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ANTIMICROBIAL, ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF DIFFERENT EXTRACTS OF VARIOUS PARTS OF *VERBESINA ENCELIOIDES.*

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The current study showed potent antibacterial efficacy of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml) against different bacterial strains, namely, *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus,* with the highest antibacterial activity against the selected pathogens in case of 100 µg/ml dose of different water extracts, in water extracts of stem and leaf (*E. coli*), Root (*B. subtilis* and *P. aeruginosa*) and fruit (*S. aureus*). Furthermore, the study showed antifungal efficacy of the plant extracts against different fungal strains, namely, *Aspergillus flavus* and *Aspergillus niger*, with the highest antifungal activity against *A. flavus* and *A. niger* in case of 100 µg/ml dose of different methanolic extracts. In addition to the antimicrobial efficacy, the study also highlights antioxidant and anti-diabetic activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml); with the highest antioxidant activity by leaf methanolic extract and flower water extract while highest anti-diabetic activity by leaf water extract and flower water extract. Cumulatively, the study provides crucial insights into antimicrobial, antioxidant and anti-diabetic activity of different parts of the plant *Verbesina encelioides.*

Keywords: Antimicrobial; Antioxidant; Anti-diabetic; Solvents; *Verbesina encelioides*.

Introduction

Verbesina encelioides, commonly known as golden crown beard, is an important medicinal plant, is native to North America and South America. In addition to these, the plant has been introduced in different geographical areas, including Asia, Australia and Africa, in order to enable adaptation of the plant to diverse environmental conditions. Rajasthan is known for its arid and semi-arid climate and harbours desert-adapted plants such as cactus, shrubs, succulents and other drought-tolerant species. *Verbesina encelioides* has been found to be well adapted to the arid environmental conditions of Rajasthan and has been found growing at many places in the state.

Different parts of the plant V*erbesina encelioides*, are documented for a number of pharmacological traits such as leaves of the plant are used to prepare herbal infusions with anti-inflammatory, antioxidant activity and relieving of respiratory and digestive disorders. Infusions made from plant roots are used as decoction for promoting urine production and aiding in the alleviation of urogenital disorders and used as a diuretic. Flowers of the plant are used as antipyretic, anti-inflammatory and treatment of skin conditions such as wounds, insect bites and cuts. In addition to these, the whole plant is known for its numerous pharmacological attributes, which include anti-inflammatory activity, antioxidant, treatment of skin conditions, antimicrobial, diuretic,

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antipyretic and several others[1]. All these pharmacological traits of the plant and its different parts may be attributed to the presence of bioactive secondary metabolites. However, different parts of the plant, *Verbesina encelioides*, harbour variable amounts of bioactive secondary metabolites, owing to which, different plant parts can be used for the treatment of several ailments. For instance, leaves being exposed to the sunlight may contain higher amounts of flavonoids and phenolics, while stems used for structural support and conduction of water and nutrients may contain higher amounts of terpenoids, alkaloids and phenolics. Similarly, other plant parts such as root, seeds and flowers also differ from each other in their composition of secondary metabolites[2-5] .

Considering this, the current study has been drafted to focus on determining antimicrobial, antioxidant and anti-diabetic activity of root, stem, leaves and flowers of *Verbesina encelioides* using different solvents, namely, water, ethanol and methanol extracts. The results of the study show potent antioxidant and antidiabetic activity of different plant extracts. In addition, the study also shows potent antibacterial as well as antifungal efficacy of different plant extracts against four different bacterial strains, including *E. coli, Bacillus, Pseudomonas* and *S. aureus* and two different fungal strains, *A. flavus* and *A. niger.*

Material And Methods

The healthy parts of the selected plant were collected from campus, University of Rajasthan, Jaipur. It was collected during May 2023. Healthy parts (root, stem, leaves and flowers) were separated and washed with running tap water to remove impurities and then shed dried. The dry plant materials were grinded to make coarse powder for further use.

Root, stem, leaves and flowers of *Verbesina encelioides* were extracted in water, methanol and ethanol by using a sonicator. Extracts were reconstituted in DMSO to make different concentrations for various activities.

Antimicrobial activity:

Well diffusion methods were used for determination of antimicrobial potential against *E. coli, B. subtilis, P. aeruginosa* and *S. aureus* (bacteria), *A. flavus* and *A. niger* (fungi). All the selected microbes were purchased from MTCC, Chandigarh. The selection of the microbes was random and on the basis of availability in the laboratory. 30 µl of each sample was loaded in 6 mm wells separately in different concentrations (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L). in a different plate, similar concentrations of standard antibiotics (streptomycin for bacteria and ketoconazole for fungi) were used. Inhibition zone (IZ-mm) and Activity index (AI) were calculated for each sample and each concentration[6].

Antioxidant activity:

For determination of DPPH radical scavenging potential of the extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) method. The mixing of 100 µl aliquot form samples was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 517 nm while methanol was used as blank.

The radical scavenging activity was determined by the ratio =

$$\frac{\left(Ab_{control} - Ab_{sample}\right)}{Ab_{control}} \times 100$$

Linear plot of concentration versus % inhibition was plotted, and by this, IC_{50} values were determined. The antioxidant potential of each extract was shown in the form of IC_{50} (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of an inhibition curve[7].

Alpha amylase inhibitory assay:

Chromogenic DNSA approach was used to perform Inhibition assay. Total assay mixture is made up of 500 µl of sodium

Figure 1: Antibacterial activity of different extracts of the selected plant parts against *E. coli.*

Figure 2: Antibacterial activity of different extracts of the selected plant parts against *B. subtilis*.

phosphate buffer 0.02 M (pH 6.9 with 6 mM NaCl), 1 ml of salivary amylase and 400 µl samples of concentration ranging from 50 to 450 µg/ml (w/v) were incubated for 10 min at 37°C. After this pre-incubation process, 580 µl of starch solution (1% w/v) was mixed to each tube and left for incubation at 37°C for 15 min. Now for the termination of reaction addition of DNSA reagent (1.0 ml) was done, then it was kept in a boiling water bath for about 5 min, cooling was done at RT, and the OD were calculated at 540 nm. The control, having no plant extracts, resulted in 100% enzymatic activity. For the elimination of the absorbance developed by plant extract, suitable controls with the sample in the reaction mixture without the enzyme were also included (negative control)[8].

