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Phytochemicals identification, anti-microbial, anti-oxidant and anti-diabetic activities of *Polygala sphenoptera* Fresen

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Polygala sphenoptera Fresen, belonging to the family Polygalaceae, is a plant traditionally used in folk medicine for various health benefits. However, its chemical composition and pharmacological properties have not been explored. This study was to prepare and optimise the ethanolic extract of *P. sphenoptera*, investigate its phytochemical composition, and evaluate its anti-microbial, *in-vitro* anti-oxidant and anti-diabetic activities. The ethanolic extract of the authenticated whole plant of *P. sphenoptera* was investigated for the presence of phytochemicals and evaluated for anti-microbial properties by determining the minimum inhibitory concentration and zone of inhibition. The anti-oxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay. The anti-diabetic potential was assessed by measuring α-amylase and α-glucosidase inhibitory activities. Phytochemical investigations indicate the presence of carbohydrates, flavonoids, tannins, alkaloids, and phenolic compounds. The phenolic content of the extract was found to be 8.733 mg GAE/g. The extract demonstrated significant anti-microbial activity against both bacterial and fungal strains. *In-vitro* studies indicated that the plant extract possesses strong anti-oxidant and anti-diabetic properties. Further detailed research will help to understand its mechanisms and validate its therapeutic potential.

Keywords: Anti-diabetic, Anti-microbial, Anti-oxidant, Phytochemicals, *Polygala sphenoptera*

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Introduction

Herbal medicine continues to cater to about 75-80% of the global population in healthcare, especially in developing nations. This preference is due to its strong cultural acceptance, better compatibility with the human body, and fewer side effects[1,2]. Plant-based traditional medicine is found to be beneficial economically and clinically and has fewer side effects than modern medications, leading to the growing demand for phytochemicals in pharmaceuticals[3]. Secondary metabolites are biosynthesised from plants such as steroids, flavonoids, alkaloids, tannins, lignans, carbohydrates, glycosides and phenolic compounds. These compounds possess a variety of biological properties, including anti-inflammatory, anti-cancer, anti-allergic, anti-diabetic, anti-microbial, and anti-oxidant actions[4,5].

Oxidative stress plays a key role in the development of many chronic conditions, such as cancer, cardiovascular diseases, and neurodegenerative disorders like Alzheimer's disease. Such stress results from an imbalance between the generation of reactive oxygen species (ROS) and the body's capacity to neutralise these reactive molecules or fix the damage caused[6]. Continuous oxidative stress can harm cells and tissues, thereby accelerating the advancement of chronic illnesses[7]. Microbial infections remain a significant global health challenge, contributing prominently to illness and death worldwide[8]. The World Health Organization (WHO) identifies diseases like tuberculosis, malaria, and lower respiratory tract infections as leading causes of mortality. The emergence of anti-microbial resistance (AMR) has compounded these issues, presenting a serious threat to public health[9]. Resistant pathogens reduce the effectiveness of conventional treatments, prolonging illnesses, escalating healthcare expenditures, and thereby elevating mortality rates. The escalation of anti-microbial resistance (AMR) has stimulated the quest for innovative anti-microbial treatments[10]. *In-vitro* research is pivotal in identifying and evaluating potential anti-microbial agents sourced from diverse origins, such as medicinal plants. Plant-based compounds have historically contributed to the

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advancement of many antibiotics and continue to offer promising avenues for discovering new anti-microbial substances[11]. Diabetes is a chronic metabolic disorder occurring due to insulin deficiency. Insulin is a pancreatic hormone essential for controlling blood sugar, and when its function is impaired, it results in high blood glucose levels, known as hyperglycaemia[12]. It can cause extensive damage to the body's nerves and blood vessels, leading to serious health problems, including cardiovascular disease, neuropathy (nerve damage), nephropathy (kidney damage), retinopathy (eye damage), foot complications, and skin conditions[13,14].

Plants belonging to the genus *Polygala* have long been utilised in traditional medicine. They are known for their ability to relieve coughs due to their antitussive properties and to help clear phlegm from the respiratory tract. Furthermore, these plants are used to enhance cognitive functions, support detoxification processes, and alleviate swelling and inflammation[15]. Phytochemical research has shown that *Polygala* species are rich in various compounds such as saponins, flavonoids, and alkaloids, with saponins being particularly prevalent. Recent studies indicate that the saponins in *Polygala* possess neuroprotective properties that are beneficial in treating neurological disorders. These effects are achieved by promoting the autophagic degradation of misfolded proteins and by exhibiting anti-inflammatory, anti-apoptotic, and antioxidative stress characteristics[16,17]. *Polygala sphenoptera* Fresen (*P. sphenoptera)* is a herb belonging to the family polygalaceae. It can be found in various woodland and grassland habitats and is used against snake poison and malaria in folklore medicine[18,19]. Furthermore, there is growing interest in alternative herbal treatments due to their potential health benefits and lower side effects compared to conventional drugs. Many plants and herbs have long been used for their medicinal properties, and modern pharmacological studies are increasingly validating these traditional uses scientifically[20]. A review of the literature on *P. sphenoptera* reveals that as the plant is a newly identified species, there is no research report on its phytochemical and pharmacological activities[21]. Hence, this present study aims to carry out a phytochemical investigation on the ethanolic extract of the whole plant and to evaluate *in-vitro* anti-microbial, anti-oxidant, and anti-diabetic activities.

