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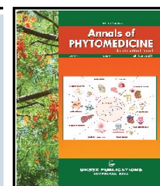

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Phytochemical profiling and bioactive potential of *Adenium obesum* (Forssk.) Roem. & Schult. leaf extracts

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Abstract

Plants are rich sources of diverse phytochemicals, including cardiac glycosides, cardenolides, pregnanes, triterpenes, flavonoids and other secondary metabolites. This research investigates the therapeutic potential of *Adenium obesum* (Forssk.) Roem. & Schult. ethanolic leaf extract (AOE), focusing on its antioxidant and antibacterial properties. GC-MS analysis showed 45 phytochemical components in the extract, comprising potential bioactive substances. An evaluation of the antioxidant activity was conducted employing the DPPH radical scavenging assay, which demonstrated significant dose-dependent free radical neutralization, comparable to ascorbic acid. AOE exhibited neutralization percentages ranging from 67.0 to 90.97 at concentrations between 20 and 100 µg/ml. Antibacterial activity was compared to *Staphylococcus aureus* (G^{+ve}) and *Escherichia coli* (G^{-ve}) using the zone of inhibition technique. The extract showed varying effectiveness against both strains, with inhibition zones reaching up to 30 mm for *S. aureus* and 25 mm for *E. coli* at higher concentrations. The presence of compounds like benzofuran, phenol and various other phytochemicals may contribute to these observed bioactivities. The *A. obesum* extract possesses significant antioxidant and antibacterial properties, suggesting its potential for developing new plant-based therapeutic agent. These findings provide a basis for upcoming investigations concerning the diverse therapeutic uses of *A. obesum*.

1. Introduction

A large number of secondary metabolites have demonstrated significant antioxidant and other activities, including flavonoids, phenolic acids, lignans, quinones, coumarins and alkaloids (Boy *et al.,* 2018). About 80% of people in developing nations are thought to treat a variety of illnesses with natural or herbal remedies (Purushotham *et al.,* 2016; Jeurkar *et al.,* 2022). To treat a wide range of health issues, native Americans and traditional healers largely rely on the medicinal qualities of plants, particularly in India which is home to the three well-known traditional healthcare structures of Ayurveda, Siddha and Unani (Seetharamu *et al.,* 2023). Plants possess a variety of pharmacological properties because of the existence of secondary metabolic products such as phenolic compounds, alkaloids, terpenoids, glycosides, steroids and flavonoids (Adiloðlu *et al.,* 2018). Through a variety oftechniques for function, including avoidance of hazardous substances, reducing reactive oxygen species and suppressing oxidative enzymes, these bioactive substances have been described to have a variety of positive outcomes in reducing the threat of illnesses brought on by reactive oxygen species (Ijaz *et al.,* 2017). Plant extracts have been shown in reports to possess a variety of biochemical qualities; for example, antioxidant, anti-inflammatory, antiallergic, antimicrobial and antibacterial activity (Pisoschi *et al.,* 2015). A known cause of chronic inflammation is oxidative damage, this is characterized as a disparity in the generation of reactive oxygen species and the defence ability to remove them. Studies have indicated that oxidative stress contributes to the cause of chronic inflammatory conditions. Furthermore, reactive oxygen species have been identified as a primary contributor to a number of inflammatory illnesses, including cardiovascular disease (Hussain *et al.,* 2016), its anti-influenza properties (Kiyohara *et al.,* 2012), antimicrobial (Hossain *et al.,* 2017) and cytotoxic efficacy (Almehdar *et al.,* 2012) have been documented earlier. It contained about 50 phytochemicals that belonged to the classes of cardenolides, flavonoids, triterpenes and pregnanes, making it an abundant supply of cardiovascular glycosides (Versiani *et al.,* 2014). In the Middle East, numerous components of this plant were utilized to address a range of ailments, such as wounds, skin conditions, joint pain and muscle soreness (Hossain *et al.,* 2011). Cardiomyogenic glycosides, which havenumerous biological activities, were abundant in *A. obesum* (AO). Many chemical components, including pregnanes, cardenolides, triterpenes, flavonoids are present in the foliage. The World Health Organization (WHO) promotes the integration of folk medicine into healthcare systems for the purpose of treating and preventing diseases, acknowledging its importance in member nations (Bamne *et al.,* 2023). The present investigation was designed to examine the ethanolic extracts of *A. obesum* leaves for its antioxidant and antibacterial properties.

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2. Materials and Methods

2.1 Chemicals

Sigma-Aldrich (St. Louis, MO) provided the DPPH, methanol and ascorbic acid (standard) were procured from Sai Scientifics, India.

Table 1:Parameters that are employed in GC-MS analysis

Parameter	Value
Instrumentation	Agilent GC 7890A / MS5975C
Column	Agilent DB5MS
Column length	30 m
Internal diameter	0.25 mm
Film thickness	0.25 ìm
Sample injection mode	Split mode
Carrier gas	Helium
Sample preparation	Extract dissolved in 100% ethanol
Compound identification	Retention time and fragmentation pattern matched with the NIST spectral library
Reference method	Kushwaha *et al*. (2019)

2.2 Plant collection

2.2.1 Extraction

The foliage of Adenium genotype Sudharsan (sample 1) (Figure 1) and another genotype Nilakaan (sample 2) (Figure 2) underwent an initial rinse under flowing water, followed by air-drying for approximately 6-7 days. Subsequently, the leaves were meticulously sectioned and pulverized individually using a grinder. The resultant powder was carefully collected and preserved in dry containers. The powdered specimens were encased within a Soxhlet apparatus and subjected to extraction using a solution comprising 150 ml of 70% ethanol and 30% water, chosen for its demonstrated superior extraction efficiency as reported (Hossain *et al.,* 2015). Following this, the resultant mixture underwent filtration employing an evaporator set at 40°C. For the purpose of further analysis easier, after being dried, the compound was kept. at –20°C. *A. obesum's* semi-solid ethanolic extract (AOE) was extracted and kept in storage at 4°C. Using the described methodology, the yield extract in percentage (%) was measured (Motadi*et al.,* 2020).

