

Phytochemical Analysis And Antibacterial Of Aqueous Leaf Extract Of Libyan Citrullus Colocynthis

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Abstract:

Medicinal plants play a significant role in the treatment of various diseases. Citrullus colocynthis (C.colocynthis) is one example, belonging to the Cucurbitaceae family. This plant has been utilized for conditions such as skin infections, constipation, cancer, diabetes, women's health problems, and respiratory illnesses. It contains numerous beneficial compounds. Researchers have extracted different chemicals from the plant's aqueous extract. The C.colocynthis leaves underwent chemical and botanical analysis. A phytochemical examination identified the presence of phenols, tannins, alkaloids, flavonoids, proteins, amino acids, cardiac glycosides, saponins, carbohydrates, and phytosteroids. Furthermore, the plant's composition was analyzed using GC-MS techniques. C. colocynthis is known for its antibacterial effects. The researchers investigated how the extract from C.colocynthis leaves interacted with three antibiotics at specific levels: tetracycline (30 mg/ml), chloramphenicol (34 mg/ml), and gentamicin (50 mg/ml). They employed the disc diffusion technique to evaluate the extract effectiveness against Escherichia coli, and Staphylococcus aureus,. The study assessed the extract capacity to inhibit growth compared to standard antibiotics.

Keywords: Citrullus colocynthis plant, Antibacterial Activity, Phytochemical, Medicinal Plants.

التحليل الكيميائي النباتي والمضاد للبكتيريا للمستخلص المائي لأوراق نبات الحنظل الليبي

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الملخص:

تلعب النباتات الطبية دورًا مهمًا في علاج العديد من الأمراض. يعد نبات الحنظل أحد الأمثلة، وهو ينتمي إلى عائلة القرعيات. تم استخدام هذا النبات لعلاج حالات مثل التهابات الجلد والإمساك والسرطان والسكري ومشاكل صحة المرأة وأمراض الجهاز التنفسي. يحتوي على العديد من المركبات المفيدة. قام الباحثون باستخراج مواد كيميائية مختلفة من المستخلص المائي للنبات. خضعت أوراق الحنظل للتحليل الكيميائي والنباتي. حدد الفحص الكيميائي النباتي وجود الفينولات والعفص والقلويدات والفلافونويد والبروتينات والأحماض الأمينية والجليكوسيدات القلبية والسابونين والكربوهيدرات والستيرويدات النباتية. علاوة على ذلك، تم تحليل تكوين النبات باستخدام تقنيات كروماتوغرافيا الغاز-مطياف الكتلة. يُعرف الحنظل بتأثيراته المضادة للبكتيريا، وقام الباحثون بدراسة كيفية تفاعل مستخلص أوراق نبات الحنظل مع ثلاثة مضادات حيوية بمستويات محددة: التتراسيكلين (30 ملغ/مل)، والكلورامفينيكول (34 ملغ/مل)، والجنتاميسين (50 ملغ/مل). وقد استخدموا تقنية الانتشار القرصي لتقييم فعالية المستخلص ضد الإشريكية القولونية والمكورات العنقودية الذهبية. وقد قيمت الدراسة قدرة المستخلص على تثبيط النمو مقارنة بالمضادات الحيوية القياسية.

الكلمات المفتاحية: نبات الحنظل، النشاط المضاد للبكتيريا، المواد الكيميائية النباتية، النباتات الطبية.

1. Introduction

Libya is home to a wealth of natural resources, and it is recognized as a nation abundant in various herbs and plants, particularly those with medicinal properties. Despite its vast land area, it has a rich plant diversity [1]. Historically, people across all continents have utilized plants for healing purposes since ancient times. The quest for remedies to viral infections began long before the identification of microbes. Initially, these efforts relied on natural substances, mainly local flora or their extracts. A significant number of these herbal treatments proved effective [2]. Research conducted by [3] centered on the initial phytochemical evaluation and biological activities of *Citrullus colocynthis* (L.) Schrad. as potential agents for controlling mosquito larvae. The main goal of this lab study was to examine the key phytochemical compounds found in the extracts of *C.colocynthis* and evaluate their effectiveness against the fourth instar larvae of *Anopheles arabiensis* Patton. The study revealed a variety of phytochemical elements, including alkaloids, saponins,