Materials and Methods

The study utilised analytical grade reagents to ensure the reliability and high quality of the results obtained. DPPH, α-amylase and α-glucosidase were purchased from HiMedia.

Collection and authentication of the plant material

P. sphenoptera plant was procured from Savadatti, Karnataka region (15047' N latitude and 750 07' E. longitude, 610 m above mean sea level) during January to April. It was authenticated by Prof Subhas N Emmi Head of the Department of Botany, K.L.E Society's Shri Kadasiddeshwar Arts College and H.S. Kadambari Science Institute Vidyanagar, Hubballi. Voucher specimen of this plant bearing Number NE0222001 is maintained in the department of Pharmacognosy K.L.E College of Pharmacy Hubballi, Karnataka, India, as shown in Fig. 1.

Physico-chemical studies[22]

Physico-chemical parameters such as total ash, acid-insoluble ash, water-soluble ash, sulphated ash, and loss on drying were determined. Different extracts were also prepared to investigate the extractive values of the plant for the study.

Phytochemical studies

Preparation of extract and its optimisation

The powdered drug (mesh size 40) was extracted by different extraction techniques such as cold maceration, hot extraction, and ultrasonic extraction using ethanol (70%v/v)[22]. The extracts were dried and weighed after being concentrated under a vacuum in a rota evaporator at 40°C[23]. The extract was examined for the presence of phytoconstituents like tannins, alkaloids, flavonoids, terpenoids, glycosides, proteins, amino acids, carbohydrates, and steroids[24].

Chromatographic studies[25]

The thin layer chromatography (TLC) of the extracts was performed using silica gel G60 as an adsorbent. The ethanolic extract of the plant was chromatographed using mobile chloroform: methanol in the ratio of 90:10, chloroform: ethylacetate in the ratio of 60:40 and Ethyl

Fig. 1 — a) Fresh plant, and b) Herbarium specimen of plant.

acetate: Formic acid: glacial acetic acid: water (100:11:11:26) as mobile phase and UV-365 nm, Dragendroff reagent (Alkaloids) and ferric chloride (Flavonoids) were used as detecting reagent respectively.

Estimation of total phenolic content[26]

Based on the preliminary phytochemical studies suggesting the presence of phenolic compounds, the TPC was estimated.

Gallic acid was dissolved in water to make a 1 mg/mL solution, then diluted to 2-10 µg/mL. Each solution received 0.5 mL of Folin-Ciocalteu reagent and stood for 15 minutes. After adding 1 mL of 10% sodium carbonate solution, the volume was adjusted to 10 mL with distilled water and left for 30 minutes at room temperature. Absorbance was measured at 760 nm. The ethanolic extract of *P. sphenoptera* was prepared at 1 mg/mL and similarly diluted[27]. A calibration curve of gallic acid concentration versus absorbance was plotted, and a linear regression equation was used to calculate the TPC as mg of gallic acid equivalent per gram of extract (mg GAE/g)[28].

Anti-microbial studies

Minimum inhibitory concentration (MIC) by serial dilution method[29]

Escherichia coli (MTCC-261) (E. coli), Staphylococcus aureus (MTCC-737) (S. aureus), Pseudomonas aeruginosa (MTCC-1948) (P. aeruginosa), Salmonella typhi (NCIM-5278) (S. typhi), Bacillus subtilis (NCIM-2010) (B. subtilis) and Staphylococcus epidermidis (MTCC-6810) (S. epidermidis) were the bacterial strains and Aspergillus niger **(**NCIM-1004) (A. niger) and Candida albicans (NCIM-3682) (C. albicans) were the fungal strains used for the current anti-microbial studies.

The tube and agar dilution methods are widely used. Prepare serial dilutions of the plant extract (2000, 1000, 500, 250, 125, and 62.5 µg/mL) along with positive, negative, and drug controls. Use Mueller Hinton broth for bacteria and potato dextrose broth for fungi, with Ciprofloxacin as the standard antibacterial drug and Amphotericin B as the standard antifungal drug. Add test organisms to all dilutions except the negative and drug controls. Seal and incubate tubes at 37°C for 24 hours. After incubation, add 40 µL of 0.2 mg/mL P-iodonitrotetrazolium violet (INT) in distilled water to each tube. Measure the intensity of the resulting red formazan at 530 nm to assess cell activity and viability[30].

Determination of Zone of inhibition (ZoI) by agar well diffusion method[31,32]

The microbial activity of plant extracts is frequently assessed using the Agar well diffusion method. The agar plate surface is inoculated by evenly applying a volume of the microbial inoculum throughout the whole agar surface. Subsequently, a sterile cork borer is used to punch a hole 6 to 8 mm in diameter aseptically. A volume (1000–2000 µL) containing the desired concentration of the anti-microbial agent or extract solution is added to the well. Then, agar plates are incubated for 24 hours at 37°C for bacteria and 72 hours at room temperature for fungi. The anti-microbial agent diffuses in the agar medium and inhibits the growth of the microbial strain to be tested.