Table 1: Abbreviations used for different samples during experiments.

Plant part name	Solvent name	Abbreviation
Root	Methanol	RM
	Methanol	RE
	Water	RW
Stem	Methanol	SM
	Methanol	SE
	Water	SW
Leaves	Methanol	LM
	Methanol	LE
	Water	LW
Flower	Methanol	FNM
	Methanol	FE
	Water	FW

Table 2: Antimicrobial activity of different extracts of the selected plant parts against *E. coli.*

Plant part	Solvent name	Antibacterial activity against *E. coli* at different concentrations (µg/ml)							
		25 µg/ml		50 µg/ml		75 µg/ml		100 µg/ml	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
V. Root	MeOH	NA	NA	8	0.26	9	0.29	12	0.37
	EtOH	NA	NA	7	0.23	8	0.25	11	0.34
	Water	7	0.29	8	0.26	9	0.29	12	0.37
V. Stem	MeOH	7	0.29	8	0.26	10	0.32	11	0.34
	EtOH	NA	NA	7	0.23	11	0.35	13	0.40
	Water	8	0.33	11	0.36	12	0.38	15	0.46
V. Leaves	MeOH	NA	NA	NA	NA	7	0.22	9	0.28
	EtOH	NA	NA	8	0.26	10	0.32	11	0.34
	Water	11	0.45	12	0.4	13	0.41	15	0.46
V. Flower	MeOH	7	0.29	8	0.26	9	0.29	11	0.34
	EtOH	8	0.33	9	0.3	10	0.32	13	0.40
	Water	9	0.37	10	0.33	12	0.38	14	0.43
Std.		24		30		31		32	

% Inhibition of alpha amylase can be calculated as follows:

% Relative enzyme activity =

$$\left(\frac{Enzyme\ activity\ in\ test\ sample\ with\ extract}{Enzyme\ activity\ in\ control}\right) \times 100$$

% Inhibition in the α-amylase activity =

$$(100 - \%\ Relative\ enzyme\ activity)$$

Results and Discussion

A standard abbreviation system for different extracts has been followed

throughout the manuscript as mentioned in table 1.

Antibacterial activity

Antibacterial activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides.*

The results in Table 2-5 and its corresponding figures (figure 1-4) show dose dependent increase in antibacterial activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml) against different bacterial strains, namely, *E. coli, Bacillus, Pseudomonas* and *S. aureus.* As per the results, the highest antibacterial activity against *E. coli, Bacillus subtilis, Pseudomonas aeruginosa* and *S. aureus* is observed in case of 100 µg/ml dose of different water extracts, namely SW and LW (*E. coli*), RW (*Bacillus subtilis*), RW (*Pseudomonas aeruginosa*) and FW (*S. aureus*).

The specific order for antibacterial activity of different extracts against *E. coli, Bacillus, Pseudomonas* and *S. aureus* is as follows:

E. coli:

SW=LW>FW>SE=FE>RM=RW>RE=SM=LE=FM>LM

Bacillus subtilis:

RW>FW>SW=LW>RM=FM>SM=LM=LE>FE>SE>RE

Pseudomonas aeruginosa:

RW>SW=LW>FW>LM>SM=FM>RM=RE=SE=LE=FE

S. aureus:

FW>SW>RW>LW>LM>SE=LE=FM>RM=RE=SM=FE

Antifungal activity

Antifungal activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides.*

Table 3: Antimicrobial activity of different extracts of the selected plant parts against *B. subtilis.*

Plant part	Solvent name	Antibacterial activity against *B. subtilis* at different concentrations (µg/ml)							
		25		50		75		100	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
V. Root	MeOH	7	0.22	9	0.25	10	0.26	13	0.31
	EtOH	7	0.22	8	0.22	9	0.23	10	0.24
	Water	11	0.35	12	0.33	18	0.47	21	0.51
V. Stem	MeOH	8	0.25	9	0.25	10	0.26	12	0.29
	EtOH	NA	NA	8	0.22	10	0.26	11	0.26
	Water	14	0.45	15	0.41	17	0.44	17	0.41
V. Leaves	MeOH	7	0.22	9	0.25	10	0.26	12	0.29
	EtOH	NA	NA	7	0.19	9	0.23	12	0.29
	Water	11	0.35	12	0.33	14	0.36	17	0.41
V. Flower	MeOH	7	0.22	9	0.25	10	0.26	13	0.31
	EtOH	8	0.25	9	0.25	10	0.26	12	0.29
	Water	13	0.41	14	0.38	18	0.47	20	0.48
Std.		31		36		38		41	