flavonoids, triterpenes, and sterols, which showed significant mortality rates and impactful biological effects. The research by [4] examined the antibacterial properties and polyphenol content of *Citrullus colocynthis*. Findings indicated that a small concentration of the extract was capable of addressing certain plant bacterial diseases. The quantity of total phenolic compounds and polyphenols was evaluated using both a plate reader and high-performance liquid chromatography (HPLC), revealing different concentrations across various extracts of *Citrullus colocynthis*. The study demonstrated antimicrobial effectiveness against *Erwinia amylovora* and the resistant bacterium *Bacillus subtilis*. Additionally, chemical analysis confirmed the presence of total phenolic compounds in *Citrullus colocynthis*. A study conducted by Falih et al. [5] sought to assess the antibacterial activity of *Citrullus colocynthis* extracts through in vitro testing. The research aimed to explore the antibacterial properties of the leaf extract of *Citrullus colocynthis*, a medicinal plant traditionally utilized for inducing abortion and treating conditions such as constipation, edema, bacterial infections, cancer, and diabetes. The investigation evaluated the antibacterial performance of *Citrullus colocynthis* (L.) against five species of pathogenic bacteria isolated from urinary tract infections: *E. Coli*, *Klebsiella pneumoniae*, *Streptococcus* spp, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

2. Materials and Methods

Collecting the Plant and preparing it for the Extraction Stage:

Citrullus colocynthis Linn (*C. colocynthis*) leaves were thoroughly harvested from the Dawun area of Tarhuna, which is situated 110 kilometers southwest of Tripoli, the capital of Libya. The leaf specimens of *C. colocynthis* were taken to Elmergib University's Faculty of Science, where the Chemistry Department is located in the picturesque town of Alkhums,

just 120 kilometers from Tripoli, Libya's capital. At this facility, the samples went through a careful sorting and washing process. Initially, they were rinsed with tap water and then treated with distilled water. Following this, the samples were gently dried and laid out in a protected area to prevent direct sunlight exposure. During the drying phase, the samples were stirred frequently to promote uniform drying. Ultimately, the drying was successfully finalized in an electric oven set at a stable temperature of 40°C for three days. After they had dried adequately, the samples were expertly ground using an electric grinder, resulting in a very fine and soft powder. This powder underwent meticulous sifting through a 500 μ sieve to ensure a uniform and high-quality outcome. To maintain the freshness of the samples, they were carefully stored in opaque plastic containers, securely closed, and kept in a cool, dry place until they were ready for use.

Process of Extraction and Measure of Productivity:

The extraction of the *C. colocynthis* plant was carried out using a technique known as maceration extraction. A precise amount of 6.00g of crude powdered plant materials was carefully gathered, and the leaves were placed individually into separate conical flasks, perfectly accommodating the 500ml size. Following this, 100ml of an appropriate solvent (75%) was introduced into the flask. The mixture was then positioned on orbital shakers, which were adjusted to spin at 150rpm for a duration of 72 hours. Once the extraction time was complete, the sample underwent filtration and was dehydrated with a vacuum rotary evaporator. It is important to note that the resulting aqueous extract had a brown tint. To evaluate how effective the extraction process was, the yield was calculated using the following formula.
$$\text{Yield \%} = \text{Weight of plant sample} / \text{Weight of crude extract} \times 100.$$

Measurement of pH of aqueous extract of leaves the plant:

Once the sample was dried from the water-based solvent, the pH of the liquid extract was determined by introducing 100 ml of water and re-dissolving it in a water bath set to 60 °C, then allowing it to cool down to 25 °C.

