Short Research Article

PHYTOCHEMICAL SCREENING OF *ARTOCARPUS ODORATISSIMUS* (MARANG) SEED EXTRACT AND ITS ANTIMICROBIAL POTENTIAL AGAINST SELECTED SOIL BACTERIA

ABSTRACT

Aims: *Artocarpus odoratissimus*, often called locally as "marang," is well-known in the Philippines for its economic and nutritional value. No phytochemical studies have been conducted on *Artocarpus odoratissimus* cultivated locally in Davao City. The purpose of this study is to establish an initial chemical profile of the species by examining the secondary metabolites found in *Artocarpus odoratissimus* seed ethanol and methanol extract and determining their antimicrobial activity against selected soil microorganisms, particularly *Staphylococcus aureus* and *Escherichia coli*.

Study design: Laboratory experiments areconductedat San Pedro College, Davao City, to assess the qualitative phytochemical components and antibacterial activity of crude ethanolic and methanolic extracts of *A. odoratissimus* against selected soil bacteria.

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Place and Duration of Study: At San Pedro College, the study was conducted in the science laboratories between December 2022.

Methodology: Seed extracts were prepared using ethanol and methanol solvents through maceration techniques. Standard methods were employed to identify the presence of various phytochemicals qualitatively.The disc diffusion method was utilized to assess the effectiveness of the extracts against specific bacterial strains, including E. coli and S. aureus. The extent of inhibition zone formation was measured to determine the antimicrobial potential.

Results: Qualitative analysis revealed the presence of various secondary metabolites in *Artocarpus odoratissimus* seed extracts, including flavonoids, tannins, phenolics, saponins, steroids, terpenoids, and alkaloids. Furthermore, the seed extracts exhibited limited antimicrobial activity against *Escherichia coli and Staphylococcus aureus* as determined by the zone of inhibition assay.

Conclusion: Seed extracts from *Artocarpus odoratissimus* showed promise due to the presence of health-promoting compounds. While initial tests against bacteria were limited, further research can improve extraction methods and explore how these compounds work together for broader antibacterial effects. Future studies should also precisely measure the compounds, test against more bacteria, and explore potential uses based on the extract's profile.

Keywords: phytochemical screening, Artocarpus odoratissimus, antimicrobial potential, soil

1. INTRODUCTION

Soilborne microorganisms are essential in many biological processes, including nutrient cycling, plant nutrition, disease suppression, water purification, and soil structure maintenance [1].

Two of the most widespread bacterial species in many soil ecosystems are Escherichia coli and Staphylococcus aureus. These two bacterial species cause invasive health-related infections in human populations [2]. Escherichia coli has been found in tropical and subtropical soils [3], of which their survival rate is influenced by high and low temperatures [4], limited moisture [5], and variation in soil texture [6]. On the other hand, Staphylococcus aureus is found in different land uses, and their concentrations are associated with varying soil properties[7].

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Natural products or secondary metabolites have been recognized ascritical active ingredients in natural medicines for treating many diseases [8]. These natural products are derived from medicinal plants and are applied to many vector-borne diseases, fungal infections, and all other life-threatening ailments [9].

Artocarpus odoratissimus, a native fruit of Southeast Asia, including the Philippines, is one potential antibacterial agent that may be useful in controlling Staphylococcus aureus and Escherichia coli infections. In some areas, the fruit is known as marang or tarap. Its fruits and leaves are antibacterial. A chemical profiling study on marang conducted in Malaysia identified several phytochemicals that have been shown to support the potency of its antibacterial properties [10]. According to a separate study, the genus Artocarpus, which includes marang, contains secondary metabolites such as flavonoids, which have some antibacterial activity [11].

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However, no phytochemical investigation of Artocarpus odoratissimus locally grown in Davao City has been reported. Therefore,developing an initial chemical profile of the species would be interesting. Moreover, with the pressing health-related problem and the availability and medical potential of secondary metabolites found in this plant, this study aims to fill the research gap by investigating the secondary metabolites found in Artocarpus odoratissimus seed extract and to determine their antimicrobial impact against selected soil microorganisms, particularly Staphylococcus aureus and Escherichia coli.

