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GC-MS Analysis, Anti-inflammatory and Antimicrobial Activity of *Verbesina encelioides* Leaves Extracts

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Abstract:
The golden crown beard or Verbesina encelioides belongs to the family Asteraceae. It is a rich source of various phyto-constituents such as terpenoids, phenols, saponins, tannins, proteins, amino acids, anthraquinones, alkaloids, and reducing sugars etc. The plant has showed antimicrobial, antiviral, anti-tumor and anti-inflammatory activities. This plant is ethnobotanically important and used in treatment of several diseases. In the present study, extracts of dried powdered V. encelioides leaves were prepared in different solvents (water, methanol and petroleum ether) and analysed for their anti-inflammatory and antimicrobial activity against selected pathogens. Water proved out to be the best solvent for extraction, though antibacterial activity was observed in methanolic extract with highest activity displayed against P. aeruginosa (AI=0.519 at 100mg/ml) and E.coli (AI=0.406 at 100mg/ml) followed by S. aureus (AI=0.316 at 100mg/ml) and B. subtilis (AI=0.366 at 100mg/ml). Similarly, the percent anti-inflammatory activity was highest in methanolic extract at 20, 40, 60, 80 and 100 mg/L concentrations was 5.17, 7.42, 10.28, 15.93, and 18.28 respectively. GC-MS results discovered presence of 43 phyto-constituents in methanolic extract such as 1-Methylene-5.alpha.-androstan-3.alpha.-ol-17; n-Hexadecanoic acid, phytol, diethyl phthalate, tetradecanoic acid, 9,12-Octadecadienoic acid (Z,Z)- were among the most abundant in methanolic extract.

Key words: Verbesina encelioides, GC-MS analysis, antibacterial activity, phytol, 9,12- Octadecadienoic acid, diethyl phthalate

Introduction

The golden crown beard, or *V. encelioides* (Cav.) Benth and Hook, is an annual wild herb that grows upright and is resistant to a wide range of climates. It is a member of the *Asteraceae* (or *Compositae*) family (Singh and Dahiya, 2017). Adequate amounts of phyto-constituents such as terpenoids, phenols, saponins, tannins, proteins, amino acids, anthraquinones, alkaloids, and reducing sugars have reportedly been found in cold and hot water extracts from different parts such as leaves, stem, and flowers of *V. encelioides* (Kaur et al., 2021). Various researches suggested that *Verbesina* possesss various toxic, allelopathic, nematocidal and antimicrobial properties. Antibacterial, antifungal, antitumor, antiprotozoal and hypoglycemic ability of the plant has been reported in earlier studies (Kaur et al. 2022). The components isolated from *V. encelioides* are natural ingredients of several medicines used for cough, cardiovascular diseases, stomach disorder, diuretic, joint pains, headache, inflammation and even used as anti-cancer and anti-diabetic drugs (Haq et al., 2021). The *V. encelioides* plant is both economically and ecologically important because different plant parts like leaf, stem and flower are valuable and known to have significant value in pharmaceutical industries. The plant showed antimicrobial, antiviral, anti-tumor, anti-inflammatory, hypoglycaemic and anti-implantation activities (Ramakrishnan et al. 2017). It is a species used in horticulture that has ethnobotanical significance from ancient times among Americans and Indians. It has been shown to be effective in treating haemorrhoids, inflammations, and bites from spiders and snakes due to the abundance of various secondary metabolites of medical importance (Jain et al., 2008). Various reports suggests that young leaves of *V.encelioides* possess more antimicrobial activity when compared to the old ones so the young leaves were used to extract anti-microbial protein (Ramakrishnan et al. 2017). *V. encelioides* leaves are rich in phyto-constituents like total soluble sugar, starch, proteins, lipid, phenols, tannins and saponins. Presence of kaempferol and terpeniol in leaves was also revealed using TLC (Nenarsia and Karnawat, 2023). The leaves are used in treatment of rheumatism and its juice possesses laxative effects (Parrota et al., 2001). Some recent studies have demonstrated that water and ethanol extracts of *V. encelioides* leaves possess significant anti-inflammatory activity by reducing pro-inflammatory cytokines and oxidative stress (Garcia et al., 2023; Kim et al., 2023; Lee et al., 2022). However, a greater portion of the accessible data is limited to traditional knowledge. These characteristics demonstrate that in order to verify its ethnomedicinal potential, a thorough and well-organized investigation of its pharmacological component is required.

