Crimson Publishers
Wings to the Research

Research Article

Preclinical Evaluation of *Asparagus Racemosus* Seed Oil Extract's *In Vitro* Anti *Salmonella* Efficacy

Niladry SG[1], Anubhav D[2]* and Mamta K[3]

[1]Faculty of Pharmaceutical Science, Assam down town University, India

[2]Department of Pharmacology, Maharana Pratap College of Pharmacy, India

[3]Department of Pharmacy, Harcourt Butler Technical University, India

ISSN: 2770-6745

***Corresponding author:** Anubhav Dubey, Assistant Professor, Department of Pharmacology, Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, India

Submission: October 14, 2024
Published: November 19, 2024

Volume 5 - Issue 1

How to cite this article: Niladry SG, Anubhav D* and Mamta K. Preclinical Evaluation of *Asparagus Racemosus* Seed Oil Extract's *In Vitro* Anti *Salmonella* Efficacy. Biodiversity Online J. 5(1). BOJ. 000603. 2024.
DOI: 10.31031/BOJ.2024.05.000603

Abstract

Background: Growing resistance to commonly used medicines makes treating a life-threatening typhoidal salmonella infection increasingly challenging. The ability of the bacteria to produce biofilm contributes to this resistance. This work examined the *in vitro* anti-salmonella actions of the essential oil of *Asparagus racemosus* seeds.

Results: The effect was that the essential oil of *Asparagus racemosus* seeds (EOAR) stopped the growth of *S. typhi* with a zone of inhibition of 12±0.2 mm, compared to 30±0.3mm for ciprofloxacin. Minimum biofilm inhibitory values were 13.5mg/mL minimum bactericidal and minimum inhibitory concentrations were 24, 50, respectively. In *S. Typhi*, EOAR produced an efflux of potassium ions, inorganic phosphate, and pyruvic acid.

Conclusion: Essential oil of *Asparagus racemosus* seeds possesses *in vitro* anti-salmonella action.

Keywords: Essential oils; *Asparagus racemosus*; *Salmonella Typhi*; Typhoid fever

Introduction

Salmonellosis is a serious challenge to the global health-care system, with alarming rates of incidence and death, particularly in Sub-Saharan Africa [1]. Although non-typhoidal salmonella species induce self-limiting diarrhea, *S. typhi* causes typhoid fever, which is a systemic, life-threatening infection of the reticuloendothelial system. The emergence of multi-drug-resistant (*S. Typhi*) strains is complicating typhoid fever therapy. Some infected people establish carrier status as a result of the production of bacterial biofilm in difficult-to-reach niches, including the gall bladder. These two critical aspects make the quest for an alternate anti-salmonella drug a high priority. Essential oils (EOs), also known as volatile oils, are byproducts of the secondary metabolism of aromatic plants. They are incredibly complex mixtures, primarily consisting of terpenoids and phenolic for many years, food processing has used EOs as flavor enhancers, preservatives, cures, and skin and hair treatments.

Currently, researchers are emphasizing EOs as potential resistance-modifying drugs due to their chemical variety, which enables them to attack microorganisms at various targets. us targets. The interaction of EO components produces synergistic, additive, or even antagonistic effects on microbes. Numerous scientific studies have shown that EOs from diverse plants have antibacterial properties against both grammeme-negative and grammeme-positive bacteria, including numerous *Salmonella* spp [2,3]. However, nothing is known about the effect of essential oil against the fatal typhoidal salmonella, *S. Typhi* [4]. *Asparagus racemosus*,

often known as shatavari, is an important plant in Ayurveda since it has the ability to treat or prevent hundreds of ailments. It reigns supreme among herbs, earning the moniker of "herb's queen." Its bioactive components include steroidal glycosides, saponins (particularly Shatavarins I, II, III, and IV), polyphenols, flavonoids, alkaloids (racemosol), and vitamins. Folk and Ayurvedic medicine frequently employ Shatavari because it contains sapogenin, a precursor to multiple pharmacologically active steroids. The most significant portions are the roots, stems, and leaves. However, the whole plant has medicinal effects. The "Rasayanas" prepared by Shatavari are great for preventing disease. Its phytochemicals make it useful for treating a variety of diseases. Shatavari contains phytochemicals that can effectively treat a wide range of ailments. Some of the many meditative has many health benefits, such as relaxing muscles, fighting inflammation, lowering blood sugar, fighting allergies, stopping malaria, protecting against cancer, boosting the immune system, helping with arthritis, reducing pain and period problems, stopping ulcers from forming, lowering stress, stopping diarrhea, fighting depression, infections. The market has demonstrated the usefulness of Shatavari extract-based medications in treating leprosy, abortion, infection, fever, and discomfort. Shatavari root, leaf, flower, and stem extracts may help with dyspepsia, mental disorders, coughs, bronchitis, throat difficulties, and female reproductive system issues. A wound is a rapid form of damage, characterized by tearing, slicing, or piercing of the skin (an open wound), or a contusion from blunt force trauma (a closed wound). In pathology, the term "wound" refers to an acute injury to the skin's surface [5,6].