$$\text{Extract yield \%} = \frac{\text{Weight of the extract after evaporating solvent and freeze drying}}{\text{Dry weight of the sample}} \times 100$$

Figure 1: Sudharsan

Figure 2: Nilakaan

2.2.2 Gas chromatography coupled with mass spectroscopy analysis

GC-MS was used to characterize secondary metabolites in the ethanol-based extract leaves of *A. obesum* using previously described techniques (Kushwaha *et al.,* 2019). The experiment was conducted utilizing Agilent GC 7890A/MS5975C using a capillary column after the extract had been dissolved in 100% ethanol before analysis. With an injector operating in split mode and helium present, the sample was introduced into the Agilent DB5MS apparatus, which has a column length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 microns. Using the NIST spectral library, the retention time and fragmentation pattern were assessed for the purpose of identifying the extract's bioactive ingredients.

2.3 *In vitro* antioxidant activity

Plant extract solution at various ratios (20, 40, 60, 80 and 100 µg/ml) was combined alongside 0.5 ml of 0.1 mm DPPH solution in methanol. Ascorbic acid (50-250 µg/ml) was utilized as the reference standard and corresponding blank samples were prepared. A control included a mixer containing 0.5 ml of methanol and 0.5 ml of DPPH solution. After 30 min in the absence of light, the reaction was performed in triplicate and the wavelength decreased was determined at 520 nm using a Microplate Reader (Elisa Reader - Bio-Rad). Using the following formula, the inhibition percentage was found:

$$\text{Inhibition } \% = \frac{Ac - As}{Ac} \times 100$$

where, Ac denotes the absorbance of the control and As denotes the absorbance of the sample

2.4 Antibacterial activity

2.4.1 Inoculum preparation

From an agar plate culture, three or five completely separate colonies of the identical structural type were chosen. The top of each colony was gently contacted by a loop and the growth was then placed into a tube holding four to five millilitres of Nutrient-rich broth or another suitable broth type. The broth culture was incubated for two to six hours at 35°C, or up to it reached or exceeded the turbidity. To achieve the desired turbidity, sterile saline or broth was added to the rapidly expanding broth culture. This yields a suspension of (G^{-ve}) *E. coli* and (G^{+ve}) *S. aureus* with a variate of approximately 1 to 2 x 108 CFU/ml (Kirby-Bauer method, 1996).

2.4.2 Inoculation of test plates

An inoculated swab of cotton was ideally inserted into the modified suspension for 15 min of the inoculum suspension's turbidity becoming adjusted. After rotating the swab a few times, press it firmly against the tube's interior wall above the fluid level. By doing this, the swab's excess inoculum will be removed. By swabbing the entire sterile agar surface, a nutrient agar plate's dried surface was inoculated. To ensure that the inoculum was dispersed uniformly, this process was repeated twice more, revolving the plate by roughly 60° each time.The final step was to swab the rim of the agar for three to 5 min, the lid may be shaken prior to implementing the drug-impregnated disks, but not longer than 15 min, to facilitate the absorption of any surplus surface moisture. A 6 mm-diameter well was made in the media, which was then punctured and filled with 20-100 µl of the sample. In addition, the petri dishes had been placed inversely in order to promote complete diffusion and zones of restriction were assessed by quantifying the diameter (mm) that developed surrounding the well following a 24 h the time of incubation at 37°C. Standard (Hi-Media) scale was used to measure the zones.

3. Results

3.1 GC-MS Analysis

Overall, 45 peaks of each (samples 1, 2) were recorded in the GC-MS graph of the extracts of *A. obesum* (Figures 3, 4), which corresponded in relation to the bioactive substances identified by assessing the duration of retention and dissection pattern with the help of the NIST the spectrum archive and GC-MS real-time evaluation. Tables 2 and 3 contain the recognized phytoconstituents. The extract's chromatogram clearly demonstrated the presence of a number of phytoconstituents that had taken place, linked to anticancer activity in various extracts. The ethanolic extract of AOE contained various compounds, including diglycerol, 1, 3-dioxane, benzofuran, benzoic acid, thiophene, butanoic acid, acetamide, oxalic acid and myo-inositol.

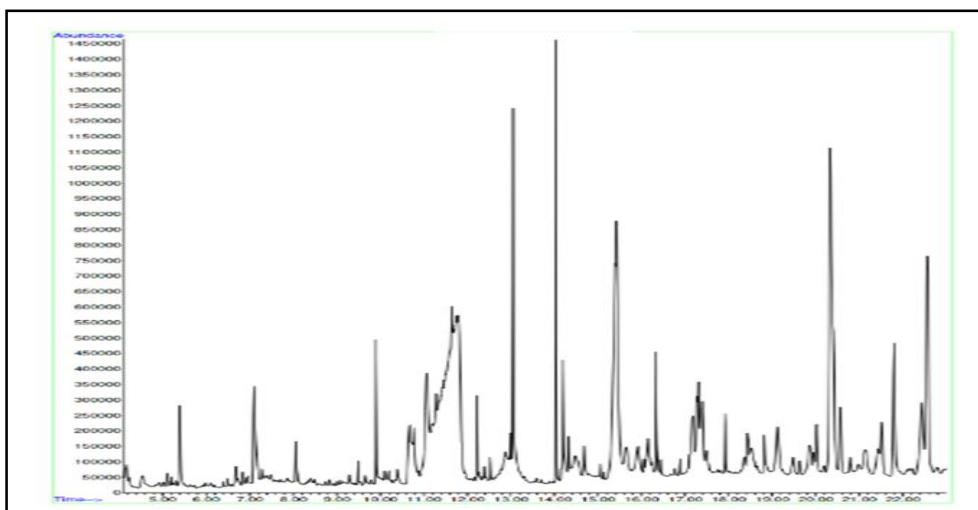

Figure 3: GC-MS chromatogram of *A. obesum* extracts (sample 1).

