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Identification of Antimicrobial Components in *Rhamphospermum nigrum* Using Gas Chromatography-Mass Spectrometry and Their Antibacterial Effect

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Abstract:

This study investigated the antimicrobial constituents of *Rhamphospermum nigrum* (black mustard) using gas chromatography-mass spectrometry (GC-MS) and evaluated its bacteriostatic effects against pathogenic bacteria. GC-MS analysis identified numerous bioactive compounds, including nonadecane (32.31%), heneicosane (cumulative 35.68%), and eicosane, alongside fatty acid esters and aromatic compounds, suggesting potential antibacterial, anti-inflammatory, and antioxidant properties. The ethanol extract exhibited significant dose-dependent inhibitory effects against *Bacillus subtilis, Escherichia coli, Corynebacterium Pseudomonas aeruginosa, and Klebsiella*, with *Corynebacterium* showing the highest sensitivity 23 mm inhibition zone at 90 mg/mL. The extract outperformed chloramphenicol (control), particularly against multidrug-resistant *P. aeruginosa*, indicating its potential to target Gram-positive peptidoglycan and biofilm-forming pathogens. Statistical analysis confirmed the positive correlation between extract concentration and antibacterial activity ($p < 0.05$). The presence of alkaloids, flavonoids, and other phytochemicals likely contributed to the observed bioactivity. These findings validate the traditional use of *R. nigrum* and highlight its promise as a source of novel antimicrobial agents, particularly for urinary tract infections. Further research is needed to isolate specific bioactive compounds and assess their cytotoxicity and mechanisms of action. This study underscores the potential of plant-derived compounds in addressing antibiotic resistance and advancing sustainable therapeutic solutions.

Keywords: Rhamphospermum nigrum, Antimicrobial, Gas Chromatography-Mass Spectrometry, Bacteriostatic Effect.

تحديد المكونات المضادة للميكروبات في نبات الخردل الأسود (*Rhamphospermum nigrum*) باستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS) وتأثيرها المضاد للبكتيريا

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الملخص:

بحثت هذه الدراسة في المكونات المضادة للميكروبات في نبات الخردل الأسود (*Rhamphospermum nigrum*) باستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة(GC-MS) ، وقيّمت تأثيراتها المضادة للبكتيريا ضد البكتيريا المسببة للأمراض. كشف تحليل كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS) عن العديد من المركبات النشطة بيولوجيًا، بما في ذلك نونايكان (32.31%)، وهينيكوسان (بنسبة تراكمية 35.68%)، وإيكوسان، إلى جانب إسترات الأحماض الدهنية والمركبات العطرية، مما يشير إلى خصائص محتملة مضادة للبكتيريا والالتهابات ومضادات الأكسدة. أظهر مستخلص الإيثانول تأثيرات مثبطة ملحوظة تعتمد على الجرعة ضد العصوية الرقيقة، والإشريكية القولونية، والوتدية الزنجارية (Pseudomonas aeruginosa)، والكلبسيلة، حيث أظهرت الوتدية أعلى حساسية لمنطقة تثبيط 23 ملمتر عند 90 ملغم

مل-[1]. تفوق المستخلص على الكلورامفينيكول (المجموعة الضابطة)، وخاصةً ضد الزائفة الزنجارية المقاومة للأدوية المتعددة، مما يشير إلى قدرته على استهداف الببتيدوغليكان موجبة الجرام ومسببات الأمراض المكونة للأغشية الحيوية. أكد التحليل الإحصائي وجود علاقة إيجابية بين تركيز المستخلص والنشاط المضاد للبكتيريا .($p < 0.05$) من المرجح أن وجود القلويدات والفلافونويدات ومواد كيميائية نباتية أخرى ساهم في النشاط الحيوي المرصود. تُثبت هذه النتائج الاستخدام التقليدي لـ*R. nigrum* ، وتُبرز أهميته كمصدر لعوامل مضادة للميكروبات جديدة، وخاصةً لالتهابات المسالك البولية. هناك حاجة إلى مزيد من البحث لعزل مركبات نشطة بيولوجيًا محددة وتقييم سميتها الخلوية وآليات عملها. تُبرز هذه الدراسة إمكانات المركبات المشتقة من النباتات في معالجة مقاومة المضادات الحيوية وتطوير حلول علاجية مستدامة.

الكلمات المفتاحية: مضاد للميكروبات، كروماتوغرافيا الغاز، مطياف الكتلة، التأثير المثبط للبكتيريا.

