}$	504	9.09
5	α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose	$C_{12}H_{22}O_{10}$	326	7.26
6	(3β,20S,22R,25R)- 26-(β-D-glucopyranosyloxy)	$C_{51}H8_4O_{23}$	1065	7.22
7	-22-methoxyfurost-5- en-3-yl β-D-xylopyranosyl-(1→3)	$C_{15}H2_6O_{13}$	414	7.12
8	-spirost-5-en-3-yl acetate	$C_{29}H_{44}O_4$	457	5.17
9	3-beta-hydroxycholest-5-ene-3,	$C_{27}H_{44}O_3$	402	4.04
10	2-decyl-2-{[(4-O-alpha-D-glucopyranosyl-	$C_{47}H_{88}O_{22}$	1005	3.52
11	Other traces			>3.0

CONCLUSION

Balanites fruit contained saponins, tannins, glycosides, flavonoids, and terpenoids, whereas garlic bulbs contained glycosides, sterols, flavonoids, resins, and terpenoids. Garlic bulbs at the concentrations of 127.78 and 25.56 mg/ml inhibited completely the formation of the *E. coli* colony after 72 hours, while the concentration of 5.11 mg/ml showed an average inhibition zone of 19.7 mm which was almost about that of the positive control cityzol (average of 19.3 mm). Balanites fruits at the concentrations of 241.71 mg/ml caused

a mean inhibition zone of 18.67 mm in *the E. coli* colony after 72 hours, which was statistically similar to that of the positive control cityzol (average of 19.3 mm). The concentrations of 48.34 and 9.67 mg/ml showed considerably high inhibition zones (more than 10.0 mm). It was clear that the garlic bulb at the tested concentrations has a potential inhibitory effect better than that of Balanites fruit on *E. coli* local strains. There was a clear positive correlation between concentrations and zones of inhibition.

GC-MS database identified sucrose, allicin, acetaldehyde, acetone, allitridin, acetic acid, and other traces from garlic bulb aqueous extract, while diosgenin, balanitin (the steroidal glycosides), Protogracillin (steroid saponins), and steroidal glycosides from Balanites fruit aqueous extract.

REFERENCES

1. O. Tenaillon, D. Skurnik, B. Picard, E. Denamur "The population genetics of commensal *Escherichia coli*". Nature Reviews: Microbiology., vol. 8, no. 3, pp. 207–217, 2010. https://doi.org/10.1038/nrmicro2298
2. CDC. "*Escherichia coli*. National Center for Emerging and Zoonotic Infectious Diseases". 2012. *https://www.cdc.gov/ecoli/general/index.html. Retrieved*.
3. R. Bentley, R. Meganathan. "Biosynthesis of vitamin K (menaquinone) in bacteria. Microbiological Reviews". vol. 46, no. 3, pp. 241–280, 1982. https://doi: 10.1128/mr.46.3.241-280.1982.
4. G. Reid, J. Howard, B. S. Gan. "Can bacterial interference prevent infection?" Trends in Microbiology. vol. 9, no. 9. pp. 424–428, 2001. DOI: 10.1016/s0966-842x(01)02132-1.
5. MayoClinic.org. "E. coli". Available at: *mayoclinic.org/diseases-conditions/e-coli/symptoms-causes/syc-20372058*, 2020.
6. Medlineplus. "*E. coli* Infections". 2021. Available at: *medlineplus.gov/ecoliinfections.html.*
7. P.B. Eckburg, E.M. Bik, C.N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, "Diversity of the human intestinal microbial flora". Science, vol. 308**,** no. 5728, 1635–1638, 2005. doi: 10.1126/science.1110591.
8. M.C. Montealegre , S. Roy, F. Böni, M.I. Hossain, T. Navab-Daneshmand, L. Caduff. "Risk Factors for Detection, Survival, and Growth of Antibiotic-Resistant and Pathogenic Escherichia coli in Household Soils in Rural Bangladesh". Applied and Environmental Microbiology, vol. 84**,** no. 24, 2018. e01978–18. doi: 10.1128/AEM.01978-18
9. A. Thompson. "E. coli Thrives in Beach Sands". Live Science. 2007.http:// www.livescience.com/health/070604_beach_ecoli.html. Retrieved 2014-07-03.
10. S. Yadav, N.A Trivedi, J. D, Bhatt. Antimicrobial activity of fresh garlic juice: An *in vitro* study. Ayu. vol. 36, no. 2. pp 203–207, 2015. doi: 10.4103/0974-8520.175548.
11. D. L. Chothani, H.U. Vaghasiya. "A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity". Pharmacogn. Rev., vol. 5 no. 9, pp 55–62. 2011. doi: 10.4103/0973-7847.79100
12. A.A. Khalifa, M.A.A. Kehail, "GC-MS and Phytochemical Screening of Garlic (*Allium sativum*) Bulbs and Ginger (*Zingiber officinale*) Rhizome". CPQ Nutrition, vol. 4, no. 1, pp. 1-7. 2019. https://www.cientperiodique.com/article/CPQNN-4-1-74.pdf
13. J.D. Dubreui, "Fruit extracts to control pathogenic *Escherichia coli*: A sweet solution". Heliyon, vol. 6, no. 2, 2020. e03410. doi: 10.1016/j.heliyon.2020.e03410.
14. A. Akbarpour, B. Kavoosi, M. Hosseinifarahi, Tahmasebi S, S. Gholipour. "Evaluation of Yield and Phytochemical Content of Different Iranian Garlic (Allium sativum L.) Ecotypes". International Journal of Horticultural Science & Technology, vol. 8, no. 4, pp. 385-400, 2021. https://search-ebscohost-com.sdl.idm.oclc.org/login.aspx?direct=true&db=awr&AN=153354031&site=eds-live.
15. A. Rizvi, B. Ahmed, M.S. Khan, H.S. El-Beltagi, S. Umar, J. Lee. "Bioprospecting Plant Growth Promoting Rhizobacteria for Enhancing the Biological Properties and Phytochemical Composition of Medicinally Important Crops". Molecules (Basel, Switzerland). vol. 27, no. 4, 2022. doi:10.3390/molecules27041407.