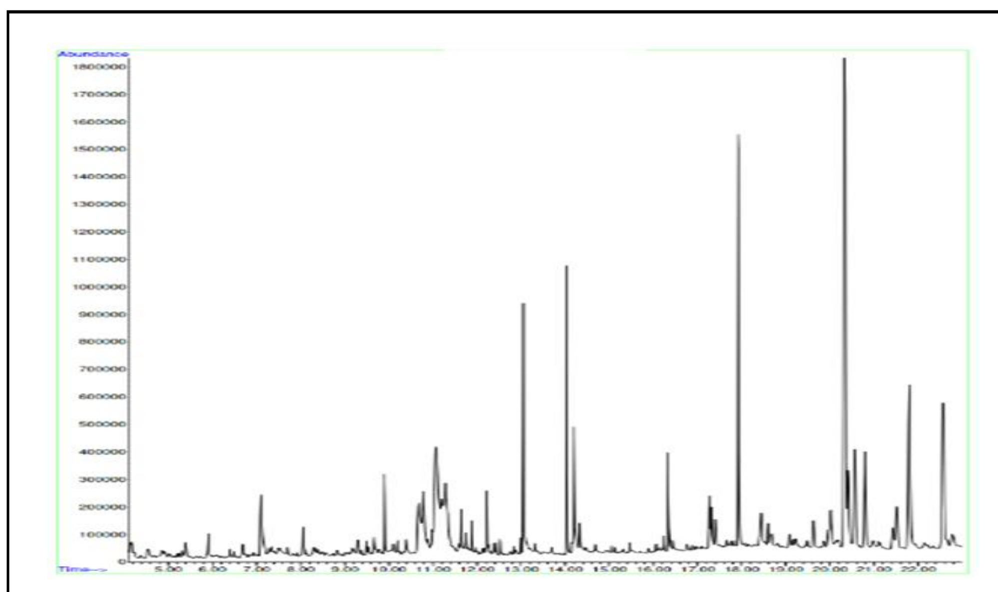

Figure 4: GC-MS chromatogram of *A. obesum* extracts (sample 2).

Table 2: List of various phytochemicals identified by GC-MS analysis that are contained in the ethanolic leaf extracts of *A. obesum* (sample 1)

Peak No.	Name of the compound	RT time	PA%	Molecular weight (g/mol)	Molecular formula
1	Diglycerol	4.542	0.58	166	$C_6H_{14}O_5$
2	1,3-Dioxane	5.398	1.17	88	$C_4H_8O_2$
3	Benzofuran	7.098	2.56	118	C_8H_6O
4	Ethanone	8.053	0.88	58	C_2H6O
5	Benzoic acid	9.897	1.46	122	$C_7H_6O_2$
6	Butanoic acid	10.675	2.44	88	$C_4H_8O_2$
7	Oxalic acid	10.764	0.91	90	$C_2H_2O_4$
8	Thiophene	11.064	4.57	84	C_4H_4S
9	3-O-Methyl-d-glucose	11.286	0.81	194	$C_7H_{14}O_6$
10	Myo-Inositol	11.642	5.75	180	$C_6H_{12}O_6$
11	Bicyclo[10.8.0]eicosane	12.219	0.71	278	$C_{20}H_{38}$
12	Beta-Amyrin	12.875	2.13	426	$C_{30}H_{50}O$
13	Diphenyl sulfone	12.986	0.78	218	$C_{12}H_{10}O_2S$
14	n-Hexadecanoic acid	13.053	5.02	256	$C_{16}H_{32}O_2$
15	Phytol	14.030	3.60	296	$C_{20}H_{40}O$
16	9,12,15-Octadecatrienoic acid	14.197	1.91	278	$C_{18}H_{30}O_2$
17	Octadecanoic acid	14.319	0.43	284	$C_{18}H_{36}O_2$
18	1,4-Dimethyl-8-isopropylidenetricyclo [5.3.0.0(4,10)] decane	14.475	1.50	204	$C_{15}H_{24}$
19	2-Heptenoic acid	14.675	0.60	128	$C_7H_{12}O_2$
20	Alpha-Amyrin	15.419	12.61	426	$C_{30}H_{50}O$
21	Tricosanoic acid	15.641	1.35	354	$C_{23}H_{46}O_2$
22	Acetamide	15.919	1.45	59	C_2H_5NO
23	Vitamin E	16.152	1.44	430	$C_{29}H_{50}O_2$