Introduction

Plants are a major source of active ingredients, including a variety of compounds, including alkaloids, phenols, and many others. These compounds play an important role in treating many different diseases and defending plants against herbivores. They also play a role in species classification [1]. Secondary metabolites include volatile oils, alkaloids, glycosides, saponins, terpenes, and phenols [2]. Anastasova noted that secondary metabolites have numerous therapeutic and medicinal effects, acting as antioxidants and defense agents against many fungal, bacterial, and other diseases. They also have therapeutic effects against diseases caused by microorganisms [3,4]. Black mustard (*Rhamphospermum nigrum*) (synonyms: *Brassica nigra* and *Sinapis nigra*) is an annual plant that grows in the cool regions of North Africa, temperate regions of Europe, and parts of Asia. It is cultivated for its dark brown to black seeds, which are a spic ،its length reaches more than 2 meters and it is branched, the lower leaves are toothed and lobed, often hairy on the underside, the upper leaves are oblong and the flowers are mostly quadrilateral and one plant produces many seeds spread in the farms. It is an erect plant, reaching 70 cm (28 in) wide and 1.2 m (4 ft) tall in moist, fertile soil [5]. Its large, stalked leaves have basal hairs, and the young stems are smooth [6]. It flowers in summer, with four-petalled, whitish-yellow flowers, up to twice the length of the sepals. Each stem has about four flowers at its apex, forming a ring around the stem [7]. Later, the plant forms long, beak-like seed pods containing spherical seeds .Studies have shown that black mustard contains many chemical compounds, including alkaloids, flavonoids, glycosides, and carotenoids. Mustard seeds are also rich in minerals, fatty acids, and some vitamins such as vitamins B and E, among other chemicals [8]. Also been confirmed that black mustard oil can be used in soap production and medical treatment. Regular use of black mustard seeds improves the body's defense mechanisms against the development of cancer [9], and can reduce the incidence of colon, lung, and bladder cancer [10,11,12,13]. It also relieves congestion by drawing blood to the surface, as in headaches. Black mustard seeds also increase blood glucose levels [14]. The study aimed to qualitatively detect by GC-Ms and test the effectiveness of the ethanol extract on some pathogenic bacterial isolates.

Material and methods

Gas Chromatography - Mass Spectrometry

The extracted samples were placed in an Agelint (USA) 7820A GC-MS. Helium gas, purity 90%, was used as the carrier gas at a flow rate of 1 ml/min. The column temperature started at 60°C and gradually increased at a rate of 10°C per minute until it reached 280°C, while the heat source temperature of the device reached 350°C. The injection port temperature was constant at 250°C, and the ionization energy value (electron volts) was 70 eV. Separation was accomplished at a 5-column MS temperature for 30 minutes, A quadrupole mass detector was used to detect the compounds through an opening in the column, and the detector temperature reached 280°C. The chemical compounds of the plant samples were compared with the compounds stored in the database of the computer linked to the GC-MS device.

Preparation of the plant extract

20 g of the powder was soaked in 100 ml of 80% methanol, according to [15], the infusion was placed in a shaking tabletop incubator for 72 hours at 20°C, and the infusion was filtered using filter paper 5cm, alcohol was evaporated in an oven at 50°C to obtain the raw extract.

Antibacterial Test

The effect of the alcoholic plant extract was tested against bacteria (*Bacillus subtilis, Escherichia coli, Corynebacterium, Pseudomonas aeruginosa and Klebsiella*), Cultures were prepared to test antifungal activity.

The samples used were Mueller-Hinton agar (MHA) medium. Concentrations were 30, 60, and 90. Three holes were made with a cork piercer and the prepared extracts were placed in them. The agar disc was incubated at 37°C for 24 hours. The agar disc was used for this test, measuring the diameter of the disc to determine bacterial growth and activity. This determined the concentration of the plant extract on the disc [16].

Statistical Analysis

The statistical analysis was performed as the arithmetic mean plus or minus the standard error. Means were compared using one-way analysis of variance (ANOVA), followed by the LSD (least significant difference) test at 0.05 for statistical significance in all statistical tests. Statistical analysis was conducted using SPSS, specifically version [17].