In the present study, various extracts (water, methanolic and petroleum ether) of *V. enceliodes* leaves were analysed for the presence of phytochemicals and chemical profile of crude extracts were performed through gas chromatography-mass spectrometric (GC-MS) analytic technique to understand the difference in composition of various extracts.

Material and Methods

Collection of plant material

The leaves of *V. encelioides* were collected from nearby areas and identified through institutional herbarium. The leaves were stored for preparation of extracts and further analysis. The leaves were thoroughly cleaned with water and dried under the shade at room temperature. Coarse powder of the dried leaves was made by using a kitchen blender and stored in an airtight container till further use.

Preparation of extracts

Leaf extract of *V. enceliodes* were prepared in solvents viz. water, methanol and petroleum ether. To prepare the extract, 1 gm of dried leaf powder was combined with 10 ml of the solvents individually in test tubes and left overnight at room temperature in a shaker. After that, tubes were centrifuged for ten minutes at 10,000 rpm, and the supernatant was gathered onto petri dishes that had been previously weighed. The solvent was allowed to evaporate on these plates without any disturbance. Petri-plates were weighed once again after full drying was noted, and the weight of extract (mg) per gram of plant material was computed. These extracts were appropriately labeled, stored in glass vials at 4° C for further use (Sukhdev et al., 2008).

$$\text{Extraction yield \%} = \frac{\text{Weight of crude extract}}{\text{Weight of powdered sample}} \times 100$$

Anti-inflammatory activities

The Inhibition of Heat-Induced Bovine Serum Albumin Denaturation Assay was used to evaluate the anti-inflammatory activities. The sample's impact on the heat-induced bovine serum albumin (BSA) denaturation assay was evaluated using a technique reported by Chandra et al. (2012) with some minor changes. PBS (phosphate buffered saline, pH 6.4) was

employed as the control, and the reaction mixes included different quantities of the material (20–100 µg/mL), as well as 1% w/v BSA and PBS individually. For twenty minutes, the reaction mixtures were incubated at 37 °C. After that, the temperature was raised to maintain the samples at 70 °C for five minutes. Using a UV-visible spectrophotometer, the turbidity was measured at 660 nm after cooling. Protein denaturation is 100% in the control. The percentage inhibition of BSA denaturation was calculated as stated below:

% Inhibition of BSA denaturation= $100\times[1-(A2/A1)]$

Where A1 = absorbance of the control, and A2 = absorbance of the test sample.

Antibacterial activities

The in-vitro antimicrobial assay was accompanied using the Agar Well Diffusion method, as outlined in standard microbial techniques. Nutrient agar (NA) plates were employed to assess antibacterial activity. For this, the test samples were diluted with dimethyl sulfoxide (DMSO) to prepare four different concentrations: 25 mg/L, 50 mg/L, 75 mg/mL, and 100 mg/mL which were evaluated for their efficacy against bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.

Petri dishes containing the nutrient agar were first sterilized and then inoculated with the test microorganisms by spreading. After a 30-minute incubation period, wells with a 6 mm diameter were created in the agar plates. The samples and a standard antibiotic (streptomycin) were applied to the wells at a volume of 30 µL each. The plates were then incubated at 37ºC for 24 hours.

The antibacterial activity was assessed by measuring the inhibition zones around each well. The diameters of these zones were compared with those produced by the commercial control antibiotic. The Activity Index was calculated using following formula to quantify the relative antibacterial effectiveness of the test samples in comparison to the standard antibiotic-

Activity Index (AI) = Inhibition zone of sample/Inhibition zone of standard

GC-MS analysis

All the standard samples and the extract were analysed by GC-MS of Hewlett- Packard 6890/5973 operated at 1000 ev ionization energy, equipped with Agilent 7890A/5975C GC HP-5. In this apparatus capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d.) was filled with Helium (He) which served as the carrier gas (split ratio 1:5). The temperature of oven was retained at 100 °C (3 min) to 280 °C at 1 to 40°C/min; temperature of detector at 250 to 280°C and carrier gas He (0.9 ml/min). Retention indices were examined by analysing retention times of samples injected under the identical chromatographic conditions. Their mass spectra and retention times were compared with published data in order to determine the components of the injected substance. Additionally, a comparison was made between it with the mass spectra found in the Wiley Library (NIST data Mankor) and the mass spectra that have already been published.