24	Hexadecanoic acid	16.330	1.46	256	$C_{16}H_{32}O_2$
25	9,9'-Biphenanthrene	17.185	2.40	378	$C_{30}H_{18}$
26	Lupeol	17.308	2.92	426	C30H50O
27	Tridecanedioic acid	17.396	2.05	244	$C_{13}H_{24}O_4$
28	Benzo[h]quinoline	17.485	0.69	179	$C_{13}H_9N$
29	2,6,10,14,18,22-Tetracosahexaene	17.919	0.68	322	$C_{24}H_{34}$
30	Benzamide	18.363	0.44	121	C_7H_7NO
31	Heptadecane	18.430	0.98	240	$C_{17}H_{36}$
32	2,5,5,6,8a-Pentamethyl-trans-4a	18.519	0.95	204	$C_{15}H_{24}$
33	2H-1-Benzopyran-6-ol	18.818	0.77	160	$C_{10}H_8O_2$
34	3-Formyl-6-(4-methoxyphenyl)-2H-thiopyran	19.118	1.97	232	$C_{13}H_{12}O_2S$
35	Gamma-Tocopherol	19.485	0.52	416	$C_{28}H_{48}O_2$
36	Phenol	19.863	1.08	94	C_6H_6O
37	Octacosyl acetate	20.018	0.91	468	$C_{30}H_{60}O_2$
38	dl-alpha-Tocopherol	20.329	11.34	430	$C_{29}H_{50}O_2$
39	Cholesta-5,20,24-trien-3-ol	20.563	1.27	384	$C_{27}H_{44}O$
40	Indeno(1,2,3-ij)isoquinoline	21.129	1.13	229	$C_{17}H_{11}N$
41	Cyclotrisiloxane	21.429	0.87	236	$C_6H_{18}O_3Si_3$
42	Campesterol	21.507	1.41	400	$C_{28}H_{48}O$
43	Stigmasterol	21.796	3.11	412	$C_{29}H_{48}O$
44	Neoisolongifolene	22.440	2.93	204	$C_{15}H_{24}$
45	Gamma-Sitosterol	22.562	5.50	414	$C_{29}H_{50}O$

Table 3: List of various phytochemicals identified by GC-MS analysis that are contained in the ethanolic leaf extracts of *A. obesum* (sample 2)

Peak No.	Name of the compound	RT time	PA%	Molecular weight (g/mol)	Molecular formula
1	3-Acetyl-dihydro-tomatidine	4.176	0.96	223	$C_{13}H_{21}NO_2$
2	Methane	4.542	0.51	16	CH_4
3	Benzeneacetaldehyde	5.387	0.39	120	C_8H_8O
4	1,6-Octadien-3-ol, 3,7-dimethyl	5.909	0.66	154	$C_{10}H_{18}O$
5	5-Methoxypyrrolidin-2-one	6.686	0.53	115	$C_5H_9NO_2$
6	Benzofuran	7.098	2.42	118	C_8H_6O
7	Divinyl sulphone	7.520	0.41	118	$C_4H_6O_2S$
8	2-Methoxy-4-vinylphenol	8.053	0.90	150	$C_9H_{10}O_2$
9	2,3-Dimethyl-3-pyrazolin-5-one	9.664	0.52	112	$C_5H_8N_2O$
10	Benzoic acid	9.897	1.34	122	$C_7H_6O_2$
11	2,3,5,6-Tetrafluoroanisole	10.197	0.34	182	$C_7H_4F_4O$
12	Coumarin	10.386	0.57	146	$C_9H_6O_2$
13	Hexanoic acid	10.675	2.98	116	$C_6H_{12}O_2$
14	Decane	10.775	1.85	142	$C_{10}H_{22}$
15	4-O-Methylmannose	11.064	8.32	194	$C_7H_{14}O_6$
16	3-Methylmannoside	11.286	4.52	194	$C_7H_{14}O_6$
17	Tetradecanoic acid	11.641	0.74	228	$C_{14}H_{28}O_2$

18	Cyclododeca-4,8-dien-1-one	11.886	0.49	178	$C_{12}H_{18}O$
19	Dodecane	12.219	0.99	170	$C_{12}H_{26}$
20	Pentadecanoic acid	13.052	4.67	242	$C_{15}H_{30}O_2$
21	Isophytol	14.030	3.95	296	$C_{20}H_{40}O$
22	9,12,15-Octadecatrienoic acid	14.197	2.99	278	$C_{18}H_{30}O_2$
23	Octadecanoic acid	14.319	0.91	284	$C_{18}H_{36}O_2$
24	15-Hydroxypentadecanoic acid	16.330	1.94	258	$C_{15}H_{30}O_3$
25	Eicosane	17.263	1.05	282	$C_{20}H_{42}$
26	Nonanoic acid	17.307	0.97	158	$C_9H_{18}O_2$
27	1-Nonadecene	17.396	0.57	266	$C_{19}H_{38}$
28	2,6,10,14,18,22-Tetracosahexaene	17.918	8.43	322	$C_{24}H_{34}$
29	17-Pentatriacontene	18.441	1.46	490	$C_{35}H_{70}$
30	Indolizine	18.596	0.46	105	C_7H_7N
31	1,2-Bis(trimethylsilyl)benzene	18.685	0.45	222	$C_{12}H_{22}Si_2$
32	Trimethyl[4-(1,1,3,3,-tetramethylbutyl) phenoxy]silane	19.085	0.35	278	$C_{17}H_{30}OSi$
33	N-Methyl-1-adamantaneacetamide	19.218	0.39	207	$C_{29}H_{48}O$
34	Gamma-Tocopherol	19.629	0.76	416	$C_{28}H_{48}O_2$
35	1-Heptacosanol	20.018	1.81	396	$C_{27}H_{56}O$
36	Vitamin E	20.329	14.69	430	$C_{29}H_{50}O_2$
37	Cholesterol	20.407	2.37	386	$C_{27}H_{46}O$
38	Androst-5	20.563	3.31	274	$C_{19}H_{30}O$
39	Crinan-3-ol	20.796	3.14	388	$C_{25}H_{40}O_3$
40	Silane	20.974	0.34	32	SiH_4
41	16-Pregnen-3,20-dione	21.429	0.84	314	$C_{21}H_{30}O_2$
42	5-Cholestene-3-ol	21.507	1.69	386	$C_{27}H_{46}O$
43	Stigmasterol	21.796	6.28	412	$C_{29}H_{48}O$
44	Pregn-5-en-3-ol	22.562	6.04	302	$C_{21}H_{34}O$
45	5-Methyl-2-phenylindolizine	22.773	0.70	207	$C_{15}H_{13}N$