Results and Discussion

Gas Chromatography Mass Spectrometry

Figure 1 and Table 1 illustrate the active components of *R. nigrum*. Gas chromatography-mass spectrometry (GC-MS) analysis of *R. nigrum* reveals a large number of bioactive compounds that may have medicinal value. Known extracts include alkanes, alkenes, fatty acid esters, and aromatic compounds, suggesting the plant's potential antibacterial, anti-inflammatory, and anti
oxidant activities

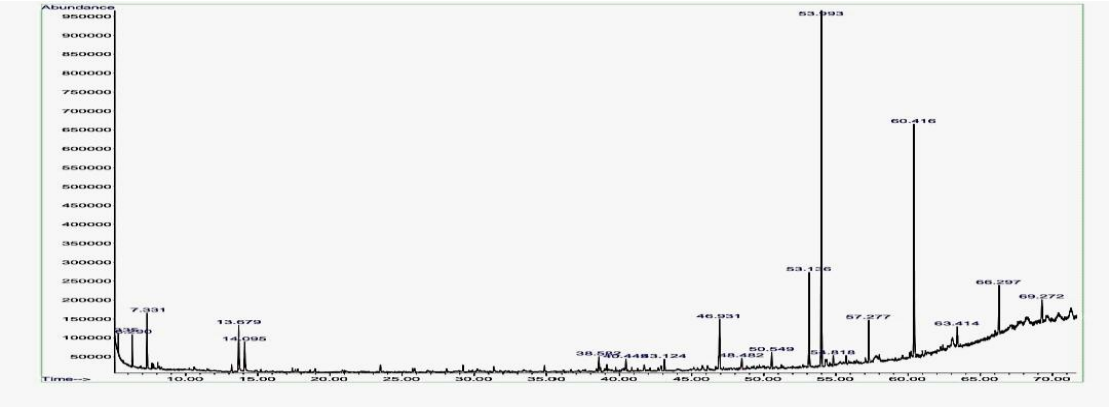

Figure 1. Spectral analysis of active ingredients in GC--mass technology

Table 1. Active ingredients in the plant extract using the GC--mass technology

Peak #	Retention Time (min)	Area Percentage (%)	Compound
1	5.333	1.02	Benzene, 2-Butenedinitrile (E)
2	6.288	1.62	Ethane, 1,1-diethoxy-
3	7.333	3.34	Toluene
4	13.677	3.49	3-Hexanol
5	14.095	2.70	2-Pentene, 2-methyl-
6	38.585	1.30	Eicosane, Pentacosane, Tetracosane
7	40.448	1.01	2-Pentene, 3-methyl-, (E)
8	43.123	1.11	Dodecane, Octadecane
9	4.929	5.38	Heneicosane, Heptacosane, Octadecane
10	50.547	1.47	Pentacosane, Octacosane, 10-Methylnonadecane
11	53.136	8.78	Heneicosane, Tridecane
12	11.993	32.31	Nonadecane
13	54.816	0.95	Methyl 11-(2,3-dideuterocyclopenta, n-1-yl) undecanoate, hexadecanoic acid, methyl ester
14	42.280	3.74	Eicosane
15	33.417	21.52	Heneicosane, Heptadecane
16	32.298	4.54	Eicosane, Docosane
17	17.270	2.65	Tetracosane, Eicosane

Nonadecane, a C19 alkane, is the most prominent compound in the plant, accounting for 32.31% of the total phytochemicals detected. Given its antimicrobial and anti-insect properties, it is not surprising that nonadecane is also used in other medicinal herbs. Its high concentration suggests that it may significantly enhance plant defense mechanisms, particularly the antibacterial defenses observed in previous experiments. Another major component

is henicosan, which appears in several peaks [peaks 9, 11, and 15] with a cumulative area percentage of 35.68% (5.38% + 8.78% + 21.52%). Henicosan, like nonadecane, is a saturated hydrocarbon known for its potential role in plant-microbial interactions. Its presence in significant quantities supports the hypothesis that the biological activity of R. nigrum may be due in part to its alkane content, which may disrupt bacterial cell membranes. In addition, eicosan [peaks 6, 14, 16, and 17] appears at varying concentrations, up to 3.74%. Eicosan has been studied for its potential role in wound healing and anti-inflammatory effects, suggesting that R. nigrum has broader therapeutic applications beyond its antimicrobial activity and presence of toluene (3.34%), a hydrocarbon known for its solvent properties and historical use in traditional medicine for its antiseptic effects, is significant. Its toxicity at higher concentrations requires further research to determine its safe limit for medicinal applications.
Fatty acid esters, such as hexadecanoic acid and methyl ester (peak 13), are also present. These compounds are known for their anti-inflammatory and antioxidant properties, often contributing to the medicinal value of plant extracts. Their presence suggests that black rhodium may have additional benefits, such as modulating oxidative stress in chronic diseases. Minor compounds such as 2-butenedinitrile (a benzene derivative, 1.02% and 1,1-diethoxy-ethane 1.62% are also present, contributing to the plant's bioactivity through synergistic interactions. Although they occur individually at low concentrations, their combined effects with other phytochemicals may enhance the overall therapeutic potential.