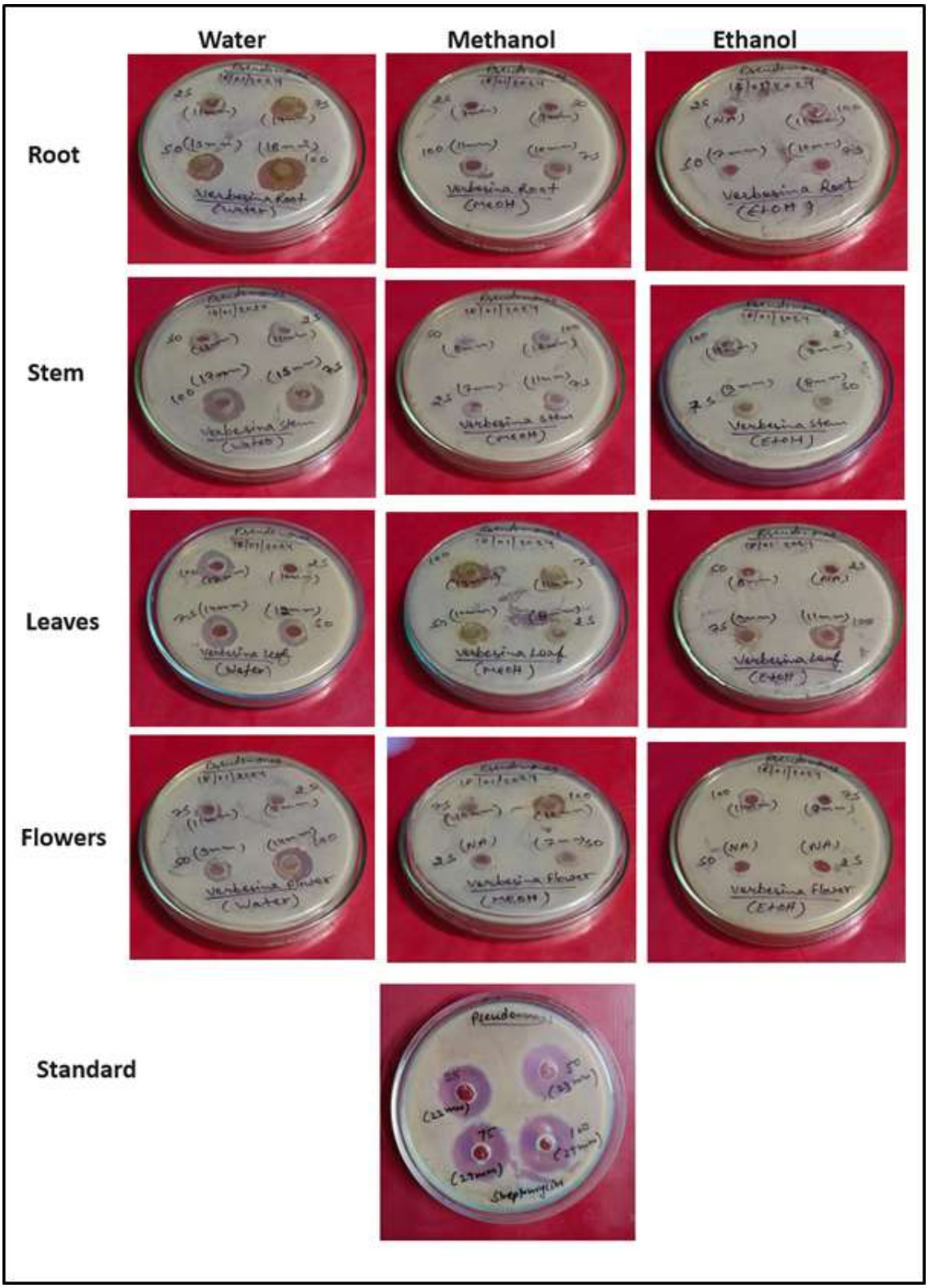

Figure 3: Antibacterial activity of different extracts of the selected plant parts against *P. aeruginosa*.

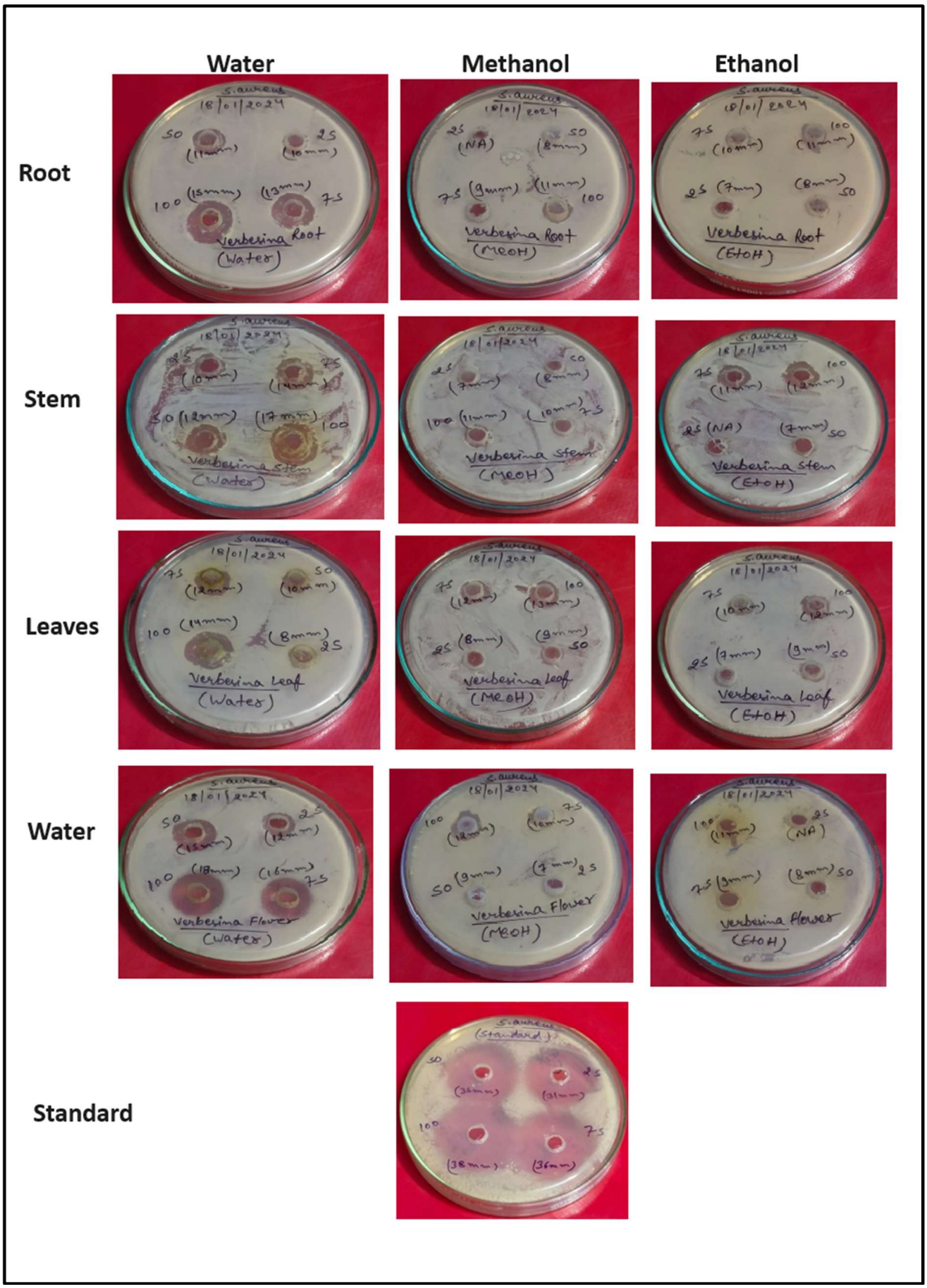

Figure 4: Antibacterial activity of different extracts of the selected plant parts against *S. aureus*.