3.2 Antioxidant activity

The adenium extract demonstrated significant DPPH-free radical neutralization capability through hydrogen donation, with varying effectiveness across different concentrations. Sample 1 exhibited neutralization percentages of 87.50%, 84.38%, 81.25%, 77.08% and 70.49%, while sample 2 showed 90.97%, 85.07%, 78.13%, 73.61% and 67.0% neutralization at levels of 20, 40, 60, 80 and 100 µg/ml respectively. Interestingly, both samples displayed higher neutralization percentages at lower concentrations, with a gradual decrease as concentration increased. Figure 5 illustrated thatin comparison to ascorbic acid, DPPH radical scavenging is grown in a manner that is subject to dose, which served asthe study's positive antioxidant control or reference. This comparison to ascorbic acid, a well-known potent antioxidant, suggests that both samples possess strong antioxidant properties.The action of eliminating increases in adosage-related way, coupled with the high neutralization percentages which indicates that these samples could potentially be valuable sources of natural antioxidants.

3.3 Antibacterial activity

Using the zone of inhibition method, the antibacterial activity of plant foliage extracts, *Escherichia coli* and *Staphylococcus aureus* was assessed, with results presented in Figures 6, 7 and 8. For *S. aureus,* sample 1 showed no inhibition (0 mm) at lower concentrations but exhibited significant activity at higher doses, with zones of 25 mm and 26 mm at 80 and 100 µg/ml, respectively. Sample 2 demonstrated a broader range of activity against *S. aureus,* with zones of 10 mm, 11 mm, 24 mm and 30 mm at 20, 40, 80 and 100 µg/ ml, respectively. Against *E. coli*, sample 1 showed no inhibition at 20 µg/ml but produced zones of 22 mm, 17 mm and 23 mm at 40, 80 and 100 µg/ml, respectively. Sample 2 exhibited consistent actions taken in opposition to *E. coli* across all concentrations, accompanied by areas of limitation 10 mm, 20 mm, 20 mm and 25 mm at 20, 40, 80 and 100 µg/ml, respectively. According to these findings, both samples may have several antibacterial activities against various strains of bacteria, both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*), based on concentration and the specific bacterial strain.

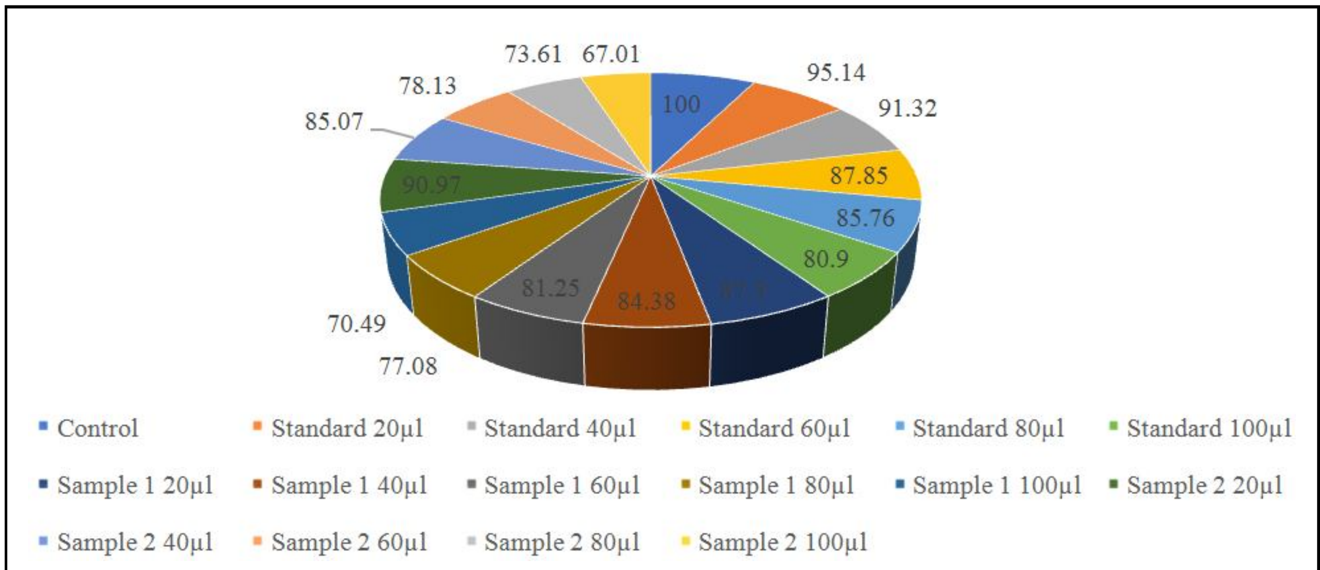

Figure 5: DPPH radical scavenging assay of *A. obesum* leaves.