Phytochemical Screening:**Qualitative Analysis**

1. **Alkaloids Test:** a) Dragendroff 's Test. b)Mayer's Test: [6].
2. **Test for Flavonoids:** a)Alkaline Reagent Test. b) Lead Acetate Test [7].
3. **Test for Tannins:** a) Ferric Chloride Solution (1%) [6].
4. **Test for Phenols:** a)Ferric Chloride Solution 10% [8].
5. **Test for Steroids:** a) **Salkowski Test** [9].
6. **Test for phytosterols:** a) Salkowski's Test
7. **Test of Protein and Amino Acid:** a) Xanthoprotein Test
8. **Test For Gum And Mucilage:** [10].
9. **Test of Carbohydrates:** a) Molisch's Test b)Benedict's Test
10. **Test for Saponins:** a) foma test [6].

Analysis by Gas Chromatography–Mass Spectrometry (GC –MS):

Using Gas Chromatography–Mass Spectrometry (GC–MS) for examination, the analysis reveals a majority of the compounds, along with their individual peak area percentages, retention times, molecular formulas, molecular weights, and notable biological effects. The analysis has identified compounds from *C.colocynthis* leave, with results presented in Table 3.3. Additionally, these results are further illustrated in Figures 3.1, 3.2, and 3.3.

Antibacterial Activity:

The antibacterial assay was performed using the agar disc diffusion method as outlined by [11] for the solvent extract. This investigation focused on gram-positive bacteria, particularly *Staphylococcus aureus*, as well as gram-negative bacteria, including *Escherichia coli*. A 100 µl inoculum (1×10^8 CFU) was added to molten Mueller Hinton Agar (Himedia) and subsequently poured into sterile Petri dishes (Himedia). In the agar disc diffusion technique, a 6mm disc (Hi-Media) was infused with 100 µl of the test compound, allowed to dry, and then positioned on the surface of the inoculated agar plate. The plates were incubated overnight at 37 °C, and microbial growth was evaluated by measuring the diameter of the zone of inhibition. Control samples for each bacterial strain were prepared using pure solvents instead of the extract. The results were obtained by measuring the diameter of the inhibition zones, with the experiment conducted in triplicate, and the mean values reported. The antibacterial activity of the plant extracts was assessed against three antibiotics at designated concentrations: tetracycline (30 mg/ml), chloramphenicol (34 mg/ml), and gentamicin (50 mg/ml). The experiment was repeated three times, with the Petri dishes incubated at 37 °C for 24 hours. Control wells containing antibiotics acted as positive controls, and the antibacterial effects were

determined by measuring the diameter of the inhibition zones after 24 hours, comparing them to the control zones.

3. Results and Discussion

3.1 Results of Color, Productivity, and pH Measurements:

TABLE 3.1 DISPLAYED THE YIELD, COLOR AND THE PH MEASUREMENTS OBTAINED FROM SAMPLES OF THE *C. COLOCYNTHIS* PLANT:

pH	Extract Color	Extract Yield (%)	Extract Aqueous
9.05	brown	14.11	Leaves

Table 3.1 shows the results of the productivity values of the aqueous extract of leaves, the results of the values of the aqueous extract of leaves, was: 14%, where the percentage of productivity were high, As shown in the Table 3.1 displays the result of the color of the aqueous extract of *C. colocynthis* plant leaves, where had the color of the aqueous extract brown . While the results of the pH values of the aqueous extract of the plant leaves, was 9.05, that was basic value.

TABLE 3.2: DISPLAYS THE RESULTS OF THE PRELIMINARY PHYTOCHEMICAL ANALYSIS OF AQUEOUS AND ETHANOL EXTRACTS OF *C. COLOCYNTHIS* (LEAVES, FRUITS, AND SEEDS:

Aqueous extract	Name of Tests	Components'
++	Dragendorff's	Alkaloids
+++	Mayer's	
+++	Alkaline	Flavonoids
++	Lead acetate	

+++	Ferric chloride solution	Tannins
+++	Ferric chloride solution	Phenols
+	Salkowski	Steroids (Terpenoids)
+++	Salkowski's	Phyto Sterols
+++	Lieberman Burchard's	
+++	Copper acetate	
+++	Xanthoprotein	Proteins and amino acids
-	Ninhydrin	
+++	Gum and Mucilage	
+++	Molisch test	Carbohydrates
++	Benedict test	
+++	Saponnins	

Key: +++ indicates a high amount, ++ indicates a moderate amount, + indicates a low amount, and – indicates the absence of an active compound.