***In-vitro* anti-oxidant activity[33,34]**

Free radical scavenging activity using 2,2-diphenyly-1-picrylhydrazyl (DPPH)

Dissolve 4 mg of DPPH in 100 mL of methanol, cover it with aluminium foil, and let it sit for 30 minutes. Dissolve 10 mg of ascorbic acid and plant extract in 100 mL of methanol to make a 100 µg/mL solution. Create 20, 40, 60, 80, and 100 µg/mL dilutions. Transfer 1 mL of each dilution into separate 10 mL flasks, add 3 mL DPPH solution, and fill to 10 mL with methanol. Measure the absorbance at 517 nm. For the control, mix 6 mL methanol with 3 mL DPPH solution and measure absorbance at 517 nm.

Percentage Scavenging Activity = Absorbance of control – Absorbance of sample/Absorbance of control X 100

Determination of *in-vitro* anti-diabetic activity

α-amylase inhibitory activity[35]

Concentrations of plant extract ranging from 100 to 500 µg/mL and equivalent concentrations of acarbose as a standard were prepared. A control without extract or drug and a blank containing phosphate buffer pH 6.9 was also set up. To each concentration of extract and standard, 1 mL of α-amylase solution (0.5 mg/mL in buffer) was added and incubated at room temperature for 10 minutes. Then, 0.5 mL of 1% starch solution (1 g starch in 100 mL buffer) was added to each test tube and incubated at 25°C for 10 minutes. Following this, 1 mL of DNSA (2,4-dinitrosalicylic acid) solution was added to each tube, and the reactions were stopped by boiling for 5 minutes. The solutions were then diluted to 10 mL with buffer. Absorbance was measured at 540 nm using a UV-visible spectrophotometer. The following formula was used to determine the percentage inhibition of α-amylase activity:

Percentage inhibition = Absorbance of control – Absorbance of sample/Absorbance of control X 100

***α-glucosidase inhibitory activity*[36]**

In this assay, α-glucosidase enzyme (1 U/mL from Yeast, SRL, Bangalore, India) was mixed with phosphate buffer (50 mM, pH 6.9) and treated with various concentrations of samples (0 to 250 µg/mL) for 10 minutes at 37°C. The reaction started with the addition of 50 µL of 5 mM p-nitrophenyl-α-D glucopyranoside in phosphate buffer and proceeded at 37°C for 30 minutes. The reaction was stopped by adding sodium bicarbonate solution (1 M), and absorbance at 405 nm was measured to assess enzyme activity. This method evaluates the ability of samples to inhibit α-glucosidase, which is important for potential therapeutic uses, especially in conditions like diabetes management. The results were expressed in terms of IC_{50} values compared to acarbose, which served as a positive control.

Results

Physico-chemical evaluation

The results of ash values, such as total ash, acid insoluble ash, water soluble ash and sulfated ash, along with the loss on drying, are shown in Table 1.

Lower water-soluble ash suggests minimal contamination with soluble inorganic impurities, reinforcing the purity of the plant material. Acid-insoluble ash values confirm the absence of significant siliceous contaminants, such as sand and soil. Sulfated ash values indicate the presence of inorganic mineral content, including possible medicinally active salts. Loss on drying (LOD) values suggest low moisture content, which is essential for enhancing the plant material's shelf life and microbial stability.

Preparation of extract and its optimisation

The plant material was extracted using ethanol (70% v/v) through various extraction techniques, including cold maceration, hot extraction, and ultrasonication. The cold maceration method has been selected for further study; despite its lower yield (7.2%) compared to hot extraction (12%) and ultrasonication (9%), cold maceration was chosen for its ability to preserve heat-sensitive bioactive compounds, prevent phytochemical degradation, and enhance solvent penetration for thorough extraction. Additionally, its traditional reliability outweighs that of the less conventional ultrasonication method, and the results are shown in Table 2.

Table 1 — Physico-chemical evaluation

Ash values	Determined value (%W/W)
Total ash	5±0.63
Acid insoluble ash	1±0.33
Water soluble ash	2.5±0.25
Sulfated ash	3±0.47
Loss on drying	1

Table 2 — Optimisation of extraction method

Extractive value	Determined value (% w/w)
Cold maceration	7.2
Hot extraction	12
Ultra sonication	9

Phytochemical studies

The ethanolic extract of *P. sphenoptera*, which was obtained through cold maceration, was subjected to preliminary phytochemical screening. The analysis confirmed the presence of various secondary metabolites, including carbohydrates, flavonoids, tannins, alkaloids, and phenolic compounds. The detailed results of this screening are presented in Table 3.