Materials and Methods

Extraction of essential oil of *Asparagus racemosus*

We got some *A. racemosus* seeds from the Vatika Agro shop in Jaipur, Rajasthan, India. The plant's validity was confirmed at Janta Postgraduate College, A.P.S. University, Rewa, Madhya Pradesh, India, where the specimen was placed in the university's herbarium under the number J/Bot/2022APS-019 as a Vocher specimen. The plant's seeds were extracted and dried in a separate oven at 450 degrees Celsius. A mechanical grinder was used to ground the dry seeds into a powder. Extracting essential oils using Soxhlet apparatus. About 50g of powdered *A. racemosus* leaves were put in the thimble and placed in the Soxhlet chamber. 500mL of n-hexane was poured in a round bottom flask and assembled for extraction. After 180 minutes of extraction at 70 degrees Celsius. The solvent was extracted under low pressure using a water bath. Ethanol was added to the crude extract and allowed to cool for 1 hour at -4 degrees Celsius. The combination was then filtered using filter paper, and the filtrate was allowed to concentrate in a water bath at 40°C to produce the essential oil of *A. racemosus* (EOAR), while the residue was concrete [7].

Salmonella Typhi

Bacterial pathogens, namely *Salmonella typhi*, were obtained from Aakaar Biotechnology Lucknow, respectively. In the aftermath of the collecting process, we transferred the isolates to McCartney bottles that had been disinfected and then brought them to the laboratory in an ice box.

Invitro anti-microbial studies sensitivity of *S. Typhi* to EOAR

Salmonella Typhi was cultivated on sterile Salmonella Shigella agar (SSA), which had just been produced. A freshly-prepared sterile Mueller-Hinton agar (MHA) plate was equally covered with a suspension of the pure culture (0.5 McFarland). A 6mm hole was drilled into the agar's middle, and then 50µL of EOAR (100 mg/mL in DMSO) was added to the well. The plate was kept at 35°C for 24 hours for incubation. The essential oil's antibacterial activity was determined by measuring the size of a distinct zone around the hole, which is known as the zone of inhibition. The positive control consisted of ciprofloxacin, whereas the negative control consisted of dimethyl sulfoxide (DMSO) [4]. Triplicates were used in the experiment.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To find the minimal inhibitory concentration of the EOAR, a slightly modified version of the broth micro-dilution technique reported by Ramalivhana [8] was used. We made the Miller-Hilton Broth (MHB) according to the manufacturer's instructions. An MHB concentration ranging from 50 to 0.39mg/mL was achieved by serially diluting the essential oil on a 96-well microtiter plate. We introduced the bacteria to each well using a micropipette, introducing exactly half of the McFarland standard suspensions, and then incubated at 37°C for 24 hours. Then, each well was incubated for 4 hours after adding 20µL of 2,3,5-triphenyl tetrazolium chloride (TTC, 5mg/mL). We monitored the color change in each well. The light-yellow TTC turns into a dark crimson formazin when infected with *Salmonella typhi*. We ran each experiment three times to ensure accuracy. Lin observed the lowest concentration of each extract that inhibited bacterial growth (i.e., wells without color change) to estimate the range of MIC for each extract. Wells that did not show any signs of bacterial growth in the MIC experiment were used to obtain the MBC values. To check for growth, 50µl of broth solution from each non-growing well was distributed on sterile MHA plates that had just been prepared. The plates were then incubated at 37°C for the night. The minimum bactericidal concentration was determined as the lowest concentration at which no observable bacterial growth could be detected [4].