Inhibitory effect on bacterial isolates

The correlation observed between the scrape concentration (30 to 90 mg/ml) and the inhibition zone for all bacteria tested was significantly positive ($p < 0.05$, LSD = 2.32 mm). The potency of bioactive compounds (like alkaloids or flavonoids) that are believed to disrupt the cell walls of Gram-positive bacteria seems to be present due to the 25 mm yield increase observed at 90 mg/ml when compared to the 16 mm yield at 30 mg/ml. *Corynebacterium* demonstrated exceptional sensitivity, most likely caused by synergism between the phytochemicals, with 2.4-fold increase (10 to 24 mg) at 90 mg/ml.
The blunted choke concentrations for parasitic phytobacteria were E. coli and Klebsiella, whose values were resistant to 20 mm at 90 mg/ml, showing the dial dependence of dose. The ethanol extract plant Chloramphenicol (the control sample) for every isolate, with the main difference being in *Pseudomonas aeruginosa* (22 mm vs. 8.8 mm) where it is considered deadliest bacteria, Pseudomonas nigricans does appear to target efflux pumps or form biofilms. *Corynebacterium subtilis* (25 mm vs. 7.5 mm), presumed to be targeting Gram-positive peptidoglycan. If its safety is confirmed in toxicity studies, this extract alone is enough to formulate ultraviolet protected product. The ethanol extracts outperformed chloroform (control sample) for all isolates, with the most significant differences observed in *Pseudomonas aeruginosa* (22 mm vs. 8.8 mm), a pathogenic bacterium known for its multidrug resistance, suggesting that Pseudomonas nigricans may target efflux pumps or form biofilms. *Corynebacterium subtilis* (25 mm vs. 7.5 mm), suggesting specificity for Gram-positive peptidoglycan, Research studies have confirmed its safety. This extract may reduce reliance on synthetic antibiotics, thus mitigating the development of resistance. LSD (5%) = 2.32 mm. Differences ≥ this value is statistically significant (e.g., 23 mm for *Corynebacterium* at 90 mg/ml versus 12 mm at 60 mg/ml). Zones of inhibition ≥ 15 mm (CLSI guidelines) indicate therapeutic potential for *B. subtilis, E. coli*, and *Klebsiella* at doses ≥ 60 mg/ml, further studies are needed to evaluate cytotoxicity in human cells to ensure that treatment durations are consistent with efficacy. This study identifies *R. nigrum* as a suitable candidate for antibiotic management, particularly against Gram-positive and biofilm-forming pathogens. By linking traditional knowledge with evidence-based science, we advocate for sustainable antimicrobial solutions that prioritize both efficacy and global health equity.

Table 2. Inhibitory effect of *Rh. nigrum* extract on bacterial isolates

Tested bacteria	Inhibition Diameter Rate (mm) Concentration of ethanol extract				Control (70 %)
Concentrations mg/ml	30	60	90		Chloramphenicol
Bacillus subtilis	16	21	25		7.5
Escherichia Coli	12	15	20		7.3
Corynobacterium	10	12	23		8
Pseudomonas aeruginosa	11	15	22		8.8
Klebsiella	12	15	20		6.3
LSD 5%	2.32				