Table 3 — Preliminary phytochemical screening

Test for Carbohydrates	
Molish's test (General test)	+
Fehling's test (Test for reducing sugars)	+
Benedicts test (Test for reducing sugars)	+
Barfoeds test (Test for monosaccharides)	+
Test for proteins	
Biuret test (General test)	-
Million's test (General test)	-
Test for steroids	
Salkowski test	-
Liebermann–Burchard test	-
Test for triterpenoids	
Salkowski test	-
Liebermann–Burchard test	-
Test for flavonoids	
Shinoda test	+
Zinc-hydrochloride test	+
Test for glycosides	
General test (Hydrolysis test)	-
Test for cardiac glycosides	-
Baljets test	-
Legals test	-
Kellarkillani test	-
Test for anthraquinone glycosides	-
Borntragers test	-
Modified Borntragers test	-
Saponin glycosides	-
Coumarin glycosides	-
Test for Tannins and phenolic compounds	
Alkaline reagent test	+
Ferric chloride test	+
Test for alkaloids	
Dragendroff test	+
Mayers test	+
Wagner's test	+
Hager's test	+

+ indicates presence - indicates absence

Table 4 — Chromatographic studies

S. No	Solvent system for TLC Stationary phase: Silica gel G 60 for TLC	No. of spots (≈)	R_f values (≈)
1	Mobile phase: Chloroform: methanol (9:1) Detecting reagent: UV-365nm and Dragendroff reagent (Alkaloids)	3	0.516 0.66 0.79
2	Mobile phase: Ethyl acetate: Formic acid: glacial acetic acid: water (100:11:11:26) Detecting reagent: UV-365nm and ferric chloride (Flavonoids)	1	0.73
3	Mobile phase: Chloroform: ethyl acetate (60:40) Detecting reagent: UV-365nm and ferric chloride (Flavonoids)	2	0.916 0.74

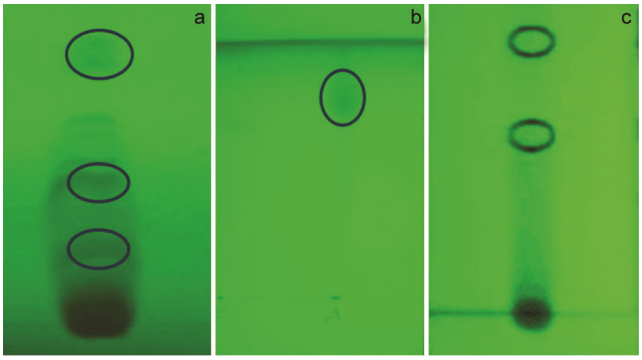

Fig. 2 — Chromatographic studies using TLC a) Mobile phase = Chloroform: methanol (90:10), b) Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26), and c) Mobile phase= Chloroform: ethyl acetate (60:40).

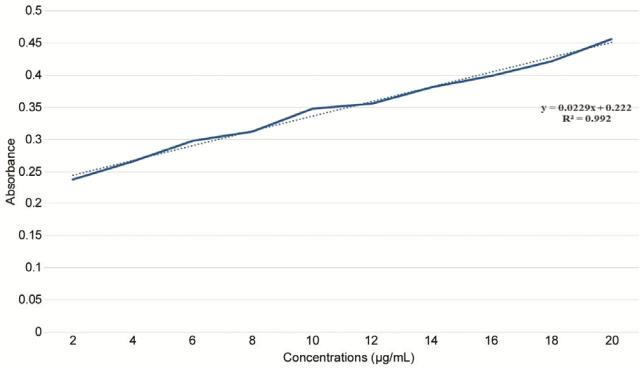

Fig. 3 — Standard calibration curve of gallic acid.

Chromatographic studies

The TLC analysis of *P. sphenoptera* ethanolic extract revealed distinct secondary metabolites such as alkaloids and flavonoids. These findings are detailed in Table 4 and Fig. 2.

Estimation of total phenolic content

The TPC of the *P. sphenoptera* ethanolic extract was estimated and found to be 8.733 mg GAE/g of extract using the Folin-Ciocalteu method. The absorbance values at different concentrations of gallic acid (Table 5) were used to construct a standard calibration curve (Fig. 3). The extract showed an absorbance of 0.420, which was interpolated from the curve to determine the TPC. This highlights the significant TPC, suggesting the strong anti-oxidant potential of the extract.

Table 5 — Total phenolic content

Sl. No	Concentrations (µg/mL)	Absorbance
1	2	0.238
2	4	0.266
3	6	0.298
4	8	0.313
5	10	0.348
6	12	0.356
7	14	0.382
8	16	0.399
9	18	0.422
10	20	0.456
Extract		0.420

Anti-microbial studies

Minimum inhibitory concentration (MIC) by serial dilution method

The anti-microbial efficacy of *P. sphenoptera* ethanolic extract was tested against various bacterial and fungal strains at different concentrations. The extract demonstrated significant anti-microbial activity, particularly at higher concentrations. The detailed results are presented in Tables 6 and 7.

Zone of inhibition (ZoI)

The ethanolic extract of *P. sphenoptera* exhibited anti-microbial activity against all tested bacterial strains, with inhibition zones increasing as the concentration of the extract increased. While Ciprofloxacin showed higher inhibition zones at lower concentrations (64 µg/mL), the extract still demonstrated significant anti-microbial effects, particularly at the higher concentration of 1000 µg/mL. Detailed results can be found in Table 8 and Fig. 4.