The results in Table 6 and 7 and Figures 5 and 6 show dose dependent increase in antifungal activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml) against different fungal strains, namely, *A. flavus* and *A .niger.* As per the results, the highest antifungal activity against *A. flavus* and *A. niger* is observed in case of 100 µg/ml dose of different methanolic extracts, namely, RM and LM (*A. flavus*) and RM, SM, LM and FM (*A. niger*).

The specific order for antifungal activity of different extracts against *A. flavus* and *A. niger* is as follows:

A. flavus:
RM=LM>FM>RE=SM=LE>SE=SW=FE>RW=FW>LW

A. niger:
RM=SM=LM=FM>RE=SE=LE>RW=LW=FE>SW=FW

Table 4: Antimicrobial activity of different extracts of the selected plant parts against *Pseudomonas aeruginosa*.

Plant part	Solvent name	Antibacterial activity against *Pseudomonas aeruginosa* at different concentrations (µg/ml)							
		25		50		75		100	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
V.Root	MeOH	7	0.31	9	0.39	10	0.37	11	0.40
	EtOH	NA	NA	7	0.30	10	0.37	11	0.40
	Water	11	0.5	13	0.56	14	0.51	18	0.66
V.Stem	MeOH	7	0.31	8	0.34	11	0.40	12	0.44
	EtOH	7	0.31	8	0.34	9	0.33	11	0.40
	Water	11	0.5	12	0.52	15	0.55	17	0.62
V.Leaves	MeOH	8	0.36	10	0.43	11	0.40	13	0.48
	EtOH	NA	NA	8	0.34	9	0.33	11	0.40
	Water	10	0.45	12	0.52	14	0.51	17	0.62
V.Flower	MeOH	NA	NA	7	0.30	10	0.37	12	0.44
	EtOH	NA	NA	NA	NA	8	0.29	11	0.40
	Water	7	0.31	9	0.39	11	0.40	14	0.51
Std.		22		23		27		27	

Antioxidant activity

Antioxidant activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides.*

The results in Table 8 and its corresponding graphs (figure 7) show dose dependent increase in antioxidant activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml. As per the results, the highest antioxidant activity is observed in case of leaf methanolic extract and flower water extract.

The order of antioxidant activity of different extracts is as follows:

200 µg/ml:
LM>FW>LE>FE>SW>LW>FM>RW>RE>RM>SE>SM

400 µg/ml:
FW>LM>LE>FE>SW>FM>LW>RW>RE>RM>SE>SM

600 µg/ml:
FW>LM>SW>FE>LW>FM>RW>LE>RE>SE>RM>SM

800 µg/ml:

LM>FW>FE>LW>SW>LE>RW>
FM>RE>SE>RM>SM

1000 µg/ml:

LM>FE>LW>FW>RW>SW>RE>
LE>FM>SE>RM>SM

Antihyperglycemic activity

Antidiabetic activity of water, ethanol and methanol extracts (figure 8) of root, stem, leaves and flowers of *Verbesina encelioides.* The results in Table 9 and its corresponding graphs show dose dependent increase in antidiabetic activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml*).* The highest antidiabetic activity in root, stem, leaves and flowers of *Verbesina encelioides* is observed in case of leaf water extract and flower water extract.

The order of antidiabetic activity of different extracts is as follows:

200 µg/ml:

LW>FW>FE>FM>LE>LM>SW>
RE>RW>SE>RM>SM

400 µg/ml:

LW>FE>FW>LE>FM>LM>SW>
SE>RE>RW>RM>SM

600 µg/ml:

FW>LW>FE>LE>FM>SW>LM>
RM>RE>SE>SM>RW

800 µg/ml:

LW>LE>FM>FW>FE>SW>LM>
RM>RE>SE>RW>SM

1000 µg/ml:

LW>LE>FM>FW>FE>SW>LM>
RM>RE>SE>RW>SM

The current study has been drafted to focus on antimicrobial, antioxidant and anti-diabetic activity of root, stem, leaves and flowers of *Verbesina encelioides* using different solvents, namely, water, ethanol and methanol extracts. The results of the study show potent antibacterial activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml) against different bacterial strains, namely, *E. coli, Bacillus, Pseudomonas* and *S. aureus,* with the highest antibacterial activity against each of the strains using water extracts, specifically, 100 µg/ml dose of different water extracts, namely SW and LW (*E. coli*), RW (*Bacillus subtilis*), RW *(Pseudomonas aeruginosa*) and FW (*S. aureus*). This may be attributed to higher efficacy of water as a solvent, to extract antibacterial secondary metabolites from the plant parts. There are several ways in which bioactive secondary metabolites from plants act as potent antibacterial agents, such as:

- Disruption of bacterial cell membrane followed by leaking of cellular components and loss of membrane integrity by phenolic acids, terpenoids and saponins.
- Inhibition of bacterial cell wall biosynthesis by alkaloids, flavonoids and terpenoids
- Inhibition of bacterial protein biosynthesis by interfering with ribosomal function, by alkaloids, phenolics and terpenoids.
- Damage to both RNA and DNA by intercalating with them, leading to faulty transcription, by quinones and alkaloids.
- Secondary metabolites such as flavonoids and phenolic acids are capable of triggering ROS production inside bacterial cells, which further damage cellular components of bacterial cells such as RNS, DNA and proteins.
- Furthermore, flavonoids and phenolics inhibit crucial signalling molecules involved in quorum sensing, as a consequence of which, bacterial communication and growth is impeded[9-10] .

A number of other studies have shown efficacy of the plant *Verbesina encelioides* in inhibition of bacterial growth in both gram positive as well as gram negative bacterial strains[11-13] .