Data analysis: All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3)±standard deviation (SD).

Results:

Extractive value

Extractive values of leaves in different solvents *viz*. water, methanol and petroleum ether were 197 mg/g, 51 mg/g and 41 mg/g respectively. Maximum extraction of *V. enceliodes* leaves was achieved in water in comparison to other solvents (Table 1).

Antibacterial activities

All the test extracts showed antibacterial activity but methanol extract showed highest activity followed by water or aqueous extract. In methanolic extract the highest activity was against *P. aeruginosa* (AI=0.519 at 100mg/ml) and *E.coli* (AI=0.406 at 100mg/ml) followed by *S. aureus* (AI=0.316 at 100mg/ml) and *B. subtilis* (AI=0.366 at 100mg/ml). In aqueous extract it was highest for *E.coli* (AI=0.375 at 100mg/ml) and *P. aeruginosa* (AI=0.370 at 100mg/ml) followed by *S. aureus* (AI=0.289 at 100mg/ml) and *B. subtilis* (AI=0.244 at 100mg/ml) (Table 2)

Anti-inflammatory activities

In present study, extracts of *V. encelioides* leaf in different solvents was evaluated for the anti-inflammatory activity at different concentrations (Table 3). The methanol extract demonstrated anti-inflammatory activity with an IC50 value of 282.18 µg/ml. The water extract exhibited an IC50 value of 283.81 µg/ml which is slightly higher than that of the methanol extract, suggesting a somewhat lower efficacy. The petroleum ether extract had the highest IC50 value of 284.15 µg/ml, indicating the lowest anti-inflammatory activity among the solvents tested.

GC-MS analysis

Methanolic extract of *V. enceliodes* leaves was analyzed through GC-MS and 44 peaks were observed in the chromatogram ((Figure 1). A total of 43 compounds were identified from it, out of which tetradecanoic acid, 3-Phenylheptanoic acid, benzylammonium salt; 9,12-Octadecadienoic acid (Z,Z)-; 1,8-Diazacyclotetradecane-2,7-dione, phytol, diethyl pthalate and Methenolone, trifluoroacetate were most abundant (Figure 2).