Determination of anti-biofilm activity of EOAR

The EOAR's anti-biofilm impact was evaluated using the same methods as [9]. The 96-well polystyrene micro-titer plates were pre-coated with 5mg/mL of cholesterol in a 1:1 mixture of ethanol and isopropanol. The plates were then left to dry at room temperature overnight to enhance the growth conditions of *S. Typhi* on the cholesterol gallstone. After following the steps outlined in MIC, the plates were left to incubate at 37°C for 48 hours without

stirring. Three washes with sterile distill water were performed after incubation to eliminate planktonic cells from each well. A 0.4% Crystal Violet (CV) solution was used to stain the adherent cells for a duration of three minutes. Two washes with sterile distill water were performed on the wells, and any extra CV was thrown away. Using an ELISA microtiter plate reader, the color intensity was determined at OD570nm after dissolving the CV from the dyed biofilms in 33% glacial acetic acid for 10 minutes. In order to determine the percentage of biofilm inhibition, the following formula:

$$Percentage\ (\%)\ inhibition = \frac{ODgrowthcontrol - ODsample}{ODgrowthcontrol} \times 100$$

The lowest concentration of the essential oil that inhibits biofilm formation was taken as the Minimum biofilm inhibition concentration (MBIC)

Assay of potassium and phosphate Ions efflux

Zhang [10] outlined a method that measured the concentration of free potassium and phosphate ions outside of cells after exposing *S. Tyhi* to EOAR. We incubated the *S.Typhi* fresh culture in sterile peptone water (0.1g/100mL) with 0 and 25mg/mL (MIC) of EOPG for 0, 30, 60, 90, and 120 minutes, respectively, for the control and test groups. We used two kits, one for measuring phosphate concentrations and another for measuring extracellular potassium, at each testing interval. The results showed the concentration of extracellular free potassium and phosphate (mmol/L) throughout each incubation period.

Measurement of pyruvic acid efflux

Following the steps described by Zou [11], the release of pyruvic acid from *S. Typhi* cultures that had EOAR added to them was carefully watched. For the control and test groups, we cultured the fresh *S. Typhi* culture in sterile peptone water (0.1g/100 mL) with 0mg/mL (MIC) of EOPG for 0 hours, 3 hours, 6 hours, and 24 hours, respectively. We took the samples at regular intervals, then spun them in a centrifuge at 6,000rpm for 15 minutes at 4°C. We froze the resulting liquid and refrigerated it until we were ready to measure. We determined the pyruvic acid content using the 2,4-dinitrophenylhydrazine technique. We swiftly combined test tubes with 0.3mL of 8% trichloroacetic acid, 0.1mL of supernatants, and 1mL of 2,4-dinitrophenylhydrazine. We heated the liquid to 37°C for 10 minutes, then added 0mL of sodium hydroxide (0.4mol/L) and stirred. We took a reading of the absorbance at 520nm. We used a pyruvic acid calibration curve to determine the pyruvic acid concentration.

GC/MS analysis of the active fraction of EOAR

We analyzed the aqueous and ethanolic AR seed extracts using GS-MS. A PerkinElmer Clarus 600 gas chromatography system is a tight match for the Rtx-5MS capillary column. We attained and sustained a steady flow rate of 1.0mL/min using helium that was 99.99 percent pure. We used an ionization energy approach to identify the GC-MS spectral lines at a temperature of 260°C, an injection volume of 1L (split ratio 10:1), an ionization energy of 70eV (electron volts), a scan duration of 0.2 seconds, and a fragment spanning from 40 to 650m/z. But the column oven was set to 500°C and left on for three minutes. Raise the temperature to 3000C by 100C every minute. We achieved the identification of plant sample components by comparing retention periods, peak areas, peak heights, and spectral line patterns to known chemical databases in the NIST and Wiley-8 libraries [5].

Statistical analysis

The data were expressed as Mean and Standard Error of Mean±SEM (n=3 for in vitro, n=4) for in vivo) animal samples and analyzed using one-way analysis of Variance (ANOVA) test using Graph Pad Prism version 5. The difference between mean of different groups were analyzed using Duncan's multiple range test at $p< 0.05$.