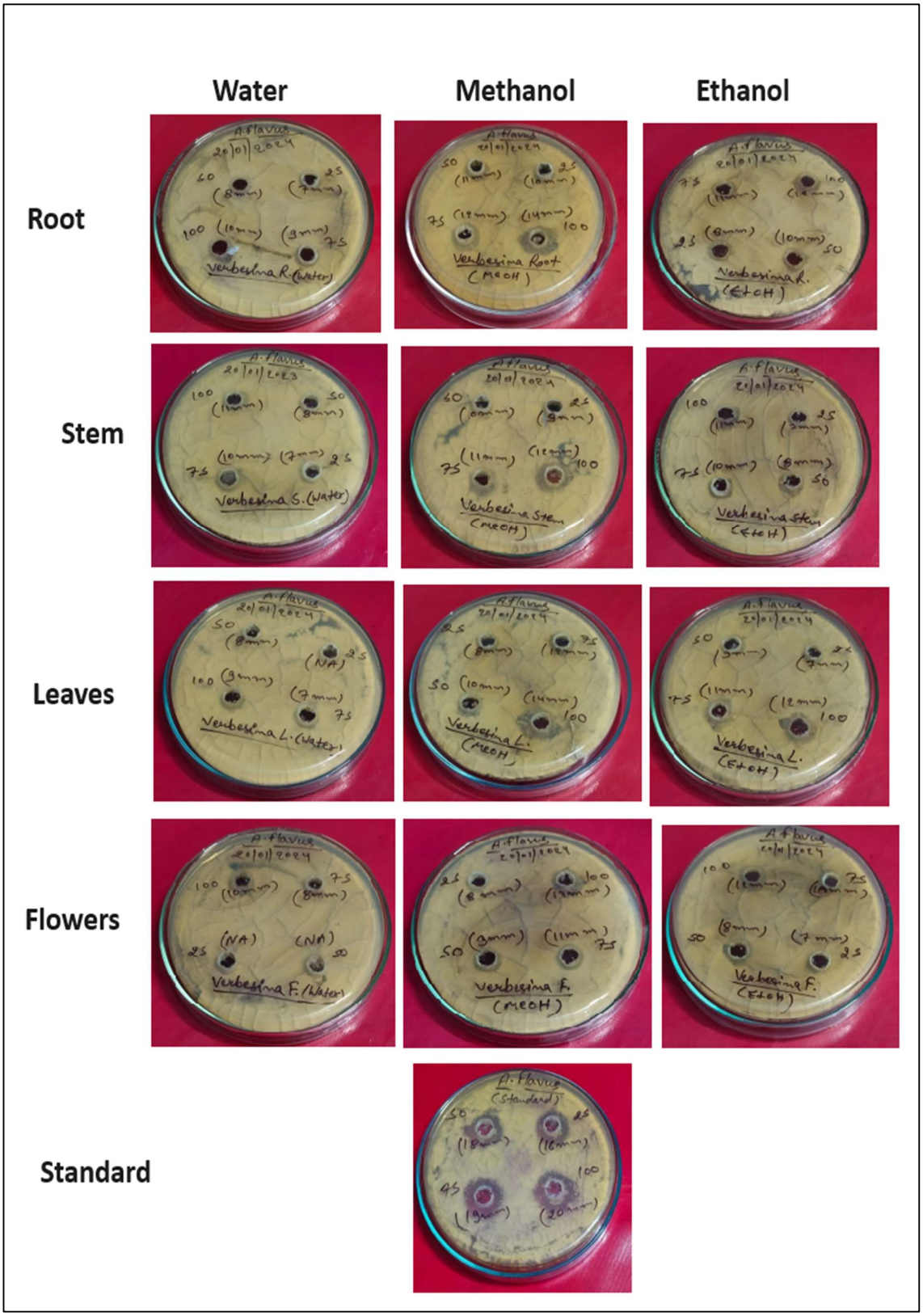

Figure 5: Antifungal activity of different extracts of the selected plant parts against *A. flavus.*