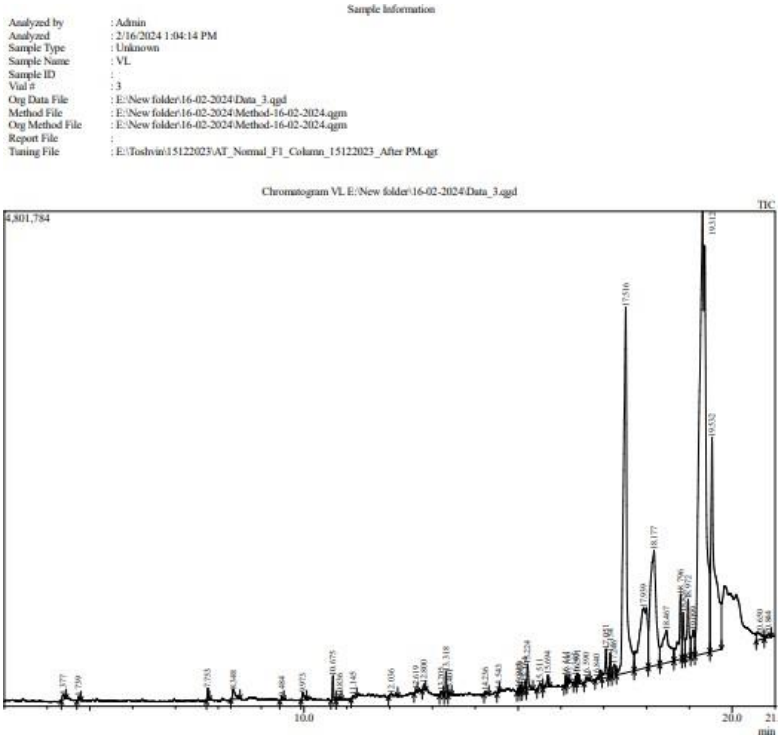

Figure 1: GC-MS chromatogram of methanolic extract of *V. enceliodes* leaves

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	4.377	4.335	4.445	160394	0.14	44384	0.27	3.61	Glycerin
2	4.739	4.695	4.795	75714	0.07	37972	0.23	1.99	Undecane
3	7.753	7.725	7.790	191395	0.17	117266	0.71	1.63	Dodecane
4	8.348	8.290	8.505	441839	0.39	97749	0.59	4.52	2-Pentanol, TMS derivative
5	9.484	9.435	9.530	72504	0.06	33053	0.20	2.19	2-Methoxy-4-vinylphenol
6	9.973	9.930	10.065	232182	0.20	71125	0.43	3.26	Phenol, 2,6-dimethoxy-
7	10.675	10.625	10.750	427869	0.37	238758	1.44	1.79	Tetradecane
8	10.836	10.750	10.875	68023	0.06	30753	0.19	2.21	Propylphosphonic acid, fluoroanhydride, 4-me
9	11.145	11.100	11.235	54031	0.05	21709	0.13	2.49	1-Hydroxymethyl-1,2,3,4-tetrahydro-naphthale
10	12.036	11.975	12.190	171100	0.15	29288	0.18	5.84	.beta.-D-Glucopyranose, 1,6-anhydro-
11	12.619	12.570	12.650	58943	0.05	26842	0.16	2.20	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4
12	12.800	12.760	12.840	112544	0.10	57520	0.35	1.96	Dodecanoic acid
13	13.205	13.165	13.255	99322	0.09	36503	0.22	2.72	Diethyl Phthalate
14	13.318	13.275	13.365	414521	0.36	230494	1.39	1.80	Hexadecane
15	13.401	13.365	13.450	63188	0.06	23111	0.14	2.73	5-Isopropenyloxymethylene-3,3-dimethyl-cycl
16	14.236	14.205	14.320	60656	0.05	26655	0.16	2.28	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-
17	14.543	14.505	14.565	58233	0.05	36000	0.22	1.62	Eicosane
18	15.015	14.970	15.040	113545	0.10	40922	0.25	2.77	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-
19	15.059	15.040	15.095	138605	0.12	62236	0.38	2.23	Hexadecane, 1-iodo-
20	15.164	15.095	15.190	268813	0.23	89334	0.54	3.01	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydr
21	15.224	15.190	15.350	640541	0.56	250598	1.51	2.56	Tetradecanoic acid
22	15.511	15.445	15.580	207150	0.18	56429	0.34	3.67	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydr
23	15.694	15.580	15.730	296001	0.26	113806	0.69	2.60	Heneicosane
24	16.111	16.075	16.140	191620	0.17	91097	0.55	2.10	Neophytadiene
25	16.166	16.140	16.210	137424	0.12	65521	0.40	2.10	2-Pentadecanone, 6,10,14-trimethyl-
26	16.345	16.295	16.365	156660	0.14	64211	0.39	2.44	
27	16.391	16.365	16.440	226343	0.20	96655	0.58	2.34	1,2-Benzenedicarboxylic acid, bis(2-methylpro
28	16.590	16.540	16.670	131630	0.11	45197	0.27	2.91	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acet
29	16.840	16.800	16.900	79176	0.07	22294	0.13	3.55	Oxalic acid, 6-ethyloct-3-yl propyl ester
30	17.051	16.960	17.110	841973	0.73	280004	1.69	3.01	Hexadecanoic acid, methyl ester
31	17.154	17.110	17.190	521394	0.45	229627	1.39	2.27	Benzenepropanoic acid, 3,5-bis(1,1-dimethylet
32	17.246	17.190	17.300	447945	0.39	110688	0.67	4.05	22-Tricosenoic acid
33	17.516	17.300	17.710	19517466	17.01	3544374	21.40	5.51	n-Hexadecanoic acid
34	17.939	17.710	18.040	7779954	6.78	586235	3.54	13.27	1-Methylene-5.alpha.-androstan-3.alpha.-ol-17
35	18.177	18.040	18.310	10635404	9.27	1111145	6.71	9.57	Methenolone, trifluoroacetate
36	18.467	18.310	18.640	3803075	3.31	310892	1.88	12.23	3-Phenyltheptanoic acid, benzylammonium salt
37	18.796	18.640	18.830	2596694	2.26	601260	3.63	4.32	9,12-Octadecadienoic acid (Z,Z)-, methyl este
38	18.857	18.830	18.900	1213584	1.06	431178	2.60	2.81	8,11,14-Eicosatrienoic acid, methyl ester
39	18.972	18.900	19.030	2188674	1.91	548310	3.31	3.99	Phytol
40	19.099	19.030	19.130	1253518	1.09	251789	1.52	4.98	Methyl stearate
41	19.312	19.130	19.480	44248258	38.56	4264418	25.75	10.38	9,12-Octadecadienoic acid (Z,Z)-
42	19.532	19.480	19.750	13597312	11.85	2045352	12.35	6.65	Octadecanoic acid
43	20.650	20.560	20.750	567370	0.49	57893	0.35	9.80	1,8-Diazacyclotetradecane-2,7-dione
44	20.844	20.750	20.910	178256	0.16	31916	0.19	5.59	3-Cyclopentylpropionic acid, 2-dimethylamino
				114740843	100.00	16562563	100.00		