***In-vitro* anti-oxidant activity**

Free radical scavenging activity using 2,2-diphenyly-1-picrylhydrazyl (DPPH)

The *in-vitro* anti-oxidant activity of the ethanolic extract from *P. sphenoptera* was assessed using 2,2-

Table 6 — Determination of MIC for bacteria

Name of the bacteria	Bacterial growth at different concentrations of *Polygala sphenoptera*ethanolic extract (µg/mL)								
	2000 µg/mL	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	+ve control	-ve control	Drug control
Escherichia coli	-	-	-	-	+	+	+	-	-
Staphylococcus aureus	-	-	-	+	+	+	+	-	-
Pseudomonas aeruginosa	-	-	-	+	+	+	+	-	-
Staphylococcus epidermidis	-	-	+	+	+	+	+	-	-
Salmonella typhi	-	-	-	+	+	+	+	-	-
Bacillus subtilis	-	-	-	+	+	+	+	-	-

+ indicates growth **-** indicates No growth
-ve control– Only Media; +ve control – Media with culture Drug control - Media with maximum concentration of extract

Table 7 — Determination of MIC for fungi

Name of the fungi	Fungal growth at different concentrations of *Polygala sphenoptera* ethanolic extract (µg/mL)								
	2000 µg/mL	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	+ve control	-ve control	Drug control
Candida albicans NCIM-3628	-	-	-	+	+	+	+	-	-
Aspergillus niger NCIM-1004	-	-	-	+	+	+	+	-	-

+ indicates growth - indicates No growth
-ve control– Only Media; +ve control – Media with culture Drug control - Media with maximum concentration of extract

Table 8 — Determination of Zone of Inhibition

Microbial strain	Concentration in well (µg/mL)	Inhibition (in mm)
Staphylococcus epidermidis (MTCC-6810)	Standard (64)	42
	P.E (500)	10
	P.E (1000)	35
	Control (DMSO)	6
Escherichia coli (MTCC-261)	Standard (64)	50
	P.E (500)	10
	P.E (1000)	30
	Control (DMSO)	5
Staphylococcus aureus (MTCC-737)	Standard (64)	38
	P.E (500)	11
	P.E (1000)	25
	Control (DMSO)	6
Salmonella typhi (NCIM-5278)	Standard (64)	58
	P.E (500)	8
	P.E (1000)	19
	Control (DMSO)	6

Standard – Ciprofloxacin 64 µg/mL; Control – DMSO; P.E - *Polygala sphenoptera* ethanolic extract

diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results detailed in Table 9 and Fig. 5, demonstrate the extract's ability to scavenge DPPH radicals compared to ascorbic acid, a well-known anti-oxidant. These findings underscore the extract's significant radical scavenging activity, suggesting its potential as an effective anti-oxidant agent.

Determination of *In-vitro* anti-diabetic activity

α-Amylase inhibitory activity

The results presented in Table 10 and Fig. 6 compare the percentage inhibition and IC_{50} values of α-amylase enzyme activity for *P. sphenoptera* ethanolic extract and acarbose using the DNSA method. The data reveal no significant difference between acarbose and the plant extract in their ability to inhibit α-amylase activity. This similarity confirms the potent anti-diabetic activity of the plant extract.

α-Glucosidase inhibitory activity

The investigation assessed the inhibitory effects of *P. Sphenoptera* ethanolic extract and Acarbose on α-glucosidase enzyme activity. Detailed results are presented in Table 11 and Fig. 7, demonstrating

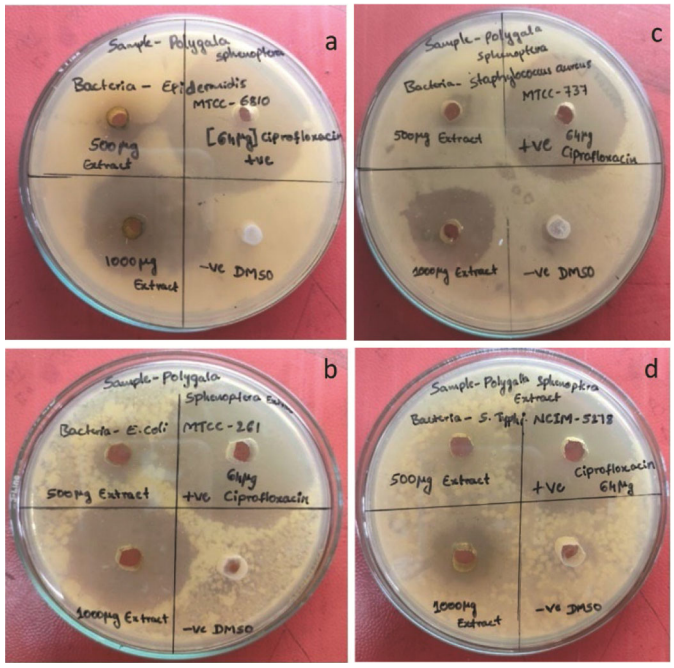

Fig. 4 — Zone of Inhibition of standard drug (Ciprofloxacin 64 µg/mL) and PS extract (500 and 1000 µg/mL) against respective strains. a) *Staphylococcus epidermidis* (MTCC-6810), b) *Escherichia coli* (MTCC-261), c) *Staphylococcus aureus* (MTCC-737), and d) *Salmonella typhi* (NCIM-5278).