As shown in Table 3.2 the results of the initial detection of metabolic products in aqueous extract of the leaves of the *C. colocynthis* plant, where the results of the aqueous extracts of the leave (alkaloids, flavonoids, tannins, phenols, steroid (terpenoids) , phytosterols, cardiac glycosides, proteins and amino acids, carbohydrates) are all present in abundance from very good to excellent, while saponnin was abundant in excellent aqueous extract, and gum and mucilage was abundant in excellent aqueous extract, and gum and mucilage was abundant in aqueous extract.

The analysis of the plant (leaves) of *C. colocynthis* extract using GC-MS yielded the following results:

Table 3.3 showcase the GC-MS analysis results of the plant leaves of *C. colocynthis* extract. Upon conducting analysis, The following Table3.3, show number of compounds, each accompanied by their respective percentage of peak area, retention time, molecular formula, molecular weight, and compelling biological activity.

TABLE 3.3: REVEALED THE RESULTS OF THE GC-MS ANALYSIS PERFORMED ON THE EXTRACT OBTAINED FROM LEAVES OF *C. COLOCYNTHIS*:

Biological Activity	Area %	Mol. Wt (g/mol)	Molecular Formula	RT (min)	Name of the compound	Comp. No.
Antioxidant, antifungal, (Kothiyal et al).	6.86	666	C ₁₈ H ₃₄ O ₉ Si ₉	44.78	Cyclononasiloxane, Octadecamethyl-	1
Antimicrobial, antihelmintic, antioxidant (Al Bratty et al.,2020).	3.32	740	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	50.51	Cyclodecasiloxane, Eicosamethyl-	2
Antimicrobial, antioxidant, anticancer(Albergoni et al.,1980).	3.17	292	C ₁₉ H ₃₂ O ₂	52.61	9,12,15Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-	3

Key: A compound number is denoted by the term "Comp. No.", while the abbreviation "RT"

signifies retention time, and "Mol. Wt." stands for molecular weight.

By analyzing the fragmentation patterns, were able to identify the organic compounds present in the extract of leaves of *C. colocynthis* the constituents that were revealed include the following:

Compound 1: Cyclononasiloxane, Octadecamethyl-

Compound 1, also known as Octadecamethyl-cyclononasiloxane or 2, 2, 4, 4, 6, 6, 8, 8, 10,10, 12,12,14,14,16,16,18,18-octadecamethyl-1,3,5,7,9,11,13,15,17-nonaoxa 2,4,6,8,10,2,4,6,8,10,12,14,16,18 nonasilacyclooctadecane. according to IUPAC, possesses a remarkable structure. As depicted in Fig 3.1, Struc. 3.1, this compound exhibits a mass spectrum and structure typical of glycerides [12], with a retention time 44.78 value, a molecular formula of $C_{18}H_{54}O_9Si_9$, a molecular weight of 666, and an area percentage of 6.86%, Compound 1 stands out as an intriguing aromatic compound. As showed in Its structure comprises Hexagonal shape where there is an silyl group (o-si-o) linked to two oxygen atoms and two methyl groups which repeted eighth times, imbuing it with biological activity. Notably, it displays antifungal, antioxidant properties, making it an exciting area of research [13].

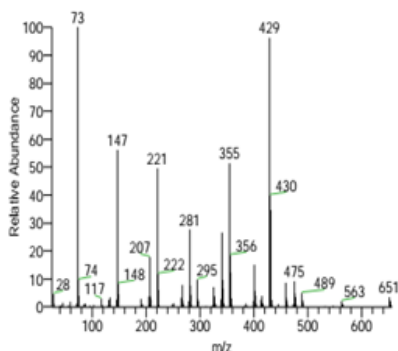
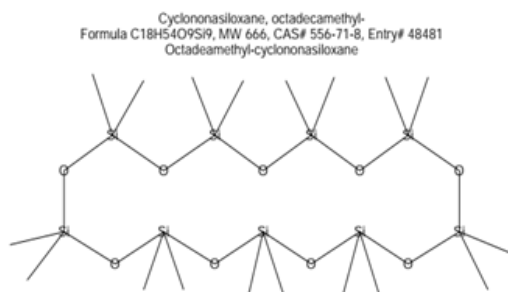