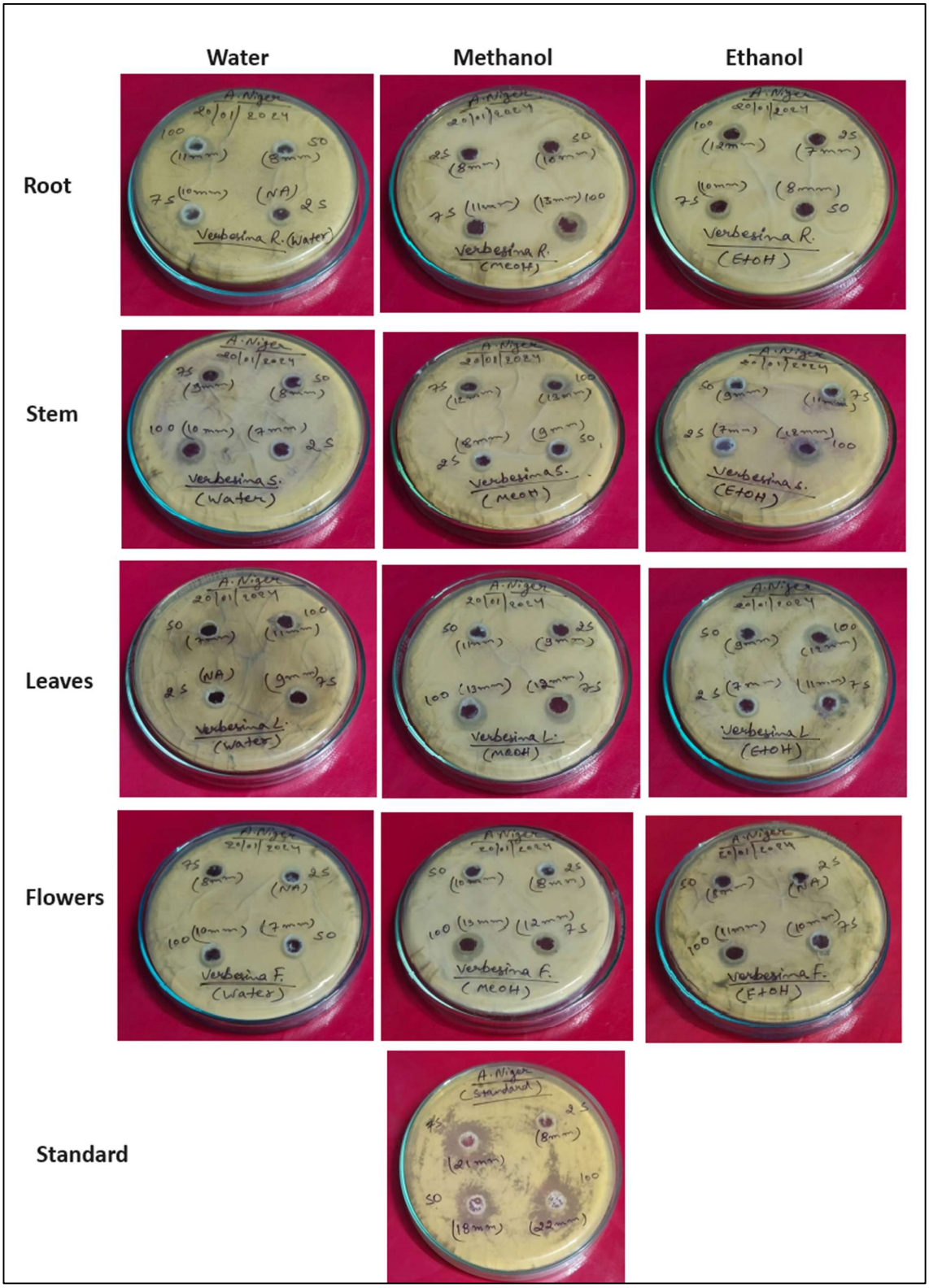

Figure 6: Antifungal activity of different extracts of the selected plant parts against *A. niger*.

The results obtained in the current study are in perfect coherence with other studies, where 2 researchers have shown efficacy of the plant *Verbesina encelioides* in inhibition of fungal growth[14-16].

Thereafter, the researchers showed antifungal efficacy of the different plant extracts, wherein, the highest antifungal activity against *A. flavus* and *A. niger* was observed in case of 100 µg/ml dose of different methanolic extracts, namely, RM and LM (*A. flavus*) and RM, SM, LM and FM (*A. niger*). The antifungal efficacy of the plant extracts from *Verbesina encelioides* may be attributed to the following reasons:

Table 5: Antimicrobial activity of different extracts of the selected plant parts against *S. aureus.*

Plant part	Solvent name	Antibacterial activity against *S. aureus* at different concentrations (µg/ml)							
		25		50		75		100	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
V. Root	MeOH	NA	NA	8	0.22	9	0.25	11	0.28
	EtOH	7	0.22	8	0.22	10	0.27	11	0.28
	Water	10	0.32	11	0.31	13	0.36	15	0.39
V. Stem	MeOH	7	0.22	8	0.22	10	0.27	11	0.28
	EtOH	NA	NA	7	0.2	11	0.30	12	0.31
	Water	10	0.32	12	0.34	14	0.38	17	0.44
V. Leaves	MeOH	8	0.25	9	0.25	12	0.33	13	0.34
	EtOH	7	0.22	9	0.25	10	0.27	12	0.31
	Water	8	0.25	10	0.28	12	0.33	14	0.36
V. Flower	MeOH	7	0.22	9	0.25	10	0.27	12	0.31
	EtOH	NA	NA	8	0.22	9	0.25	11	0.28
	Water	12	0.38	15	0.42	16	0.44	18	0.47
Std.		31		35		36		38	

Table 6: Antimicrobial activity of different extracts of the selected plant parts against *A. flavus.*

Plant part	Solvent name	Antifungal activity against *A. flavusr* at different concentrations (µg/ml)							
		25		50		75		100	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
V. Root	MeOH	10	0.62	11	0.61	12	0.63	14	0.7
	EtOH	8	0.5	10	0.55	11	0.57	12	0.6
	Water	7	0.43	8	0.44	9	0.47	10	0.5
V. Stem	MeOH	9	0.56	10	0.55	11	0.57	12	0.6
	EtOH	7	0.43	8	0.44	10	0.52	11	0.55
	Water	7	0.43	8	0.44	10	0.52	11	0.55
V. Leaves	MeOH	8	0.5	10	0.55	12	0.63	14	0.7
	EtOH	7	0.43	9	0.5	11	0.57	12	0.6
	Water	NA	NA	7	0.38	8	0.42	9	0.45
V. Flower	MeOH	8	0.5	9	0.5	11	0.57	13	0.65
	EtOH	7	0.43	8	0.44	10	0.52	11	0.55
	Water	NA	NA	NA	NA	8	0.42	10	0.5
Std.		16	1	18		19		20	1

- Disruption of fungal cell membrane followed by leaking of cellular components and loss of membrane integrity by polyphenolic compounds such as flavonoids, tannins and alkaloids.
- Inhibition of fungal cell wall biosynthesis by restricting ergosterol

biosynthesis by polyphenols and azoles[17].

- Degradation of fungal cell wall comprising of chitin and glucan, by chitinases and glucanases produced by certain plant species.
- Interference with fungal cell division by restricting cell cycle regulators, essential cell cycle proteins and microtubule inhibition by plant alkaloids, terpenoids and flavonoids[18].
- Production of ROS by flavonoids and phenolic acids inside fungal cells, which further damage cellular components such as RNS, DNA and proteins.
- Disruption of cell-to-cell signalling and quorum sensing in fungi, causing inhibition of fungal cell growth by flavonoids and terpenoids[19].

Table 7: Antimicrobial activity of different extracts of the selected plant parts against *A. niger*.

Plant part	Solvent name	Antifungal activity against *A. niger* at different concentrations (µg/ml)							
		25		50		75		100	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
V. Root	MeOH	8	1	10	0.55	11	0.52	13	0.59
	EtOH	7	0.87	8	0.44	10	0.47	12	0.54
	Water	NA	NA	8	0.44	10	0.47	11	0.5
V. Stem	MeOH	8	1	9	0.5	12	0.57	13	0.59
	EtOH	7	0.87	9	0.5	11	0.52	12	0.54
	Water	7	0.87	8	0.44	9	0.42	10	0.45
V. Leaves	MeOH	9	1.12	11	0.61	12	0.57	13	0.59
	EtOH	7	0.87	9	0.5	11	0.52	12	0.54
	Water	NA	NA	7	0.38	9	0.42	11	0.5
V. Flower	MeOH	8	1	10	0.55	12	0.57	13	0.59
	EtOH	NA	NA	8	0.44	10	0.47	11	0.5
	Water	NA	NA	7	0.38	8	0.38	10	0.45
Std.		8		18		21		22	

Table 8: % Free radical scavenging activity of different extracts of the selected plant parts.