Figure 2: Peak Report of GC-MS analysis of methanolic extract of *V. enceliodes* leaves

Discussion

For extraction of natural compounds from different plant parts, various conventional and nonconventional methods can be used (Chemat et al., 2017). In present study, three different solvents (water, methanol and pertoleum ether) were used for isolation and identification of phytochemicals from the leaves of *V. enceliodes*. Maximum extraction was achieved from water in comparison to other solvents. Amount and variety of phytochemical extraction varies according to the depending on the solvent used for their extraction. Water has a polar nature so as a solvent it is more efficient in isolation of phenolic compounds (Venkataramanamma et al., 2016).

In present study, all the three extracts (water, methanolic and petroleum ether) of *V. encelioides* leaves showed prominent antibacterial activities against all the pathogens tested at all concentrations though the maximum activity (calculated as AI) was observed for methanol extracts at 75mg/ml and 100mg/ml in comparison to aqueous and petroleum ether extracts. Although the values were lower in comparison to Streptomycin which was used as standard. All the test extracts showed antibacterial activity but methanol extract was remarkably active against *P. aeruginosa* (AI=0.519 at 100mg/ml) and *E.coli* (AI=0.406 at 100mg/ml) followed by *S. aureus* (AI=0.316 at 100mg/ml) and *B. subtilis* (AI=0.366 at 100mg/ml). Earlier too, the methanol extract of *V. encelioides* have shown appreciable activity against *K. pneumoniae* and *E. arerogenes* (Moussa and Ali, 2022). Results of present study are in accordance with previous report highlighting the toxic effect of *V. encelioides* on various microorganisms such as the hydroalcoholic extracts of the aerial parts and roots of this plant have demonstrated cytotoxicity against *S. aureus*, *B. subtilis* and *E. coli* (Kuete et al., 2012). It has been reported that extracts of aromatic plants with high amount of one single constituent have shown promising anti-quorum sensing properties to battle plant diseases (Camele et al., 2019). The higher AI observed for methanol extract can be explained by the fact that organic solvents like ethanol and methanol are noted for their ability to extract a broader range of phytochemicals, including essential oils and flavonoids, which contribute to higher antimicrobial activity as compared to water (Ncube et al., 2008; Siriwong et al., 2021). Conversely, water, a polar solvent, primarily extracts hydrophilic compounds but may be less effective in extracting non-polar antimicrobial agents. For example, in a study on *Verbesina encelioides*, ethanol and methanol extracts demonstrated stronger antimicrobial activity against bacterial strains like *Staphylococcus aureus* and *Escherichia coli* than aqueous extracts (Sati et al., 2012). This suggests that the choice of solvent not only impacts the extraction yield but also the spectrum and potency of antimicrobial activity. Similarly, Gupta et al. (2011) reported that methanol extracts of *Verbesina encelioides* showed significant inhibitory effects against fungal pathogens, highlighting the solvent's role in enhancing the antimicrobial properties of plant extracts. Thus, the effectiveness of plant extracts as antimicrobial agents is closely linked to the solvent used during extraction, influencing both the quality and extent of their therapeutic potential.