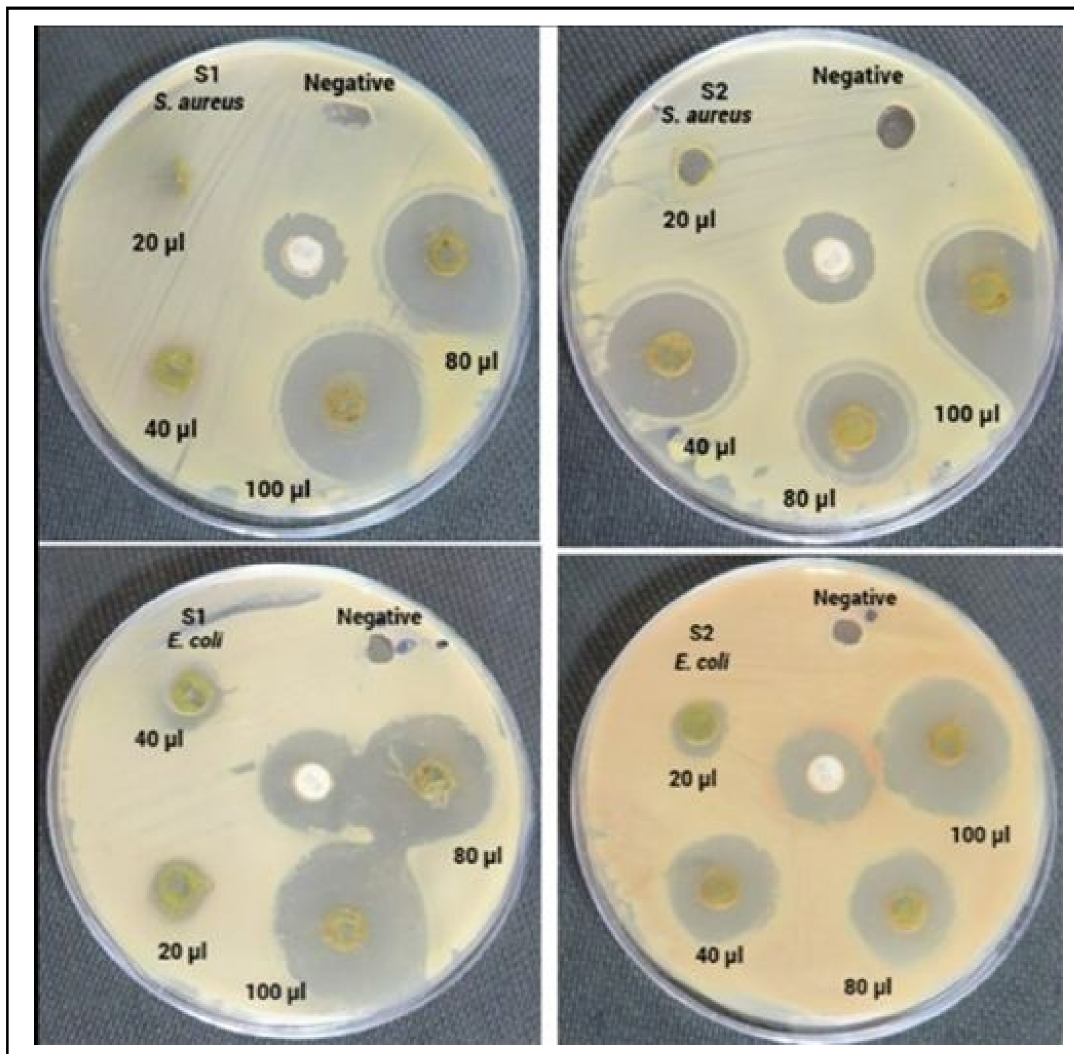

Figure 6: Antibacterial activity of *A. obesum* leaves.