This study utilizes the antimicrobial activity of aloe vera extract, which is commonly associated with urogenital tract infections, tonsillitis, scarlet fever, rheumatic fever, gastrointestinal tract infections, and wound infections. These potent herbal remedies have made significant progress in fungal infection treatments and are safe for use, especially in immunocompromised patients [18]. The presence of saponins, tannins, alkaloids, lectins, and anthraquinones in aloe vera extract may play an important role in the antifungal activity, since the antibacterial and antifungal actions of these phytochemicals have been well documented [19]. Furthermore, as a result of confirming its common use, the validated result of this study shows that this plant extract may represent a good, non-toxic, and less expensive alternative to allopathic drugs and a new source of antibacterial and antifungal activity. To investigate and isolate these compounds and to study their principles and mechanism of action [20].
Humans have long turned to medicinal plants for healing, and this ancient wisdom is gaining renewed attention, especially for common ailments like UTIs. While antibiotics have been a cornerstone of modern medicine, the growing crisis of antibiotic resistance demands that we uncover new, non-antibiotic treatments. This pressing need has ignited a global quest for bioactive compounds from plants that can effectively combat bacterial infections, particularly UTIs [21]. These plant-derived discoveries offer a hopeful path to developing potent new medications that can overcome microbial resistance, paving the way for more sustainable human healing, The methanol extract of *Rhamphospermum nigrum* showed strong antibacterial activity, particularly against *Corynebacterium* and all Gram-negative bacteria, even at low concentrations. This suggests the extract might be more effective against Gram-negative bacteria. This finding aligns with the principle that antibacterial agents with lower Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) are more effective [22].
The differences in bacterial susceptibility observed could be due to variations in the microorganisms' inherent tolerance or the specific physicochemical properties of the phytochemicals within the plant extract. For example, Gram-negative bacteria possess phospholipid membranes containing lipopolysaccharide (LPS) structural components [23], which can make their cell walls less permeable to antimicrobials. While the overall antibacterial effect of the methanol extract is likely driven by its most common chemical compounds, it's important not to underestimate the therapeutic potential of compounds present in lower concentrations or those without previously identified pharmacological activities in scientific studies [24,25].

Conclusion

This study confirms the significant medicinal value of *Rhamphospermum nigrum* and highlights its rich array of phytochemicals with diverse therapeutic potential. We demonstrated the potent antibacterial effects of the extract, likely due to these identified chemicals. This makes *Rh. nigrum* a promising source of innovative pharmaceutical compounds, particularly for the treatment of urinary tract infections, thus legitimizing its traditional use. Our ongoing discovery programs are now focused on isolating specific bioactive compounds for further evaluation, a process that could be significantly accelerated by artificial intelligence in the future to open up new avenues for human healing**.**

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

References

1. Agneta R, Möllers C, Rivelli A. Horseradish (Armoracia rusticana), a neglected medical and condiment species with a relevant glucosinolate profile: a review. Genet Resour Crop Evol. 2013; 60:1923–43. doi: 10.1007/s10722-013-0010-4.
2. Anastasova I. Thirty-three herbs make the magic of Karaisen wormwood wine. Zemya. 2000 Feb 16; XI (30):3.
3. Anastasova I. Wormwood from Karaisen – the miracle of miracles. Zemya. 2001 Feb 14; XII (31):1-2.
4. Bakalov N. Layed wormwood wine – an inclined to remember tradition. Viticulture and Winemaking. 2011; LVIX (1):26-7.
5. Animashaun TO, Adeoye-Isijola MO, Naidoo KK, Coopoosamy RM, Olajuyigbe OO. Gas chromatography-mass spectrometry analysis, phytochemical screening, antioxidant and antibacterial activity of methanol root extract of Rhaphiostylis beninensis (Hook. f.) Planch. [family Icacinaceae] against Uropathogens. Biomed Pharmacol J. 2023;16(2):1055-79.
6. Sathya PM, Mohan H, Park JH, Seralathan KK, Cho M, Oh BT. Bio-electrochemical degradation of carbamazepine (CBZ): A comprehensive study on effectiveness, degradation pathway, and toxicological assessment. J Environ Manag. 2024; 360:121161.
7. Baziz K, Maougal RT, Amroune A. An ethnobotanical survey of spontaneous plants used in traditional medicine in the region of Aures, Algeria. Eur J Ecol. 2020;6(2). Available from: [https://doi.org/10.17161/eurojecol.v6i2.14669.