For instance, in a recent study, ethanolic and methanolic extracts of *V. encelioides* displayed significantly stronger antimicrobial activity against certain Gram-positive and Gram-negative bacteria in comparison to aqueous extracts (Moussa et al., 2022). Findings of present study

are consistent with the work of Ahmed et al. (2020), who demonstrated that methanol extracts of *V. encelioides* exhibited potent antibacterial and antifungal properties, highlighting the effectiveness of organic solvents in enhancing antimicrobial activity. Ramakrishnan et al., (2017) also explained that presence of some proteins in the young leaves of this plant is responsible for moderate level antimicrobial activity against various bacterial and fungal species. The strong antibacterial activity of methanol extract can also be attributed to the presence of bioactive compounds observed in its GC-MS analysis which showed abundance of phytol, diethyl phthalate, tetradecanoic acid, 3-Phenylheptanoic acid, benzylammonium salt, 9,12-Octadecadienoic acid (Z,Z)-, 1,8-Diazacyclotetradecane-2,7-dione, and Methenolone, trifluoroacetate. The presence of these compounds suggests a multifaceted mechanism contributing to the antibacterial activity of the extract. Tetradecanoic acid, or myristic acid, is a saturated fatty acid known for its antimicrobial properties. It disrupts microbial cell membranes and alters their permeability, which can inhibit bacterial growth (Joudeh & Linke, 2019). The presence of myristic acid in the methanolic extract indicates that it likely contributes to the extract's antibacterial activity by compromising bacterial cell wall integrity. Another compound 3-Phenylheptanoic acid, a phenylalkanoic acid derivative, has been associated with antimicrobial and anti-inflammatory activities. This compound can inhibit bacterial enzyme systems and modulate immune responses, enhancing the overall antibacterial efficacy of the extract (Arai et al., 2020). Its significant presence suggests that it plays a role in the extract's ability to target and suppress bacterial growth. Similarly, Benzylammonium salts found in GC-MS analysis in the present study are known for their antimicrobial properties, particularly due to their ability to disrupt microbial cell membranes and interfere with cellular functions (Saini et al., 2021). Therefore, it can be said that the presence of benzylammonium salt in the extract likely contributes to its antibacterial activity by impairing the structural integrity of bacterial cell membranes, thereby exerting a bactericidal effect.

9,12-Octadecadienoic acid (Z,Z)- also known as linoleic acid, an essential fatty acid, exhibits both antimicrobial and anti-inflammatory properties. It is known to affect bacterial membrane fluidity and integrity, enhancing the extract's ability to combat bacterial infections (Miller et al., 2016). The presence of linoleic acid in the *V. encelioides* extract suggests that it may aid in reducing oxidative stress and bacterial cell damage, contributing to the observed antibacterial effects. Another phyto-compound 1,8-Diazacyclotetradecane-2,7-dione, or cyclen, is less commonly studied for its antimicrobial properties, but its derivatives have

shown potential in various biological activities. It can chelate metal ions, which may influence microbial growth and stability (Rao et al., 2018). Its presence in the extract may suggest a role in modulating bacterial growth by interacting with essential metal cofactors. Likewise, methenolone, a synthetic anabolic steroid, is primarily known for its anti-inflammatory and immunomodulatory effects. Although less traditional in the context of antimicrobial activity, its presence might enhance the extract's overall therapeutic profile by modulating immune responses and reducing inflammation (Sahin et al., 2022). Another compound DEP (diethyl phthalate) has been shown to have minimal direct antibacterial activity against common pathogens. However, it can affect microbial growth indirectly. DEP can disrupt cell membranes and interfere with the uptake of nutrients, potentially influencing the growth of microorganisms (Yoo et al., 2021). Some studies suggest that DEP may affect biofilm formation, which is crucial in chronic infections. Its presence could alter the adhesion properties of bacteria, potentially impacting their ability to form biofilms (Ali et al., 2019). Phytol is a terpene alcohol with a structure that facilitates its interaction with lipid membranes. It has been recognized for its potential antimicrobial and therapeutic properties, due to its ability to disrupt bacterial cell membranes and influence membrane fluidity. Similar to results of present study, previous studies have demonstrated that plant extract containing phytol can inhibit the growth of various Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Hussein et al., 2022). This phenomenon lies on the unique property of Phytol which involves the disruption of microbial cell membranes, which compromises the integrity and function of the bacteria, leading to their death. It is lipophilic in nature, allowing it to integrate into lipid layers of bacterial membranes, disrupt membrane integrity, and enhance permeability. This disruption can lead to leakage of intracellular contents and cell death (Reddy et al., 2020). Quantity and type of the extracted phytochemicals varies according to the solvent used for their extraction. The phyto-component profile of any extracts and their biological activity depend critically on the type of solvent used (Tamborlin et al., 2020). Water or its hydroalcoholic mixtures have been suitable for isolation of phenolic compounds due to their polar nature (Venkataramanamma et al., 2016; Singh et al., 2014). The final chemical profile of the extract is determined by the type of extraction solvent utilised during the process. As a result, the extracted substances' bioactivity will be greatly impacted by both their absolute and relative concentrations (Sumere et al., 2018; Rostagno et al., 2013).