Fig 3.1: Mass Spectrum of Compound
Cyclononasiloxane, Octadecamethyl



Struc. 3.1: Cyclononasiloxane, octadecamethyl

Compound 2: Cyclodecasiloxane, Eicosamethyl-

Compound 2, also known as Eicosamethyl Cyclodecasiloxane or 2, 2,4,4,6,6,8 8,10,10, 12,12,14,14,16,16,18,18,20,20-Icosamethyl-1,3,5,7,9,11,13,15,17,19-Decaoxa-2,4,6,8, 10,12,14,16,18,20-

Decasilacycloicosane according to IUPAC, possesses a remarkable structure. As depicted in Fig 3.2, Struc. 3.2 this compound exhibits a mass spectrum and structure typical of glycerides. [12] with a retention time 50.51 value, a molecular formula of $C_{20}H_{60}O_{10}Si_{10}$, a molecular weight of 740 and an area percentage of 3.32%, Compound 2 stands out

as an intriguing aromatic compound. Its structure comprises Hexagonal shape where there is an silyl group (o-si-o) linked to two oxygen atoms and two methyl groups which repeated nine times, contains biological activity. particularly, it displays antimicrobial, antihelminthic antioxidant, properties, making it an exciting area of research [14].

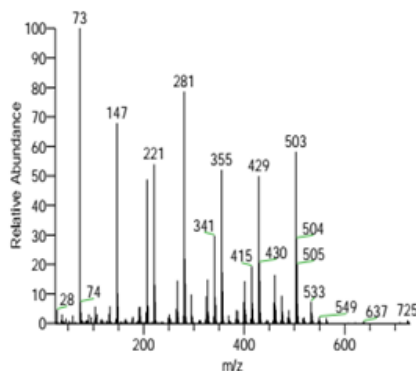
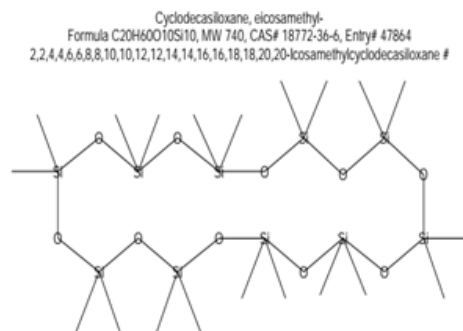


Fig 3.2: Mass Spectrum of Compound
Cyclodecasiloxane, Eicosamethyl-



Struc. 3.2: Cyclodecasiloxane, Eicosamethyl

Compound 3: 9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-

Compound 3, also known as 9,12,15-Octadecatrienoic acid, Methyl Ester or Methyl Octadeca-9,12,15-Trienoate according to IUPAC, possesses a remarkable structure. As depicted in Fig 3.3, Struc. 3.3, this compound exhibits a mass spectrum and structure typical of fatty acid esters. [15] with a retention time 52.61 value, a molecular formula of $C_{19}H_{32}O_2$ a molecular weight of 292, and an area percentage of 3.17%, Compound 3 It is an

aliphatic compound containing a methyl group and a functional group of ester, and there are three pair bonds in carbon atoms No. 9,10,15. includes biological activity. especially, it displays antimicrobial, antioxidant, anticancer properties, making it an exciting area of research [16].