2. MATERIAL AND METHODS

Study Design. This laboratory experiment evaluated the qualitative phytochemical components and the antibacterial activity of the crude ethanolic and methanolic extracts of *Artocarpus odoratissimus*against a selection of soil bacteria. The microbiology laboratory at San Pedro Hospital is where the test bacteria were obtained. At San Pedro College, the study was conducted in the science laboratories.

Ethanol Extraction of Plant Extract. 85 grams of powdered dried seeds were used to make the extracts, which were then macerated with 500 mL of 95% ethanol in an Erlenmeyer container, covered with aluminum foil, and allowed to sit for 4 days while being stirred intermittently. The filtrate (1) and residue were then produced by filtering the mixture through paper. The residue was then macerated once more (remacerated) with 250 mL of 95% ethanol, and the erlenmeyer was then covered with aluminum foil and left for 2 days while being stirred occasionally. The material underwent filtration to create filtrate after two

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days (2). In order to obtain a thick extract, the filtrates (1) and (2) were combined and evaporated using a rotary evaporator [12].

Methanol Extraction of Plant Extract. Plant seeds were mashed after being sun-dried. Each seed sample was macerated, and 200 g was extracted with 500 ml of methanol and placed in a mechanical shaker for 48 hours. The extract was filtered with No.1 Wattman filter paper. The resulting extracts were concentrated, dried in a rotary evaporator, and stored in the refrigerator for future use [13].

Phytochemical Analysis. Using standard methods [14], [15],[16] to qualitatively determine the contents the presence of flavonoids, tannins, phenolics, saponin, steroids, terpenoids, and alkaloids were analyzed in the seed extracts of *Artocarpus odoratissimus*.

Test for Flavonoids: Approximately 1 ml of extract and a few drops of 10% NaOH were added. Flavonoids were present because of the orange color.

Test for Tannins: 20 ml of water was used to boil the extract before it was filtered. FeCl3 was added in a few drops to the sample. If the color appears greenish-brown or black-blue, tannin was said to react favorably.

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Test for Phenolic: The extract is diluted with distilled water to a volume of 5 ml. Add a few drops of the neutral, 5% FeCl3 solution afterward. The dark green color indicates the presence of phenolic compounds.

Test for Saponin:The extract was cooked in a water bath with 20 mL of water. After shaking, the extract was let to stand for 15 minutes. The development of a stable foam revealed positive samples with saponin content.

Test for Steroids: 2 ml of extracts with 2 ml of conc, 2 ml of acetic anhydride was added. To create a deeper layer, H2SO4 was added. Changing from violet to blue, the color indicated the presence of steroids.

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Test for Terpenoids: A small amount of conc. was added to a mixture of 5 ml of extract, 2 ml of chloroform, 2 ml of acetic anhydride, and a few drops. Added was H2SO4. Terpenoids were present, as evidenced by the reddish violet color.

Test for Alkaloids: After treating the 5 ml of extract with Dragondroff's reagent and 2 ml of diluted hydrochloric acid, an orange-brown precipitate that formed indicated the presence of alkaloids.

Test Microorganisms. Gram-negative (E. coli) and Gram-positive (S. aureus) organisms were used in this study. The microorganism stock culture was obtained from San Pedro Hospital.