Our results revealed the anti-inflammatory potential of methanolic extract of *V. enceliodes* leaves with lowest IC50 value among the solvents tested, indicating that methanol is the most effective solvent for extracting anti-inflammatory compounds from *V. encelioides* leaves. The efficacy of methanol aligns with its known ability to extract a broad range of polar and semi-polar compounds, which are often responsible for significant biological activities (Jadhav et al., 2016). Water, being a less polar solvent compared to methanol, may not dissolve all the anti-inflammatory agents as effectively, which could account for the marginal increase in IC50. The results are consistent with other studies where water extracts have shown moderate anti-inflammatory activity due to the limited range of compounds extracted (Poudel et al., 2014). Petroleum ether is a non-polar solvent, which is less effective at extracting polar anti-inflammatory compounds compared to polar solvents like methanol and water. This finding is supported by studies showing that non-polar solvents typically yield extracts with lower biological activity due to their inability to dissolve a wide range of bioactive compounds (Singh et al., 2018).

It has been suggested that plant extracts contain anti-inflammatory qualities and can be used to treat hemorrhoids or gum sores (Shluker 2002). Essential oil of *V. macrophylla* showed anti-inflammatory potential by lowering levels of pro-inflammatory cytokines TNF-α and IL-1β, as well as ear edema in concentration dependent manner (Oliveira et al., 2021). Petroleum ether extract of *V. encelioides* revealed the presence of β-sitosterol glucoside and β-sitosterol galactoside and various studies reported the anti-inflammatory potential of sitosterol and sitosterol glycoside (Abbas et al., 2016).

It can be said that the results obtained in present study are indicative of positive correlation between the extract concentration and the diameter of inhibition annulus. The rise in the inhibition zone as well as AI was undoubtedly proportionate to the amount of the extract applied. It has also been observed that the choice of solvent in extracting plant compounds significantly influences the antimicrobial efficacy of plant extracts.

Also, it is anticipated that by gaining a more profound comprehension of the bioactive components found in extracts of leaves of *V. enceloides* can be used for medicinal purposes due to their antioxidant and anti-inflammatory properties. The present study revealed the presence of major bioactive constituents in different extracts of it which may serve as the basis in determining the possible biological, pharmacological importance and as a source of

valuable goods and can unlock new avenues for novel therapeutics.

Conclusion

The plant *V. encelioides* is an invasive plant with economic and ecological importance. The plant showed antimicrobial, antiviral, anti-tumor, anti-inflammatory and hypoglycaemic activities. In present study three solvents (water, methanol, and petroleum ether) were used to prepare the extract from *V. encelioides* leaves and maximum extraction values were observed in water extract. Methanol extract showed maximum antibacterial potential against *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis* in comparison to other extracts. The GC-MS analysis of methanolic extracts revealed the presence of various phytocomponents with medicinal importance such as diethyl phthalate, phytol etc. The study suggests that *V. encelioides* leaves can be used for medicinal purposes due to their antioxidant and anti-inflammatory properties. Also, there are compounds identified which confirms that its leaves can serve as source of valuable goods and can unlock new avenues for novel therapeutics and cosmetics. However, additional research is requisite to understand the full potential of these compounds, variation in the extract component on the varietal basis, consideration for the exploration and effective utilization of various levels of functional and bioactive constituents for the designing and improvement of food, pharmaceutical and cosmetic products.

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