Results

Table 1: Biofilm inhibition potential of crude extract and essential oil of *P. guajava* in *S. Typhi.*

EOAR (mg/mL)	% Biofilm Inhibition	Ciprofloxacin (mg/mL)	% Biofilm Inhibition
50	7	10	8.92
25	5.58	5	5.38
12.5	2.84	2.5	4.02
6.25	NI	1.24	0.77
3.13	NI	0.62	NI
1.56	NI	0.3	NI
0.78	NI	0.17	NI
0.39	NI	0.07	NI

According to Figure 1a, the zone of inhibition for the growth of *S. typhi* by EOAR was 12±0.2mm, whereas the zone for ciprofloxacin was 30±0.3mm. In Figure 1b, we can see that the minimum inhibitory concentration (MIC) of ciprofloxacin is 0.313±0.00mg/mL, and that of EOAR is >50mg/mL, while the MBC of ciprofloxacin is 2.5mg/mL. If we compare the lowest inhibitory concentration of EOAR to ciprofloxacin, which is 1.25mg/mL, we find that it is 10 times higher at 13.5mg/mL (Table 1). Compared to the control group, the concentration of inorganic phosphate and potassium ions outside of bacterial cells increased significantly after 60 minutes of exposure to 25mg/mL of EOAR. When 25mg/mL of EOAR was added to S. Typhi during the incubation phase, the level of pyruvic acid outside of cells rose significantly ($P<0.05$). Figure 2a-2c shows that the total pyruvic level in the control group stayed at 1.7mM/L for 6 to 24 hours after exposure.

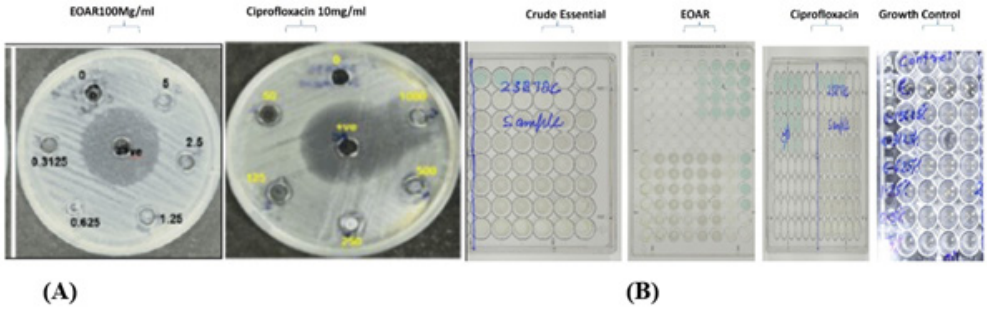

Figure 1: In vitro activities of essential oil of *Asparagus racemosus* (EOAR) against *Salmonella Typhi*.

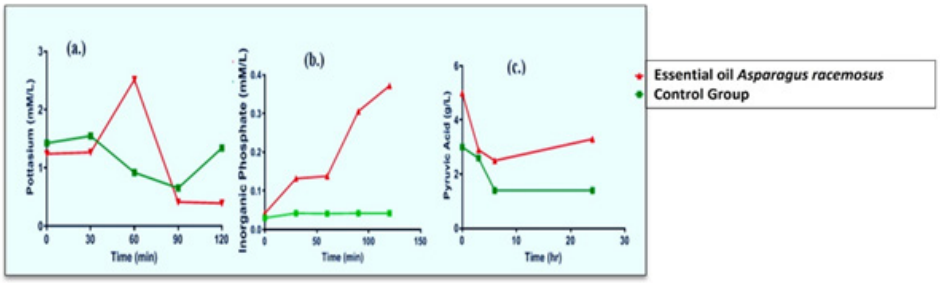

Figure 2: Time course efflux of
a) potassium ion
b) inorganic phosphate
c) pyruvic acid from *S. Typhi* exposed to EOAR

Compounds identified in active fraction of EOAR

Twenty-seven Compounds were identified in active fraction of EOAR that showed anti-salmonella activities. (Table 2).

Table 2: Compounds identified in active fraction of EOAR.