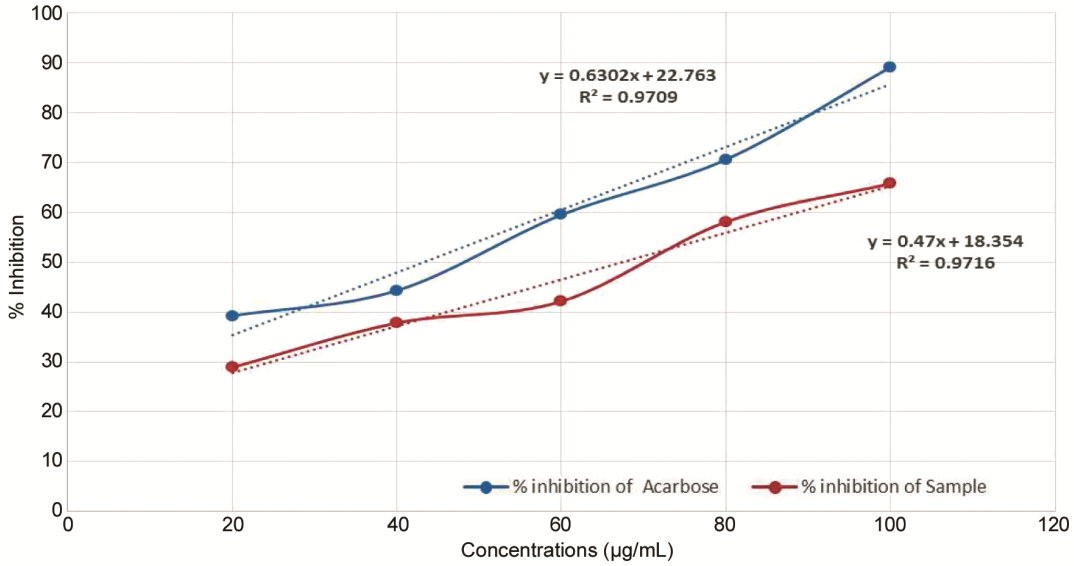

Fig. 5 — DPPH Radical scavenging activity.

Table 9 — Determination of *In-vitro* anti-oxidant activity

Percentage inhibition by ascorbic acid			Percentage inhibition by *Polygala sphenoptera* ethanolic extract		
Concentrations (µg/mL)	Absorbance	% Inhibition	Concentrations (µg/mL)	Absorbance	% Inhibition
20	0.252	39.27	20	0.295	28.91
40	0.231	44.33	40	0.258	37.83
60	0.168	59.51	60	0.240	42.16
80	0.122	70.6	80	0.153	58.09
100	0.045	89.15	100	0.142	65.78
IC_{50} = 43.21 µg/ mL			IC_{50} = 67.33 µg/ mL		

Table 10 — *In-vitro* anti-diabetic activity by alpha-amylase inhibition

Percentage inhibition by acarbose			Percentage inhibition by *polygala sphenoptera* ethanolic extract		
Concentrations (µg/mL)	Absorbance (660 nm)	% Rate of inhibition	Concentrations (µg/mL)	Absorbance (660 nm)	% Rate of inhibition
100	0.462	51.5	100	0.525	33.33
200	0.451	55.21	200	0.502	39.44
300	0.432	62.03	300	0.472	48.3
400	0.425	64.7	400	0.463	51.18
500	0.39	79	500	0.428	63.55
IC_{50} = 106.34 µg/ mL			IC_{50} = 339.35 µg/ mL		

Table 11 — *In-vitro* anti-diabetic activity by alpha-glucosidase inhibition

Percentage inhibition by Acarbose			Percentage inhibition by *polygala sphenoptera* ethanolic extract		
Concentrations (µg/mL)	Absorbance (660 nm)	% Rate of inhibition	Concentrations (µg/mL)	Absorbance (660 nm)	% Rate of inhibition
10	1.681	21.66	10	1.77	17.52
20	1.247	41.89	20	1.426	33.55
30	0.967	54.93	30	1.147	46
40	0.693	67.7	40	0.925	56.89
50	0.324	84.9	50	0.76	64.58
IC_{50} =27.26 µg/ mL			IC_{50} = 35.35 µg/ mL		