8. Boborov EG, Bush NA. Flora of the USSR, vol. 8: Capparidaceae, Cruceriferae and Resedaceae. Leningrad: Akademia Nauk; 1939. 524 p.
9. Bussmann RW, Paniagua Zambrana NY, Sikharulidze S, Kikvidze Z, Kikodze D, Tchelidze D, et al. A comparative ethnobotany of Khevsureti, Samtskhe-Javakheti, Tusheti, Svaneti, and Racha-Lechkhumi, Republic of Georgia (Sakartvelo), Caucasus. J Ethnobiol Ethnomed. 2016; 12:43. Available from: https://doi.org/10.1186/s13002-016-0110-2
10. Bussmann RW, Paniagua Zambrana NY, Sikharulidze S, Kikvidze Z, Kikodze D, Tchelidze D, Batsatsashvili K, Hart RE. Ethnobotany of Samtskhe-Javakheti, Sakartvelo (Republic of Georgia), Caucasus. Indian J Tradit Knowl. 2017;16(1):7–24.
11. Bussmann RW, Batsatsashvili K, Kikvidze Z, Paniagua-Zambrana NY, Khutsishvili M, Maisaia I, et al. *Sinapis arvensis* L. In: Batsatsashvili K, Kikvidze Z, Bussmann RW, editors. Ethnobotany of mountain regions far Eastern Europe. Cham: Springer International Publishing; 2020. Available from: https://doi.org/10.1007/978-3-319-77088-8_127-2
12. Shaheen G, Akram M, Jabeen F, Muhammad S, Shah A, Munir N, et al. Therapeutic potential of medicinal plants for the management of urinary tract infection: A systematic review. Clin Exp Pharmacol Physiol. 2019;46(7):613–24.
13. Al-Badr A, Al-Shaikh G. Recurrent urinary tract infections management in women: a review. Sultan Qaboos Univ Med J. 2013;13(3):359-62.
14. Alteri CJ, Hagan EC, Sivick KE, Smith SN, Mobley H. Mucosal immunization with iron receptor antigens protects against urinary tract infection. PLoS Pathog. 2009;5(9): e1000586.
15. Pitart C, Marco F, Keating TA, Nichols WW, Vila J. Activity of ceftazidime-avibactam against fluoroquinolone-resistant Enterobacteriaceae and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2015;59(6):3059-65.
16. Sweileh WM, Al-Jabi SW, Sa'ed HZ, Sawalha AF, Abu-Taha AS. Global research output in antimicrobial resistance among uropathogens: A bibliometric analysis (2002–2016). J Glob Antimicrob Res. 2018; 13:104-14.
17. Habte TM, Dube S, Ismail N, Hoosen AA. Hospital and community isolates of uropathogens at a tertiary hospital in South Africa. S Afr Med J. 2009;99(8).
18. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect. 2016;22(5):416-22.
19. Kumar A, Sangwan NS. Metabolomics: Towards Understanding. In: *Plant-microbiome Interactions for Climate-resilient Agriculture*. [Publisher?]; 2025. p. 229. *(Note: This reference needs more complete book details like publisher and city of publication)*
20. Erdem I, Ali RK, Ardic E, Omar SE, Mutlu R, Topkaya AE. Community-acquired lower urinary tract infections: Etiology, antimicrobial resistance, and treatment results in female patients. J Glob Infect Dis. 2018;10(3):129.
21. Sathya PM, Mohan H, Park JH, Seralathan KK, Cho M, Oh BT. Bio-electrochemical degradation of carbamazepine (CBZ): A comprehensive study on effectiveness, degradation pathway, and toxicological assessment. J Environ Manag. 2024; 360:121161.
22. Wintola OA, Olajuyigbe AA, Afolayan AJ, Coopoosamy RM, Olajuyigbe OO. Biological activities and chemical composition of essential oil from *Erythrina caffra* Thunb. used in treatment of infections and complications associated with gastrointestinal infections in South Africa. Heliyon (Elsevier). 2021;7(6): e07244.
23. Adeoye-Isijola MO, Jonathan SG, Coopoosamy RM, Olajuyigbe OO. Molecular characterization, gas chromatography mass spectrometry analysis, phytochemical screening and insecticidal activities of ethanol extract of *Lentinus squarrosulus* against *Aedes aegypti* (Linnaeus). Mol Biol Rep. 2021;48(1):41-55.
24. Fagbemi KO, Aina DA, Adeoye-Isijola MO, Naidoo KK, Coopoosamy RM, Olufunmiso Olusola Olajuyigbe OO. Bioactive compounds, antibacterial and antioxidant activities of methanol extract of *Tamarindus indica* Linn. Sci Rep. 2022; 12:9432.
25. Animashaun TO, Adeoye-Isijola MO, Naidoo KK, Coopoosamy RM, Olajuyigbe OO. Gas chromatography-mass spectrometry analysis, phytochemical screening, antioxidant and antibacterial activity of methanol root extract of *Rhaphiostylis beninensis* (Hook. f.) Planch. [family Icacinaceae] against Uropathogens. Biomed Pharmacol J. 2023;16(2):1055-79.