Peak	R. Time	Area	Area%	Name	Molecular Formula	MW
1	12.061	54726	1.64	2,4-Ditert-Butylphenyl 5-Hydroxypentanoa	$C_{19}H_{30}O_3$	306
2	12.142	28747	0.86	Decanoic Acid, 8-Methyl-, Methyl Ester	$C_{12}H_{24}O_2$	200
3	12.742	81212	2.44	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256
4	13.017	55520	1.67	1,2Benzenedicarboxylic Acid, Diethyl Ester	$C_{12}H_{14}O_4$	222
5	13.282	34876	1.05	1,2,4-Trimethoxy-5,[(1e)-Propenyl]Benzene	$C_{12}H_{14}O_4$	208
6	13.733	24792	0.75	Cyclopentaneacetic Acid, 3-Oxo-2-Pentyl-, Methyl Ester	$C_{13}H_{22}O_3$	226
7	13.92	54254	1.63	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate	$C_{15}H_{22}O_2$	234
8	14.15	11843	0.36	4-Nonanol	$C_9H_{20}O$	144
9	14.498	20265	6.09	Methyl Tetradecanoate	$C_{15}H_{30}O_2$	242
10	14.99	467360	14.05	Tetradecanoic Acid	$C_{14}H_{28}O_2$	228
11	16.525	16598	0.5	7,9-Ditert-Butyl-1-Oxaspiro[4.5]Deca-6,9-Diene	$C_{17}H_{24}O_3$	276
12	16.616	23141	6.95	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	270
13	17.052	24149	7.26	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256
14	18.251	33296	10.01	14-Methyl-8 Hexadecyn-1-Ol	$C_{17}H_{32}O$	252

15	18.309	626587	18.83	9-Octadecenoic Acid (Z)-, Methyl Ester	$C_{19}H_{36}O_2$	296
16	18.367	42346	1.27	9-Octadecenoic Acid (Z)-, Methyl Ester	$C_{19}H_{36}O_2$	296
17	18.543	101350	3.05	Octadecanoic Acid, Methyl Ester	$C_{19}H_{38}O_2$	298
18	18.748	57026	1.71	(8z)-14-Methyl-8-Hexadecen-1-Ol	$C_{17}H_{34}O$	254
19	19.118	84757	2.55	9,11-Octadecadienoic Acid, Methyl Ester, (E,E)-	$C_{19}H_{34}O_2$	294
20	19.662	11377	0.34	Chloromethyl 2-Chlorododecanoate	$C_{13}H_{24}Cl_2O_2$	282
21	20.066	140641	4.23	Myristic Acid Glycidyl Ester	$C_{17}H_{32}O_3$	284
22	20.679	61613	1.85	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337
23	21.152	39825	1.2	9-Octadecenoic Acid,1,2,3-Propanetriyl Ester, (E,E,E)-	$C_{57}H_{104}O_6$	884
24	21.544	22872	6.87	Linoleyl acetate	$C_{20}H_{36}O_2$	308
25	21.738	47141	1.42	Glycidyl Palmitate	$C_{19}H_{36}O_3$	312
26	22.007	34377	1.03	Phthalic Acid, Di(6-Methylhept-2-Yl) Ester	$C_{24}H_{38}O_4$	390
27	22.253	13261	0.4	(2,6,6-Trimethylbicyclo [3.1.1] Hept-3-Yl) Methenamine	$C_11H_{21}N$	167

Discussion

A major worldwide concern that is causing typhoid treatment failures is the emergence of multidrug-resistant *S. typhimurium* strains. There have been reports of *S. typhi* developing resistance to first-line antimicrobial medications, which means that these treatments are becoming less effective in controlling typhoid fever [12]. Iseppi [13] found that essential oils had powerful antioxidant, antiviral, antiparasitic, and antibacterial properties, especially against drug-resistant bacteria and viruses. The n-hexane-soluble part of the leaves has some anti-salmonella activity, so this study looked at whether the essential oil from Asparagus racemosus seeds could also stop *Salmonella typhimurium* from growing. The fact that salmonella did not grow around the agar well showed that the essential oil of *Asparagus racemosus* seeds (EOAR) had a strong anti-salmonella effect in the lab. Zone of Inhibition (ZI) experiments measure an antibiotic agent's capacity to suppress bacterial growth in a solid growth medium [14]. Even though the ZI is only 13±0.2 mm, which is much lower than the 31±0.3 mm found for ciprofloxacin, this might be because the essential oil is hydrophobic and hence has little diffusion in the water-based growth medium. Some infected people have chronic carriage of *S. typhi*, and biofilm is a crucial factor in this process. In most cases, the gallbladder is home to this bacterial colony of sessile bacteria. In addition to its anti-salmonella efficacy, EOAG may be able to inhibit biofilm development in vitro. aromatherapy blend. To keep their internal environment stable, bacteria rely on their membrane's permeability barrier to let certain chemicals enter and exit the cell. Blazek [4] assert that even minor changes to the structural integrity of a cell membrane can significantly impact cellular metabolism, ultimately resulting in cell death. The abundant potassium within cells, around 100 times higher than the levels outside of cells, impacts multiple physiological processes [15].