Plant part	Solvent name	% Free radical activity of extracts at different concentrations (µg/ml)					IC_{50} (µg/ml)
		200	400	600	800	1000	
V. Root	MeOH	6.49±0.38	9.88±0.87	12.37±0.78	16.63±1.29	19.46±0.66	**2882.05±227.01**
	EtOH	8.32±2.47	10.24±2.60	15.31±1.67	19.29±2.12	24.39±2.88	**2363.28±466.47**
	Water	9.23±1.71	12.28±3.32	17.92±2.25	22.52±3.33	25.73±5.66	**1545.82±193.40**
V. Stem	MeOH	5.10±2.46	7.16±2.64	12.01±3.16	15.43±3.55	18.88±4.94	**1976.65±180.24**
	EtOH	6.05±1.03	9.01±0.65	13.93±0.93	16.84±2.09	19.49±2.10	**2776.17±463.75**
	Water	11.20±3.65	15.36±5.34	19.89±4.66	23.23±4.74	24.89±4.28	**2395.08±368.78**
V. Leaf	MeOH	13.96±5.18	16.27±3.22	20.21±3.10	24.85±1.99	28.74±0.46	**2209.43±397.81**
	EtOH	12.26±3.76	16.03±2.41	17.59±2.49	22.54±4.53	24.07±4.52	**1871.25±177.74**
	Water	11.19±1.34	14.01±0.59	18.64±2.19	24.02±3.61	27.94±5.32	**1727.54±190.88**
V. Flower	MeOH	11.01±6.41	14.51±8.37	18.14±9.26	21.39±10.24	23.54±11.99	**2989.17±125.64**
	EtOH	11.37±0.52	15.79±0.75	19.13±0.93	24.27±0.49	28.25±2.13	**2054.56±220.22**
	Water	12.28±3.38	16.55±2.57	20.38±2.79	24.32±3.03	27.05±2.21	**2230.10±245.29**

**Experiments were done in triplicate. Values are represented in the form of Mean ± SD. Calculations were done in MS Excel.*

In order to find out the probable reason behind antimicrobial activity of the plant extracts, the researchers tested the antioxidant activity of different plant extracts, wherein dose dependent increase in antioxidant activity of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* was observed at different doses (25, 50, 75 and 100 µg/ml*;* with the highest antioxidant activity in case of leaf methanolic extract and flower water extract. Looking at the results, it can be observed that highest antioxidant activity, in general, was demonstrated by water extracts whereas ethanolic and methanolic extracts showed somewhat lesser antioxidant potential. The antioxidant activity of the plant extracts may be attributed to ability of bioactive secondary metabolites such as phenolic acids, flavonoids and carotenoids to scavenge free radicals and prevent ROS mediated cellular damage. Furthermore, phenolics are able to chelate crucial metal ions such as copper and iron, which further causes inhibition of Fenton and Haber-Weiss reactions, inhibiting the generation of deleterious ROS species[20]. Certain secondary metabolites also inhibit crucial enzymes involved in ROS biosynthesis such as xanthine oxidase and NADPH oxidase while also simultaneously augmenting the activity of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase and therefore aid in circumventing the ROS production[21]. The increased biosynthesis of antioxidant enzymes may occur through polyphenols mediated activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which further triggers biosynthesis of antioxidant machinery. Furthermore, to add to the already existing antioxidant state, certain secondary metabolites prevent membrane damage to cells by inhibition of lipid peroxidation, and therefore contribute to overall plant health and longevity[22].

Furthermore, the researchers have shown dose dependent antidiabetic efficacy of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides* at different doses (25, 50, 75 and 100 µg/ml*)*, with the highest antidiabetic activity in case of leaf water extract and flower water extract. The antidiabetic activity of plant extracts may occur due to several reasons, the most prominent one being, overcoming of insulin resistance and augmentation of insulin sensitivity in peripheral tissues such as muscle, liver, and adipose tissue by activation of insulin receptors and maintenance of optimum glucose metabolism. Furthermore, certain secondary metabolites such as saponins and berberine have been known to stimulate insulin secretion by interacting with pancreatic cells. Furthermore, polyphenols inhibit alpha-amylase and alpha-glucosidase enzymes, thereby impeding the digestion of carbohydrates in intestine, and a consequence slow release of carbohydrates leading to lower blood sugar spike post meal. Furthermore, flavonoids and terpenoids interact with GLUT transporters, thereby inhibiting uptake of glucose by cells coupled with activation of the enzyme AMP-activated Protein Kinase (AMPK), as a consequence of which, cells are efficiently able to utilize glucose molecules. A number of other studies have also highlighted antidiabetic efficacy of *Verbesina encelioides* by improvement of insulin sensitivity and improved glucose metabolism[23-25] **.**

Conclusions

Concluding the results of the study in a nutshell, the study highlights potent antibacterial, antifungal, antioxidant and antidiabetic activity of different doses of water, ethanol and methanol extracts of root, stem, leaves and flowers of *Verbesina encelioides.* The study is novel in the sense that it highlights the variability in potency of extracts from different plant parts (using three types of extraction solvents; water, ethanol and methanol) for addressing different issues such as growth of different bacterial and fungal strains, oxidative

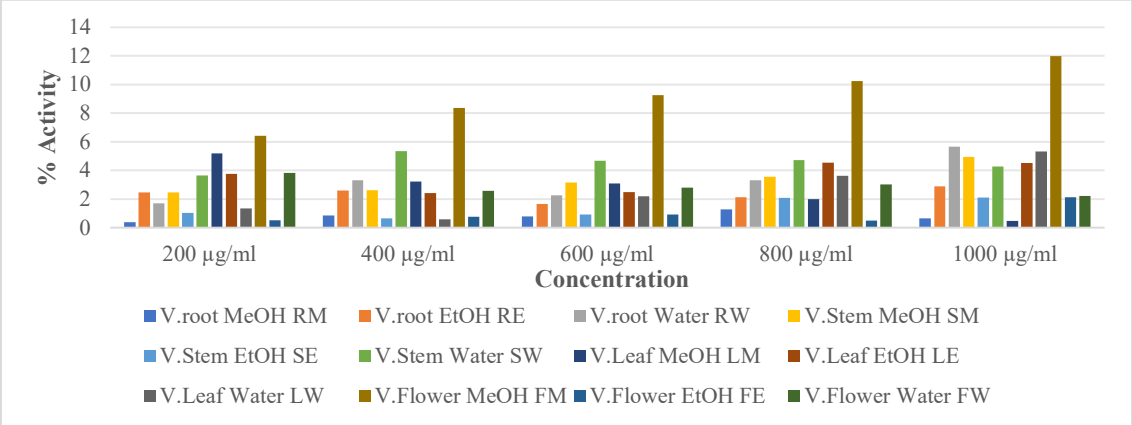

Figure 7: DPPH Free radical scavenging activity of different extracts of the selected plant parts.