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Disc diffusion method. A stock solution was prepared by dissolving 0.1 g of the extract with 100 mL of their respective solvents (distilled water and absolute ethanol) to produce a final 100 mg/mL concentration. The stock solution was diluted to 50 and 100 mg/mL of extract. 20 μL of each dilution was impregnated into sterile, 6 mm in diameter blank discs. Distilled water and ethanol-loaded discs were used as negative controls for aqueous and ethanol extracts, respectively. Ampicillin for *E.coli* and oxacillin disc for *S. aureus*are positive controls. All discs were fully dried before the application on the bacterial lawn. The susceptibility of the test organisms to Artocarpus odoratissimus seed extract was estimated by measuring the diameter of zone inhibition in millimeters. The values are an average of three replicates (NARMS, C., 2008). Tables of the American Society for Microbiology, 2016

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were used to obtain a standard qualitative report of sensitive (S), intermediate (I), or resistant (R).

3. RESULTS AND DISCUSSION

Qualitative Phytochemical Profile of *Artocarpus odoratissimus* (Marang) seed extract.

By utilizing methanol and ethanol extracts from the Artocarpus odoratissimus(Marang) seed, a qualitative phytochemical screening process was used to detect the presence or absence of several secondary metabolites. As shown in Table 1, the Marang seed extract's rich phytochemical profile is suggested by flavonoids, tannins, polyphenols, saponins, steroids, terpenoids, and alkaloids.

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Phytochemicals	Methanol Extract	Ethanol Extract
Flavonoids	+	+
Tannins	+	+
Phenolic	+	+
Saponin	+	+
Steroids	+	+
Terpenoids	+	+
Alkaloids	+	+
(+) - indicates presence of secondary metabolites		
(-) - indicates absence of secondary metabolites		

Table 1. Phytochemical constituents of Artocarpus odoratissimus seed extract

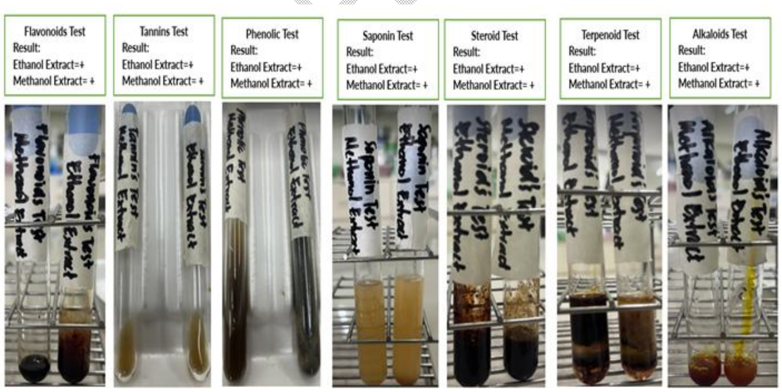

Figure 1. Experimental Results of the Phytochemical constituents of Artocarpus odoratissimus seed extract

The investigation findings are supported by literature indicating abundant secondary metabolite chemicals in Artocarpus plants [17]. Some Artocarpus species were reported to possess high concentrations of flavonoids, phenols, steroids, tannins, saponins, and triterpenoids [18].

The phytochemical and antioxidant properties of the Artocarpus odoratissimus (Marang) fruit's seed and flesh were investigated [19]. The investigation results indicated that the Marang peel had a higher total phenolic content than the meat. Furthermore, the total flavonoid concentration of the Marang seed was higher than that of the flesh. Many microorganisms, including food-related diseases and clinically significant bacteria, fungi, and protozoa, can have their growth and activity inhibited by plant-derived phenolics, such as phenolic acids, flavonoids, and tannins [20].

Flavonoids are potent antioxidants detected in both extracts, play a crucial role in protecting cells from oxidative stress, and may contribute to the seed extract's potential health benefits. Many flavonoids have additionally demonstrated anti-infective properties by forming complexes with various proteins inside bacterial cell walls or extracellular proteins, eventually killing [21].

Tannins in the seed extract indicate the metabolite's astringent and antibacterial properties. Previous research has examined tannic acid's effects on various bacterial species, including Gram-positive (mostly Staphylococcus aureus) and Gram-negative (primarily Escherichia coli). The potential of tannins to penetrate bacterial cell walls and reach the interior membrane, along with their ability to disrupt cell metabolism and ultimately cause the cell's death, account for the antibacterial properties of these compounds [22]. Supporting those as mentioned earlier, a study using chestnut tannin on chicken performance discovered that 1,000 mg/kg of tannins reduced the number of harmful bacteria in the small intestine, including Escherichia coli. Furthermore, it has been demonstrated that tannins decrease the quantity of bacteria in the small intestine and colon of chickens [23].