This includes acid-base homeostasis, fluid and electrolyte balance, glucose metabolism, and membrane potential [16]. Energy transfers, protein activation, and the metabolism of carbon and amino acids all rely on phosphates, which are inorganic phosphates [17]. One of the most crucial metabolic intermediates is pyruvic acid. Besides connecting glycolysis and the tricarboxylic acid cycle, pyruvic acid is also very important for the ability of *S. typhi* to infect and spread. When exposed to EOAR, the cells efflux potassium ions, inorganic phosphate ions, and pyruvic acid, indicating bacterial cell membrane impairment. In line with these results, Bouyhaya [18] found that the essential oils from Origanum compactum made parts of the cytosol of Escherichia coli and Bacillus subtilis leak out. Components of essential oils have a hydrophobic property that allows them to partition into the bacterial cell membrane's lipids. This disrupts the cell structures, makes them more permeable, and causes lysis and intracellular compound leakage. Bioautography is a less time-consuming and expensive way to connect the bioactivity and fractionation of natural chemicals. GCMS detected 27 chemicals in the active fractions. Oxadiazole and aromatic derivatives are among those that have shown promise as antibacterial agents. Furthermore, the bacteria are Salmonella. Typhi-rich bile flow can cause infections in the intestines, which can lead to enteritis and sores at Peyer's patches in the small intestine's ending ileum, caecum, and ascending colon [19]. In this study, the histology evaluation of the small intestine revealed perforations, vacuolations across the intestinal villi and the muscularis layer [20-22], and a few ulcerations (spot ulceration, deep ulcer, and red coloration) in the control group but only mildly in the treated groups. Given that *S. Typhi* may be the cause of these injuries, it is plausible that EOPG therapy played a role in alleviating them. Liver histological studies show that the treated groups had normal central venules that weren't swollen in a big way, while the untreated groups had big changes in the shape and number of cells, which could mean they had liver hepatitis. Ritu reported hepatomegaly in 55% of patients with typhoid fever [23-25].

Conclusion

This research demonstrated *in vitro* anti-salmonella activity for the essential oil of *Asparagus racemosus* seeds (EOAR). This provides support for the long-standing practice of using *Asparagus*

racemosus as a traditional remedy for typhoid. This research also investigated the essential oil's anti-salmonella action and a few phytochemicals found in it. As an antibacterial agent, EOAR may provide a minimal risk of microbial resistance development due to its diverse chemical profile. The ability of EOAR to combat biofilms could potentially address the emergence of carriers. The oil is potentially useful in the treatment of typhoid and for future medication development. It contains phytochemicals.

Acknowledgement

Thanks to the college administration, the writers had access to the resources they needed to complete the project.

Data Availability Statement

The corresponding author has access to all the data supporting the presented research results upon request.

Conflict of Interest

There is no perceived conflict of interest, according to the writers.

Ethical Approval

At this time, there is no publication, submission to another journal, or review of the paper.

Supplementary File

None.

Credit Authorship Contribution Statement

All authors participate equally.