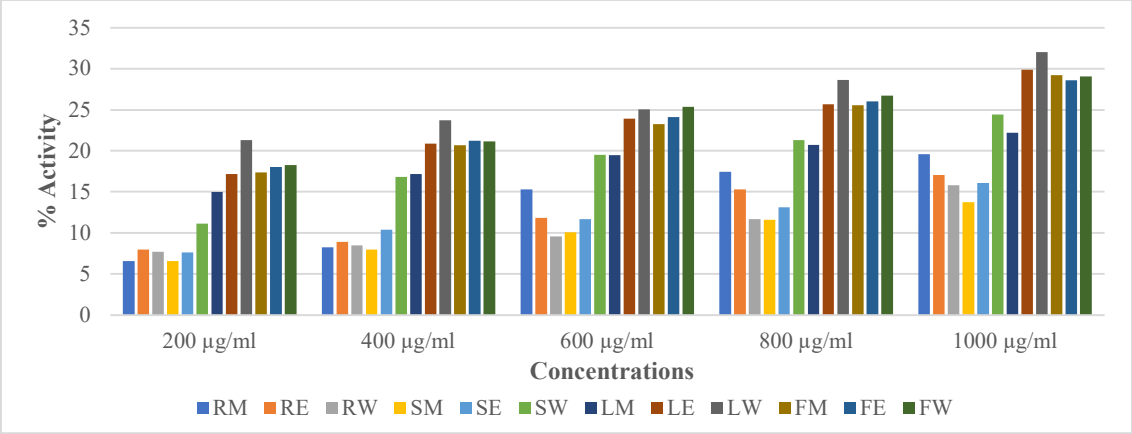

Figure 8: % alpha amylase inhibitory activity of different extracts of the selected plant parts.

Table 9: % Anti-hyperglycaemic activity of different extracts of the selected plant parts.

Plant part	Solvent	% Anti-hyperglycaemic activity of extracts at different concentrations (µg/ml)					IC50 (µg/ml)
		200	400	600	800	1000	
V. Root	MeOH	6.54±0.25	8.25±0.75	15.32±0.41	17.45±1.81	19.59±2.51	2711.25±36.53
	EtOH	8.00±0.75	8.88±0.78	11.84±1.50	15.32±0.25	17.07±1.14	3705.71±37.69
	Water	7.70±0.20	8.51±0.57	9.55±1.12	11.68±2.36	15.78±1.59	4788.18±92.83
V. Stem	MeOH	6.54±0.25	7.97±1.74	10.08±3.86	11.61±4.67	13.74±4.24	6528.50±395.88
	EtOH	7.63±1.34	10.38±4.97	11.70±5.71	13.10±6.80	16.10±8.32	7528.45±632.51
	Water	11.13±2.72	16.81±3.11	19.54±4.92	21.29±5.90	24.45±8.00	2914.07±105.03
V. Leaf	MeOH	14.98±0.36	17.20±0.68	19.45±2.29	20.75±2.30	22.19±3.24	4695.14±37.03
	EtOH	17.17±2.76	20.88±1.02	23.94±0.64	25.69±0.66	29.89±0.57	2401.53±37.61
	Water	21.32±0.32	23.72±1.10	25.06±0.52	28.62±0.87	32.01±0.26	2421.28±11.68
V. Flower	MeOH	17.35±1.81	20.71±1.84	23.26±2.78	25.58±2.46	29.19±4.33	2559.41±55.48
	EtOH	18.04±0.37	21.21±0.65	24.12±1.40	25.99±3.06	28.56±1.72	2742.80±57.24
	Water	18.29±1.76	21.14±0.68	25.36±1.00	26.71±1.57	29.05±1.76	2526.24±25.30

**Experiments were done in triplicate. Values are represented in the form of Mean ± SD. Calculations were done in MS Excel.*

outbursts as well as diabetes and its associated complications. Different plant parts harbour varying amounts of secondary metabolites, which is in accordance with the role assigned to each plant part in maintenance of lant physiology. For instance, leaves harbour higher amount of antioxidant molecules such as flavonoids and phenolics to prevent them from action of direct sunlight, while, roots contain higher amount of alkaloids and saponins, to aid in allelopathy and contribute to plant defense against soil borne microbes. Furthermore, presence of environmental conditions such as temperature, light, soil composition, humidity and others also influence concentration of secondary metabolites. In addition, other factors that contribute to variability in amount of secondary metabolites in different plant parts include developmental age and stage of the plant, genetic variation among plant species as well as variability arising due to difference in harvesting and processing stage. The results demonstrated in the current study are a reflection of the crucial role of solvent in determining the antimicrobial, antioxidant and antidiabetic activity of plant extracts. The profile of secondary metabolites extracted from different parts may significantly vary from each other in accordance with the physico-chemical properties such as temperature, concentration, extraction time and polarity of the used solvent. Polar solvents are better for extraction of polar metabolites such as flavonoids and phenolics, whereas non-polar solvents are better for extraction of non-polar metabolites such as alkaloids and terpenoids. Furthermore, correct choice of the solvent coupled with carefully executed extraction process ensures minimal degradation of the extracted metabolite. Talking about the solvents used in the study, the current study used methanol, water and ethanol as solvents, wherein, all three of them differ from each other in terms of polarity, with highest polarity in water>methanol>ethanol. Furthermore, water requires higher temperature for extraction process in comparison to the other two organic solvents. Nonetheless, irrespective of the choice of the solvent, the study highlights potent antioxidant, antidiabetic and antimicrobial efficacy of root, stem, leaves and flowers of *Verbesina encelioides.*

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