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The extract contains terpenoids, which have been shown to have a variety of biological purposeshave reported that these metabolites possess antibacterial solid activity, demonstrating bacteriostatic and bactericidal effects on various infections [24].

Alkaloids and polyphenols have demonstrated potent antibacterial action among the secondary metabolites examined [25]. Promising results have been obtained from research investigating the antibacterial mechanism of natural alkaloids derived from Artocarpus plants, indicating their potential efficacy in treating bacterial infections. Alkaloids, nitrogen-containing chemicals found in plants, have antibacterial properties by impeding the creation of bacterial cell walls, modifying the permeability of cell membranes, disturbing bacterial metabolism, and interfering with the synthesis of nucleic acids and proteins [26]. Research has demonstrated the antibacterial properties of Artocarpus extracts against Methicillin-Resistant Staphylococcus aureus (MRSA) strains, suggesting its effectiveness in fighting against bacteria resistant to treatment [27].Furthermore, the presence of steroids and saponins in the seed extract promotes the antibacterial activity and mechanism of the Artocarpus odoratissimus(Marang) fruit.

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Antimicrobial Activity of Artocarpus odoratissimus seed extract.

The Zone of Inhibition (ZOI) was the basis for determining the bacteria's susceptibility, and the antimicrobial potential of *A. odoratissimus* seed extract against soil microorganisms particularly *Escherichia coli* and *Staphylococcus aureus*.

Table 1 showed the susceptibility of *Escherichia coli* to the various treatments impregnated in the sensitivity discs. The sensi disc impregnated with Artocarpus odoratissimus seed methanol and ethanol seed extracts produced an average of 10.33- and 9.33-mm zone of inhibitions against E. coli, indicating resistance. While ampicillin sensitivity disc had the highest average zone of inhibition against the test organism (20.6 mm), it was interpreted as susceptible. The disc loaded with distilled water as the negative control has a 0 mm zone of inhibition and a resistant interpretation. The result is supported by the study that most Escherichia coli species are resistant [28]. Escherichia coli multidrug resistance is a concerning global issue due to the bacterial species' remarkable ability to acquire resistance genes, primarily through horizontal gene transfer [29].

Treatments impregnated in the SensiDisk	Zone of Inhibition (in millimeters)				
	Plate1	Plate2	Plate3	Average	Interpretation
A.odoratissumus methanol seed extract	10	12	9	10.33	Resistant
A.odoratissumus ethanol seed extract	10	8	10	9.33	Resistant
Ampicillin (Positive Control) 30ug	19	22	21	20.6	Susceptible
Distilled Water (Negative Control)	0	0	0	0	Resistant

Table 2. Susceptibility of *Escherichia coli*

The susceptibility of Staphylococcus aureus, the test soil microorganism, to the various treatments impregnated in the sensitivity discs was shown in Table 3. Sensi discs containing Artocarpus odoratissimus seed methanol and ethanol extracts have an average zone of inhibition of 11.67- and 12 mm, respectively, indicating that the test microorganisms are resistant to the extract. The positive control, oxacillin sensi disc, demonstrated the most significant zone of inhibition, 15.13mm long, and is susceptible to Staphylococcus aureus. The disc with distilled water showed a 0 mm zone of inhibition, indicating that the test microorganisms resist the negative control.

Although the level of bacterial inhibition is low, the flavonoid content of the seed extract still contributes to its antimicrobial potential because the following mechanisms are well known for the effectiveness of flavonoids as antibacterial agents against a variety of pathogenic microorganisms: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and formation of biofilms [30].