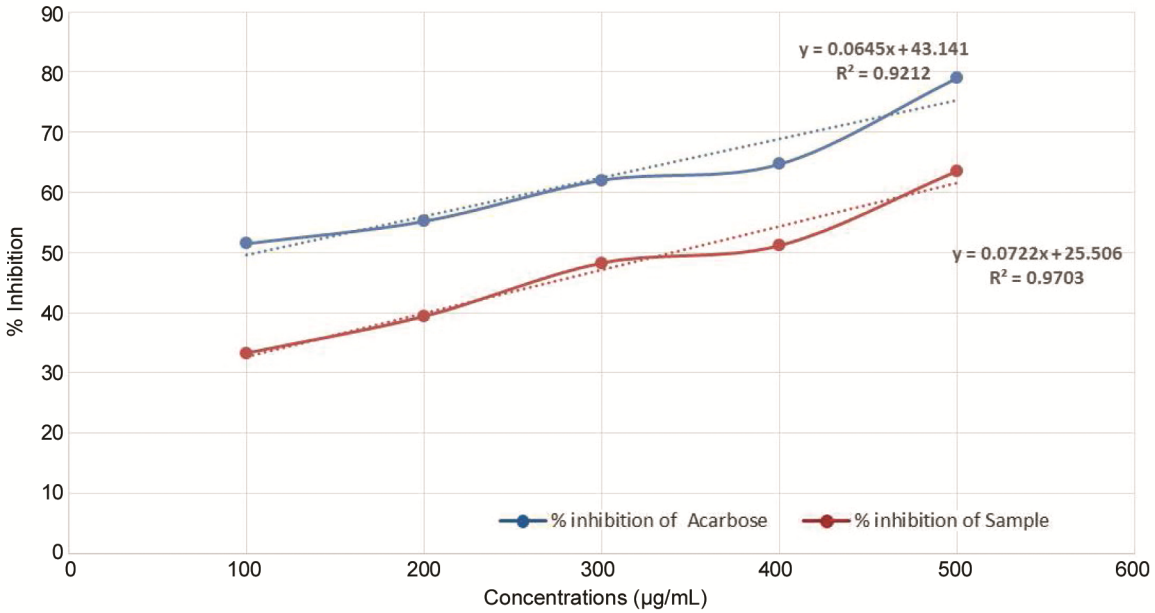

Fig. 6 — Percentage rate of inhibition of α-Amylase.

significant inhibition by both substances. A comparison of the data indicates no substantial difference between Acarbose and the plant extract, suggesting that the extract possesses promising anti-diabetic potential comparable to Acarbose.

Discussion

The preliminary phytochemical analysis, combined with *in-vitro* and *in-vivo* studies, highlights the therapeutic potential of *P. sphenoptera* Fresen. The identified bioactive compounds are linked to the plant's anti-microbial, anti-oxidant and anti-diabetic properties. These results validate the traditional use of the plant in herbal medicine and suggest the need for further research to isolate specific compounds and understand their mechanisms of action. Future research should include detailed pharmacological investigations, safety evaluations, and clinical trials to establish *P. sphenoptera* Fresen as a therapeutic agent.

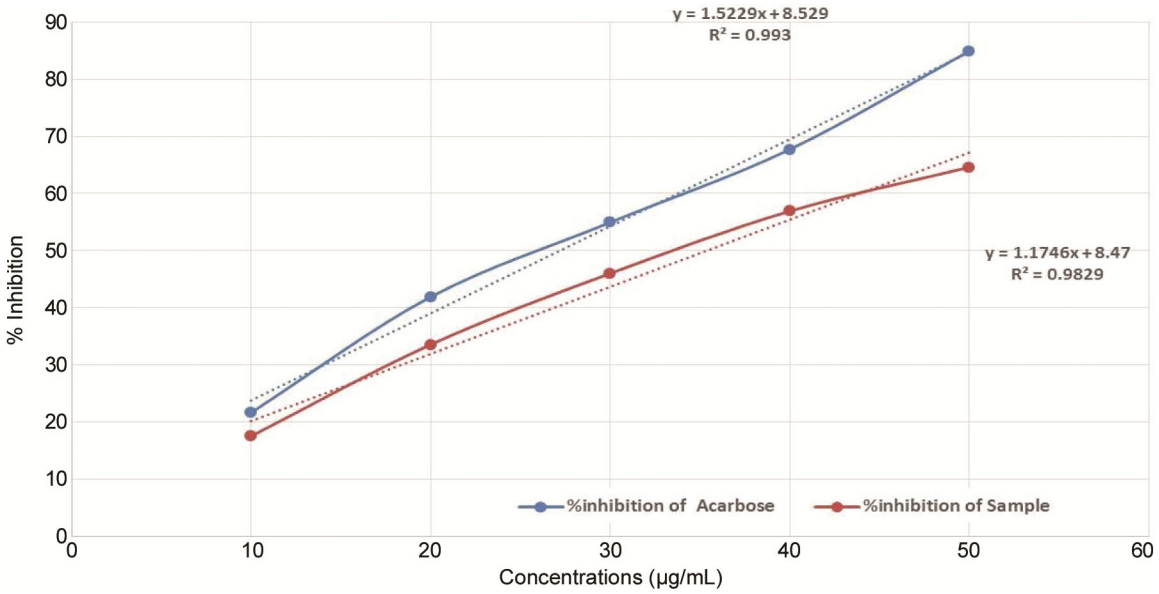

Fig. 7 — Percentage rate of inhibition of α-Glucosidase.

The results of the ash values show that the lesser water-soluble ash value indicates that the plant material is not contaminated with inorganic material, and the acid-insoluble ash value indicates that it is not contaminated by sand and other debris. Sulfated ash values indicate the presence of inorganic mineral content, including possible medicinally active salts. Loss of drying values suggests low moisture content, which is essential for enhancing the shelf life and microbial stability of the plant material.

The study opted for cold maceration, even though it has a lower extractive yield than hot extraction, to avoid the degradation of heat-sensitive phytoconstituents. This approach preserves the natural structure and biological activity of the bioactive compounds, which is essential for accurately assessing their therapeutic potential.