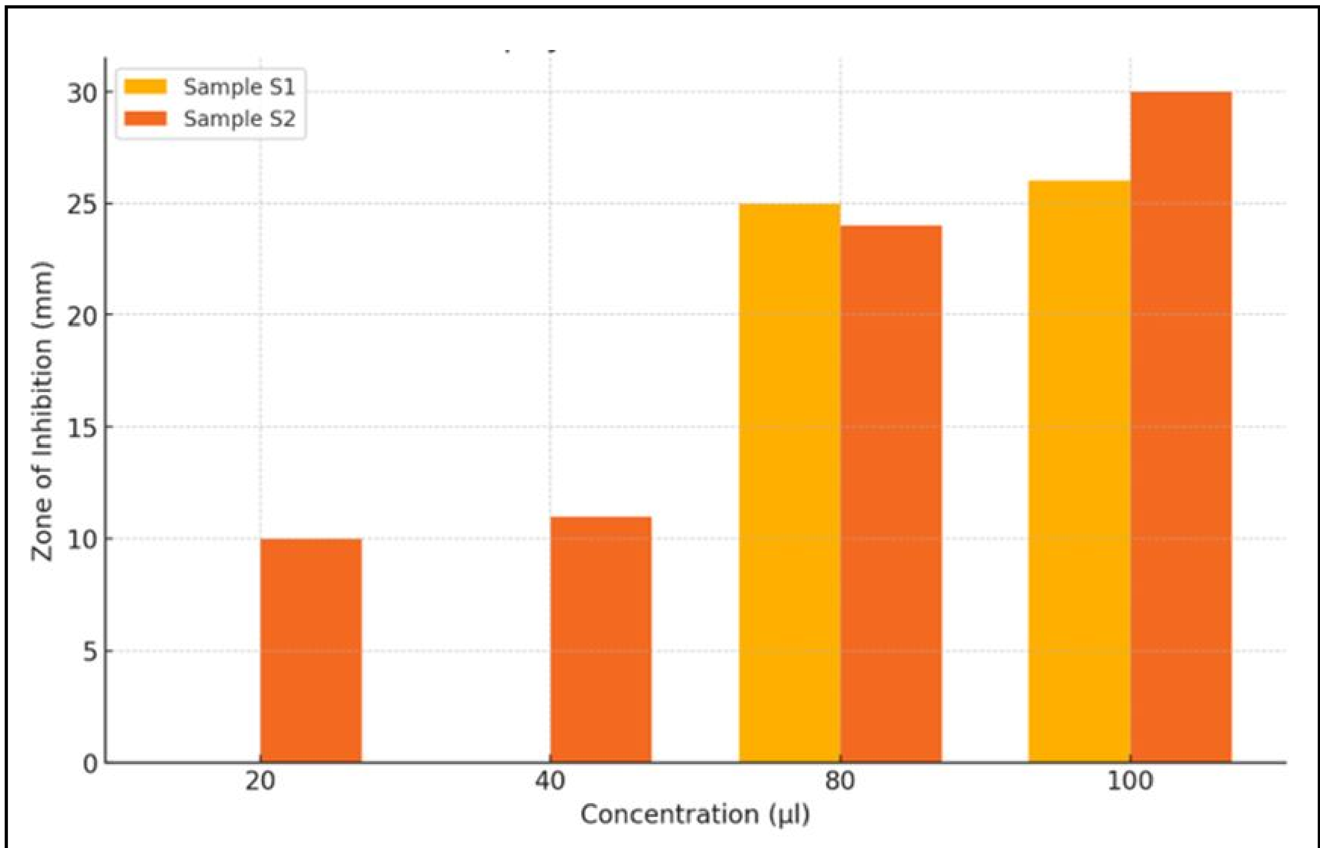

Figure 7: Antibacterial activity of *A. obesum* leaves (Gram-positive).

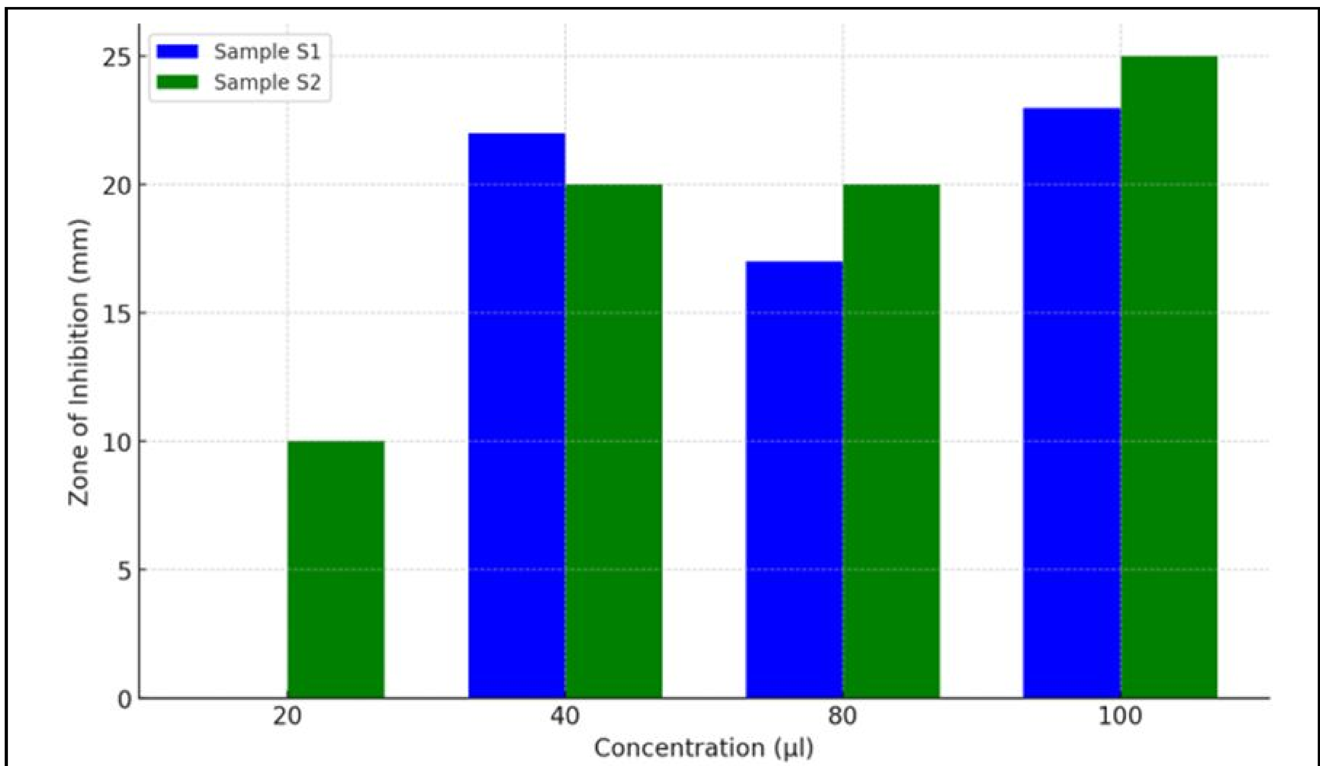

Figure 8: Antibacterial activity of *A. obesum* leaves (Gram-negative).

4. Discussion

Chemicals found in medicinal plants have therapeutic properties and serve as building blocks for chemical and pharmaceutical semi-synthesis (Srinivasarao *et al.,* 2015). Many different medical conditions were treated with plants and the variety of products they produced. Medicinal plants were used extensively in most countries' traditional medical systems to treat basic ailments. Thus, since ancient times, developed nations have utilized plant-based herbal remedies to treat a wide range of medical ailments. Herbal medicine derived from a variety of plants was a good source of medicinal compounds. The medications were either pharmaceutical drugs or plant-based products that were sold worldwide constituting plant-based active ingredients (Abalaka *et al.*, 2013; Ahmed *et al.,* 2017). These medically significant plants were used by the drug-producing industries to create new medications. Scientists from all over the world have already conducted numerous biological and pharmacological studies on a wide range of constituents. Thus, the current focus was on screening the chosen plant species and portions to verify their application in complementary and alternative medicine (Ali *et al.,* 2019).

The ethanolic extracts from *A. obesum* leaves analysed in this study indicated the existence of severalplant-based constituents, including terpenoids, prenylated flavonoids, carbohydrates and cardiac glycosides. This work has examined the anti-inflammatory, anticancer and antioxidant characteristics of the extracts from *A. obesum* leaves. These plant-based biologically active constituentsconfer the ethanolic extracts of *A. obesum* their therapeutic qualities. Forty-five phytochemical compounds were found in *A. obseum* leaf extracts by GC-MS analysis, connecting to the therapeutic qualities of this plant species.