References

1. Marchello CS, Carr SD, Crump JA (2020) A systematic review on antimicrobial resistance among salmonella typhi worldwide. The American Journal of Tropical Medicine and Hygiene 103(6): 2518 2527.
2. Habibi H, Ghahtan N, Morammazi S (2018) The effects of some herbal essential oils against salmonella and escherichia coli isolated from infected broiler flocks. Journal of World Poultry Research 8 (3): 74-80.
3. Helal IM, El Bessoumy A, Al Bataineh E, Joseph M, Rajagopalan P, et al. (2019) Antimicrobial efficiency of essential oils from traditional medicinal plants of asir region, Saudi Arabia, over drug resistant isolates. BioMed research international 8928306.
4. Blazek AD, Paleo BJ, Weisleder N (2015) Plasma membrane repair: A central process for maintaining cellular homeostasis. Physiology 30(6): 438 448.
5. Dubey A, Basak M, Dey B, Ghosh N (2023) Queen of all herbs (Asparagus racemosus): An assessment of its botany, conventional utilization, phytochemistry and pharmacology. Research Journal of Biotechnology18(6): 146-154.
6. Dubey A, Ansari MV, Sahu VK, Mishra A (2024) Assessment of in-vitro antimicrobial and antioxidant activity of essential oil obtained from Artemisia Argyi. Cahiers Magellanes-NS 6(2): 2961-2975.
7. Dubey A, Ghosh NS, Singh R (2023) An in-depth and in vitro evaluation of the antioxidant and neuroprotective activity of aqueous and ethanolic extract of Asparagus racemosus linn seed. Research Journal of Chemistry and Environment 27(10): 46-66.
8. Ramalivhana JN, Obi CL, Samie A, Iweriebor BC, Uaboi EP, et al. (2014) Antibacterial activity of honey and medicinal plant extracts against gram negative microorganisms. African Journal of Biotechnology 13(4):616-625.
9. González JF, Alberts H, Lee J, Doolittle L, Gunn JS (2018) Biofilm formation protects salmonella from the antibiotic ciprofloxacin in vitro and in vivo in the mouse model of chronic carriage. Scientific reports 8(1): 222.
10. Zou L, Hu YY, Chen WX (2015) Antibacterial mechanism and activities of black pepper chloroform extract. Journal of Food Science and Technology 52(12): 8196 8203.
11. Iseppi R, Mariani M, Condò C, Sabia C, Messi P (2021) Essential oils: A natural weapon against antibiotic-resistant bacteria responsible for nosocomial infections. Antibiotics 10(4): 417.
12. Bhargav HS, Shastri SD, Poornav SP, Darshan KM, Nayak MM (2016) Measurement of the Zone of Inhibition of an Antibiotic. IEEE 6th International Conference on Advanced Computing pp. 409-414.
13. Gründling A (2013) Potassium uptake systems in Staphylococcus aureus: new stories about ancient systems. mBio 4(5): e00784-13.
14. Anubhav D, Mamta K (2024) Antimicrobial activity, phytochemical screening of crude extracts, and essential constituents of Syzygium Aromaticum, Tymus Vulgaris and Eucalyptus Globulus on selected pathogens, Microbial Bioactives 7(1): 1-5.
15. Dick CF, Dos SAL, Meyer FJR (2011) Inorganic phosphate as an important regulator of phosphatases. Enzyme research 103980.
16. Bouyahya A, Abrini J, Dakka N, Bakri Y (2019) Essential oils of Origanum compactum increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. Journal of Pharmaceutical Analysis 9(5): 301-311.
17. Jaroni D, Zhu L, Olsen C, Mc Hugh T, Friedman M, et al. (2014) Apple, carrot, and hibiscus edible films containing the plant antimicrobials carvacrol and cinnamaldehyde inactivate Salmonella Newport on organic leafy greens in sealed plastic bags. Journal of food science 79(1): M61-M66.
18. Baby J, Mini PR (2010) In vitro antimicrobial activity of Psidium guajava L. Leaf essential oil and extracts using agar well diffusion method. International Journal of Current Pharmaceutical Research 2(3): 28-32.
19. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 6(2): 71-79.
20. Dewanjee S, Gangopadhyay M, Bhattabharya N, Khanra R, Dua TK (2014) Bioautography and its scope in the field of natural product chemistry. Journal of Pharmaceutical Analysis 5(2): 75-84.
21. Dubey A, Ghosh NS, Mishra A, Kumari M, Sahu VK (2024) Preclinical evaluation of Asparagus racemosus seed oil extract's wound healing efficacy. International Journal of Pharmaceutical Science and Medicine 2(2): 40-43.
22. Lu Y, Liu Y, Yang C (2017) Evaluating in vitro DNA damage using comet assay. Journal of Visualized Experiments 128: 56450.
23. Olaniyi TD, Awodugba TM, Adetutu A (2021) Ethnobotanical survey and evaluation of anti-salmonella potentials of commonly used plants for typhoid treatment in Ogbomoso, Oyo state, Nigeria. Journal of Complementary and Alternative Medical Research 15(1): 1-15.
24. Stautz J, Hellmich Y, Fuss MF, Silberberg JM, Devlin JR, et al. (2021) Molecular Mechanisms for Bacterial Potassium Homeostasis. Journal of molecular biology 433(16): 166968.
25. Zhang J, Ye KP, Zhang X, Pan DD, Sun YY, et al. (2017) Antibacterial activity and mechanism of action of black pepper essential oil on meat-borne escherichia coli. Frontiers in Microbiology 7: 2094.