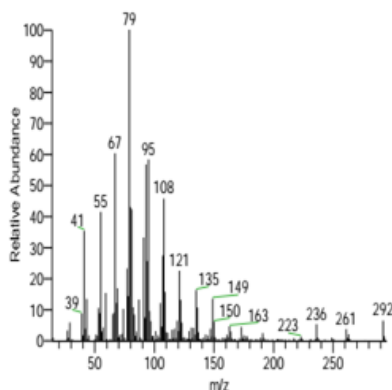


Fig 3.3: Mass Spectrum of Compound 9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-

9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
Formula C19H32O2, MW 292, CAS# 301-00-8, Entry# 53064
Linolenic acid, methyl ester



Struc. 3.3: 9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-

When conducting GC-MC analysis of the study plant (leaves), the results obtained for the *C. colocynthis* plant showed the presence of 3 different compounds in the leaves. The bioactive compounds found in the leaves are classified from glycerides, fatty acid esters, alkaloids, and steroids. Comparing all the compounds obtained by GC-MC analysis of active and biological found in leaves, which are thought to have the same activities in that they are antimicrobial, antiviral, antifungal, anti-cancer and antioxidant. That tannins its importance to prevent the development of microorganisms by precipitating microbial protein and making nutrition proteins are not available to them [17]. Flavonoids display a remarkable array of biochemical and pharmacological actions viz. anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombotic, antiviral and anti-carcinogenic activities. Flavonoids are also shown to inhibit microbes

which are resistant to antibiotics by [18]. It has also been found that alkaloids are important in detoxification and hypertension. properties because alkaloids are known to be effective for this purposes [19,20]. shown that saponins are active antifungal agents [17]. The secondary metabolites identified in the *C. colocynthis* could be responsible for antimicrobial activity exhibited by this plant.

TABLE 3.4: ANTIBACTERIAL ACTIVITY OF THE *C. COLOCYNTHIS* EXTRACT AGAINST *ESCHERICHIA COLI*, AND *STAPHYLOCOCCUS AUREUS*:

Aqueousextract	Microorganisms
8 mm	<i>E. coli Escherichia coli</i>
9 mm	<i>Staphylococcus aureus</i>

concluded that in the antibacterial activity experimental (effective against bacteria staphylococcus aureus and Escherichia coli . of the plant exhibited activity comparable to action of commercial antibiotics (tetracycline, chloramphenicol, and gentamicin). So, some of secondary metabolites identified in the leaves, of *C. colocynthis* plant may be responsible for the antibacterial activity.

4. Conclusion

To sum up we can say it can be inferred that the analysis of the leaves of the *C. colocynthis* plant using GC-MS revealed the presence of bioactive substances. These substances are thought to have properties that are antimicrobial, antifungal, anticancer, antioxidant and antiviral activities making them valuable to humans. Given the promising findings from the leaf's aqueous extract, the plant's value as a medicinal resource is recognized for its ability to hinder the growth of specific harmful bacteria. Its

abundance of secondary metabolites allows it to function as a natural antibiotic.

5. Recommendations

In this research study, we suggest the following:

- It is recommended to separate the chemical substances derived from leaves in this study, extract them, and verify their biological effects by performing tests against various harmful bacteria and fungi. This could be advantageous for the pharmaceutical industry through the use of these biochemical compounds.
- Also, draw attention to the conduct of laboratory analyzes of leaves, extract aqueous and organic solvents other against harmful bacterial strains other than those referred to in this study, extracts against any type of bacteria, and to make antibiotics suitable for these harmful strains.
- It was also recommended to study and analyze heavy elements and metals and to know their concentration.

References:

- [1] K. N. Watanabe, A. Kikuchi, Z. K Shinwari, Z. I Khattak & S. A. Gilani, "Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta* Decne. Phytotherapy Research", *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, vol.21, no.4, pp. 301-307, 2007.
- [2] A. Sofowora, "Screening plants for bioactive agents," *Medicinal plants and traditional medicinal in Africa*, Spectrum Books, Ibadan, 156pp, 1993.
- [3] A. A., Satti, & A. E. Edriss, "Preliminary Phytochemical Screening and Activities of *Citrullus colocynthis* (L.) Schrad. as Mosquito Larvicides," *World Journal of Pharmaceutical Research*, vol.3, no.10, pp. 1705-1720, 2014.
- [4] A. M., Mehni, S., Ketabchi, & G. H. S. Bonjar, "Antibacterial activity and polyphenolic content of *Citrullus colocynthis*," 2014.