Treatments impregnated in the SensiDisk	Zone of Inhibition (in millimeters)				
	Plate1	Plate2	Plate3	Average	Interpretation
A.odoratissumus methanol seed extract	14	11	10	11.67	Resistant
A.odoratissumus ethanol seed extract	13	12	11	12	Resistant
Oxacillin (Positive Control) 30ug	14	17	15	15.33	Susceptible
Distilled Water (Negative Control)	0	0	0	0	Resistant

Table 3. Susceptibility of *Staphylococcus aureus*

4. CONCLUSION

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents that are known to exhibit medicinal as well as physiological activities. The presence of phytochemicals such as flavonoids, tannins, phenolics, saponin, steroids, terpenoids, and alkaloids was found through analysis of the plant extracts.

Based on the zone of inhibition, seed extracts from Artocarpus odoratissimus exhibited limited antibacterial activity against soil microorganisms, such as Staphylococcus aureus and Escherichia coli. Both soil bacteria, nevertheless, continue to be resistant to the extract. The impact can arise from secondary chemicals present in the extract. Clearly, more research is required to assess the extract's effectiveness in various indications, including the presence of particular phytochemicals. Other researchers could use different test species to compare the antibacterial capabilities of the extract.

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REFERENCES

[1] Filip Z (2002) International approach to assessing soil quality by ecologically-related biological parameters. Agric EcosystEnvironat some place 88: 169–174.

[2] Poolman, J. T., & Anderson, A. S. (2018). Escherichia coli and Staphylococcus aureus: leading bacterial pathogens of healthcare associated infections and bacteremia in older-age populations. Expert review of vaccines, 17(7), 607–618. https://doi.org/10.1080/14760584.2018.1488590

[3] Solo-Gabriele, H. M., M. A. Wolfert, T. R. Desmarais, and C. J. Palmer.2000. Sources of Escherichia coli in a coastal subtropical environment. Appl. Environ. Microbiol.66:230-237.

[4] Ishii, S., Ksoll, W. B., Hicks, R. E., & Sadowsky, M. J. (2006). Presence and growth of naturalized Escherichia coli in temperate soils from Lake Superior watersheds. Applied and environmental microbiology, 72(1), 612-621.

[5] Byappanahalli, M. N., R. L. Whitman, D. A. Shively, M. J. Sadowsky, and S. Ishii.2006. Population structure, persistence, and seasonality of autochthonous Escherichia coli in temperate, coastal forest soil from a Great Lakes watershed. Environ. Microbiol.8 [Online.

[6] Desmarais, T. R., H. M. Solo-Gabriele, and C. J. Palmer.2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Appl. Environ. Microbiol.68:1165-1172.

[7] Gerken, T., Wiegner, T. N., & Economy, L. M. (2022). A comparison of soil Staphylococcus aureus and fecal indicator bacteria concentrations across land uses in a Hawaiian watershed (Vol. 51, No. 5, pp. 916-929).

[8] Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. Chinese medicine, 13, 20. https://doi.org/10.1186/s13020-018-0177-x

[9] Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Rizza MD. Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria spp. Chil J Agri Res. 2011;71(2):231–9.

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[10] Hakim, E.H., Achmad, S.A., Juliawaty, L.D., Makmur, L., Syah, Y.M., Aimi, N., et al., 2006. Prenylated flavonoids and related compounds of the Indonesian Artocarpus (Moraceae). J. Nat. Med. 60, 161184.

[11] Biotropia,"ANTIMICROBIAL and HERBICIDAL ACTIVITIES of the FRUTICOSE LICHEN RAMALINA from GUIMARAS ISLAND, PHILIPPINES." BIOTROPIA, vol. 26, no. 1, 31 Jan. 2019, 10.11598/btb.2019.26.1.836. Accessed 17 Apr. 2021.