Benzofuran, benzoic acid, 9,12,15-octadecatrienoic acid, octadecanoic acid, vitamin E, 2,6,10,14,18,22-tetracosahexaene, gamma-tocopherol and stigmasterol are all compounds commonly present in both samples 1 and 2. In sample 1, the compound alpha-amyrin had the highest peak area, followed by dl-alpha-tocopherol at 12.61% and 11.34%, respectively, while octadecanoic acid had the lowest at 0.43%. In sample 2, vitamin E had the highest peak area at 14.69%, followed by 2,6,10,14,18,22-tetracosahexaene at 8.43% while 2,3,5,6-tetrafluoroanisole had the lowest at 0.34%. However, the differences in the dominant compounds and their concentrations between the two samples suggest potential variations in their biological activities and therapeutic potential.

The extract (ethanol) of *A. obesum* foliage contained heptadecane, an unstable ingredient of *Spirulina platensis* that has been demonstrated to possess antiproliferative effectiveness towards HepG2 cells, which are cancerous cells found in the liver of humans. (Wu *et al.,* 2005). Similarly, extracts from the endophytic fungus *Talaromyces purpureo* genus were subjected to GC-MS analysis, which also demonstrated dodecane's anticancer properties. This worked well against HPV18 +-infected HeLa cells used to treat human cervical cancer (Kumari *et al.,* 2018). According to past chemical studies, *A. obesum* is a potential medicinal plant that includes a variety of compounds, including flavonoids, pregnancynes, cardiac glycosides, cardenolides and triterpenes (Versiani *et al.,* 2014). A crop's ability to produce secondary metabolites is dependent on favourable weather, which includes elements like soil moisture, temperature and sunlight. Similar results have been documentedin *Hibiscus tiliaceus* (Nandagopalan *et al.,* 2015), in *Tephrosia purpura* (Kavitha *et al.,* 2018)and in *Cassia alata* (Kavipriya *et al.,* 2018). It has been demonstrated that phytol, which was currently observed in the ethanolic extract of *A. obesum,* possessed antimicrobial, anti-inflammatory, anticancer and diuretic attributes (Vijayalingam *et al.,* 2019).

The test for DPPH was first employed to look into the antioxidant possibilities of AOE and how it interacts with DPPH and crude extracts. The colour of the DPPH changes to α, α-diphenyl-β-picrylhydrazine following the response with DPPH. Dose-dependent DPPH radical scavenging was observed indicating that AOE possessed strong antioxidant properties. The AOE extract demonstrated significant dosage-related DPPH scavenging of radicals, ranging from 70.49% to 90.97% at concentrations between 20 and 100 µg/ml. In the analysis, sample 2 showed the highest inhibition at 90.97%, followed by sample 1 at 87.50%, both at a range of 20 µg/ml. This antioxidant activity was similar to the ascorbic acid standard antioxidant, which was employed as a positive control. The ability of AOE to neutralize free radicals and exhibit antioxidant properties could contribute to its potential therapeutic effects, as oxidative stress and free radicals were implicated in various chronic diseases, including cancer. In order to generate the greatest number of free radicals, plant extracts, in particular those from *C. quadrangularis* leaves, *E. abyssinica* bark and *A. multiflorum*, demonstrated strong antioxidant and radical scavenging capabilities in a related study (Marume *et al.,* 2017). Hence, the application of *A. obesum* ininjury treatment, where it inhibits additionaltissue damage and encourages recovery and restoration, can be explained by its proven free radical scavenging and antioxidant qualities.

Two bacterial strains, (Gram-positive) *Staphylococcus aureus* and (Gram-negative) *Escherichia coli*, were used to test the AOE extract's antibacterial activity. The AOE extract demonstrated antibacterial activity against both strains, as demonstrated by the results. The extract's potential to stop the growth of these bacterial strains was offered by the extract's increasing zone of inhibition at higher extract ranges. In the analysis against *S. aureus*, sample 2 showed the maximum zone of inhibition at 30 mm with a focus on 100 µg/ml, succeeded by sample 1 at 26 mm. Similarly, against *E. coli*, sample 2 also displayed the largest zone of inhibition at 25 mm, followed by sample 1 at 23 mm, both at 100 µg/ml. The broad-spectrum bioactivities of compounds with a benzofuran nucleus (a bicyclic ring system) included antimicrobial activities (Kamal *et al.,* 2011). It was recently found that a benzofuran glucoside in ethyl acetate extract is a novel and strong antimicrobial drug that inhibits the expansion of two species of fungal and one species of Gram-positive and Gram-negative bacteria. Phenol was recognized to possess antimicrobial properties (Bennett *et al.,* 1959). Phenolic substances prevent the development of bacteria by rupturing the bacterial membrane, preventing the production of viral elements like toxins and enzymes, as well as inhibiting the development of biofilms (Miklasińska-Majdanik *et al.,* 2018), which was prominently present in the *A. obesum* leaf exact.

5. Conclusion

The research provided convincing proof of the various therapeutic applications of the AOE, demonstrating its efficacy in multiple areas of bioactivity. The research findings highlight AOE's significant antibacterial properties, showcasing its capacity to prevent Gram-positive and Gram-negative bacteria from its growth and further

proliferation. Notably, it demonstrated robust antioxidant capabilities when assessed through *in vitro* methods, particularly in the DPPH free radical scavenging assay, these findings provide hope for the creation of novel, plant-based therapeutic agents derived from AOE and lay the groundwork for future research and contribute significantly to the ongoing advancement and diversification of herbal medicine.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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