- [5] H. Y., Falih, Z. F., Shaker, & H. H., Abed, "Evaluation Of The Antibacterial Activity Of Citrullus Colocynthis Extracts (In vitro study)," *Al-Qadisiyah Journal of Pure Science*, vol. 26, no. 4, pp.175-180, 2021.
- [6] N. Savithramma, M. L., Rao, & D. Suhurulatha, "Screening of medicinal plants for secondary metabolites. Middle," *East Journal of Scientific Research*, vol. 8, no. 3, pp.579-584, 2011.
- [7] A. Soni, & S. Sosa, "Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts," *Journal of Pharmacognosy and phytochemistry*, vol. 2, no.4, pp.22-29, 2013.
- [8] N. Tamilselvi, P. Krishnamoorthy, R. Dhamotharan, P. Arumugam, & E. Sagadevan, "Analysis of total phenols, total tannins and screening of phytocomponents in Indigofera aspalathoides (Shivanar Vembu) Vahl EX DC," *Journal of chemical and pharmaceutical research*, vol. 4, no. 6, pp.3259-3262, 2012.
- [9] R. Sheel, K. Nisha, & J. Kumar, "Preliminary phytochemical screening of methanolic extract of Clerodendron infortunatum," *IOSR Journal of Applied Chemistry*. Vol.7, no. 1, pp.10-13, 2014.
- [10] G.E. Trease, & W. C. Evans, "Pharmacognosy," London, 1983.
- [11] A. W. Bauer, W. M. M. Kirby, J. C. Sherris, M. Turck, "Antibiotic susceptibility testing by a standardized single disk method," *Am. J. Clin. Pathol*, vol.45, pp.493-496, 1966.
- [12] M. Elshaier, "Identifying Females of Three Carder Bee Species of Genus Anthidium (Hymenoptera: Megachilidae) Using Chemical Compounds of Cuticle," *Egyptian Journal of Chemistry*, vol. 65, no. 9, pp.431-437, 2022.
- [13] G. Kothiyal, K. Kishor, R., Kamal & N. Kunar, "GC-MS Analysis For Determination of Bioactive Compounds of Phellinus Pectinatus," *A Species of Wild Mushroom from Uttarakhand Himalaya, India*, 2024.
- [14] M. AlBratty, H. A. Makeen, H. A. Alhazmi, S. M. Syame, A. N. Abdalla, H. E. Homeida & A. Khalid, "Phytochemical, cytotoxic, and antimicrobial evaluation of the fruits of miswak plant, *Salvadora persica* L," *Journal of Chemistry*, 2020.
- [15] F. Khan, M. G. Magaji, I. Abdu-Aguye, I. M. Hussaini, A. Hamza, A. B. Olorukooba & I. M. Maje, "Phytochemical profiling of the bioactive principles of *Alysicarpus glumaceus* (Vahl) DC. aerial parts," *Istanbul Journal of Pharmacy*, vol. 51, no. 2, pp.228-238, 2021.

- [16] V. Albergoni, E. Piccinni, & O. Coppelotti, "Response to heavy metals in organisms—I. Excretion and accumulation of physiological and non-physiological metals in *Euglena gracilis*. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, vol. 67, no. 2, pp.121-127, 1980.
- [17] O. A. Sodipo, M. A. Akanji, F. B., Kolawole, & A. A. "Odutuga, Saponin is the active antifungal principle in *Garcinia kola*, heckle seed," *Biosci. Res. Commun*, vol. 3, pp.171, 1991.
- [18] M. Iinuma, H. Tsuchiya, M. Sato, J. Yokoyama, M. Ohyama, Y. Ohkawa, & T. Fujii, "Flavanones with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*," *Journal of Pharmacy and pharmacology*, vol. 46, no. 11, pp.892-895,1994.
- [19] W. C . Trease Evans, "Pharmacognosy," *Baillene Tindall, London*, pp.735-738, 1982.
- [20] R. Y. Zee-Cheng, "Anticancer research on Loranthaceae plants," *Drugs of the Future*, vol. 22, no. 5, pp.519-530, 1997.