[12] Torar, G.M. J., Lolo, W.A., and Citraningtyas G., Uji AktivitasAntibakteriEkstrakEtanolBijiPepaya (Carica papaya L.) TerhadapBakteri Pseudomonas Aeruginosa dan Staphylococcus aureus. PharmaconJurnalIlmiah Farmasi, 6(2), 14–21.

[13] Anowi, F. C., Ike, C. H. I. B. E. Z. E., Ezeokafor, E. M. M. A., & Ebere, C. H. U. K. W. U. E. N. W. E. I. W. E. (2012). The phytochemical, antispamodic and antidiarrhoea properties of the methanol extract of the leaves of Buchholzia coriacea family Capparaceae. International Journal of Current Pharmaceutical Research, 4(3), 52-55.

[14] Shah, M. D., & Hossain, M. A. (2014). Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant Merremiaborneensis. Arabian Journal of Chemistry, 7(6), 1034-1038.

[15] Lohidas, J., Manjusha, S., & Jothi, G. G. G. (2015). Antimicrobial activities of Carica papaya L.

[16] Ikalinus, R., Widyastuti, S. K., & Setiasih, N. L. E. (2015). Skriningfitokimiaekstraketanolkulitbatangkelor (Moringa oleifera). Indonesia Medicus Veterinus, 4(1), 71-79.

[17] Kamal, T., Muzammil, A., Abdullateef, R. A., & Omar, M. N. (2012). Investigation of antioxidant activity and phytochemical constituents of Artocarpus altilis. Journal of Medicinal Plants Research, 6(26), 4354-4357.

[18] Sivagnanasundaram, P., & Karunanayake, K. O. L. C. (2015). Phytochemical screening and antimicrobial activity of Artocarpus heterophyllus and Artocarpus altilis leaf and stem bark extracts.

[19] Bakar, M. F. A., Mohamed, M., Rahmat, A., & Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). Food chemistry, 113(2), 479-483.

[20] Li, A. N., Li, S., Zhang, Y. J., Xu, X. R., Chen, Y. M., & Li, H. B. (2014). Resources and biological activities of natural polyphenols. Nutrients, 6(12), 6020-6047.

[21] Kilani-Jaziri S, Frachet V, Bhouri W, Ghedira K, Chekir-Ghedira L, Ronot X. Flavones inhibit the proliferation of human tumor cancer cell lines by inducing apoptosis. Drug Chem Toxicol. 2011;35:1–10.

[22] Belhaoues, S., Amri, S., &Bensouilah, M. (2020). Major phenolic compounds, antioxidant and antibacterial activities of Anthemis praecox Link aerial parts. South African Journal of Botany, 131, 200-205.

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[23] Jamroz, D., Wiliczkiewicz, A., Skorupińska, J., Orda, J., Kuryszko, J., &Tschirch, H. (2009). Effect of sweet chestnut tannin (SCT) on the performance, microbial status of intestine and histological characteristics of intestine wall in chickens. British poultry science, 50(6), 687-699.

[24] Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A. L., Chong, C. M., Chong, C. W., ... & Lai, K. S. (2019). Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules, 24(14), 2631.

[25] Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Frontiers in microbiology, 10, 911.

[26] Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics, 10(3), 318.

[27] Prastiyanto, M. E., Azizah, I. H., Haqi, H. D., Yulianto, B. D., Agmala, A. B., Radipasari, Z. D., ... & Putri, A. R. (2020). In-vitro antibacterial activity of the seed extract of three-member Artocarpus towards Methicillin-Resistant Staphylococcus aureus (MRSA). JurnalTeknologiLaboratorium, 9(2), 128-135.

[28] Sabir, S., Anjum, A. A., Ijaz, T., Ali, M. A., & Nawaz, M. (2014). Isolation and antibiotic susceptibility of E. coli from urinary tract infections in a tertiary care hospital. Pakistan journal of medical sciences, 30(2), 389.

[29] Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial resistance in Escherichia coli. Microbiology spectrum, 6(4), 10-1128.

[30] Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current medicinal chemistry, 22(1), 132-149.