The preliminary phytochemical screening of the *P. sphenoptera* ethanolic extract showed the presence of different types of secondary metabolites like carbohydrates, Flavonoids, tannins, alkaloids and phenolic compounds and the absence of steroids, triterpenoids, proteins and glycosides. These phytochemicals have pharmacological effects that include alkaloids as anti-inflammatory, anti-cancer, analgesic, local anaesthetic and pain relief, antibacterial, and antifungal activity[37]. Flavonoids reduce inflammation, protect against oxidative stress, are cardio-protective and decrease the chance of developing chronic illnesses[38]. Carbohydrates: anti-inflammatory, cardioprotective, wound healing, immunomodulatory and antibacterial properties are exhibited by some complex carbohydrates and polymers[39]. Tannins are anti-inflammatory, anti-microbial, anti-oxidant, lowering blood pressure, lowering serum cholesterol levels, and modulation of immune responses[40]. Phenolic compounds have strong anti-oxidant properties that enable phenolic compounds to decrease oxidative damage and counteract free radicals. Anti-cancer, cardioprotective, antibacterial and anti-inflammatory[41].

The TLC analysis of *P. sphenoptera* ethanolic extract revealed distinct secondary metabolites with varying Rf values. Using chloroform: methanol (9:1) as the mobile phase and detecting with UV-365 nm and Dragendorff reagent, three alkaloid spots were identified with Rf values of 0.516, 0.66, and 0.79. Flavonoids were detected using two different solvent systems; ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) showed one spot with an Rf of 0.73 and chloroform: ethyl acetate (60:40) revealed two spots with Rf values of 0.916 and 0.74. These results demonstrate the diverse phytochemical constituents present in the extract.

The TPC was estimated using a linear regression equation: $Y = 0.0229x + 0.0222$, with a correlation coefficient 0.992. Based on this equation, the amount of phenol content present in the extract was determined to be 8.733 mg GAE/g of extract. The plant extract highlights the significant phenolic content, suggesting the strong anti-oxidant potential of the extract.

The anti-microbial efficacy of *P. sphenoptera* ethanolic extract was tested against different bacterial strains at various concentrations (2000, 1000, 500, 250, 125, and 62.5 µg/mL). The results indicate bacterial growth inhibition or reduced viability at these concentrations. The ethanolic extract of *P. sphenoptera* demonstrates significant anti-microbial activity, particularly at higher concentrations. MIC and ZoI studies revealed stronger antibacterial activity against Gram-negative bacteria. The extract effectively inhibited *E. coli* (30 mm) and *S. typhi* (19 mm) at 1000 µg/mL, with lower MIC values (≤250 µg/mL), suggesting better penetration through the outer membrane. Against Gram-positive bacteria, it inhibited *S. epidermidis* (35 mm) and *S. aureus* (25 mm) but required a higher MIC for *S. epidermidis* (partial resistance at 500 µg/mL). Although the extract was less potent than the standard antibiotic (Ciprofloxacin, 64 µg/mL), it exhibited notable anti-microbial activity, particularly at higher concentrations. These findings highlight the potential of *P. sphenoptera* as a natural anti-microbial agent, especially against Gram-negative bacteria. Further isolation and optimisation of active constituents are necessary, and these will be explored in future research.

The antifungal properties of *P. sphenoptera* ethanolic extract were evaluated against different fungal strains at various concentrations (2000, 1000, 500, 250, 125, and 62.5 µg/mL). *A. niger* and *C. albicans* were the fungal strains used in the study. MIC of *A. niger* and *C. albicans* was found to be 500 µg/mL. Hence, the study highlights the limited antifungal potency of *P. sphenoptera* ethanolic extract in its crude form compared to Amphotericin B. However, it underscores the need for further purification and isolation of bioactive compounds to enhance their efficacy as a potential source of antifungal agents.

The ethanolic extract of *P. sphenoptera* demonstrated anti-microbial activity against all tested bacterial strains, with the inhibition zones increasing in a dose-dependent manner. While Ciprofloxacin (standard antibiotics) show higher inhibition zones at lower concentrations (64 µg/mL), the extract exhibits moderate anti-microbial activity rather than high potency, particularly at the higher concentration of 1000 µg/mL. This is likely due to its composition as a crude mixture containing multiple compounds. In contrast, the standard antibiotic is a purified compound with optimised bioactivity. Further isolation and characterisation of active constituents may enhance the extract's potency and therapeutic potential.

The graph presented shows how the ethanolic extract of *P. sphenoptera* scavenges DPPH radicals in comparison to ascorbic acid, a common anti-oxidant. Plotting the percentage suppression of DPPH radicals against ascorbic acid and extract concentrations (µg/mL). The ethanolic extract of *P. sphenoptera* exhibited a 65.78% inhibition rate, whereas ascorbic acid demonstrated an 88.15% inhibition rate at a concentration of 100 µg/mL. Using the linear regression analysis, the IC_{50} values were determined to be 43.21 and 67.33 µg/mL for the ethanolic extract and ascorbic acid, respectively. These findings indicate that the extract possesses significant radical scavenging activity.

Percentage inhibition of α-amylase enzyme activities for *P. sphenoptera* ethanolic extract and acarbose by DNSA method was shown in Fig. 4. IC_{50} values of standard and *P. sphenoptera* ethanolic extract were found to be 106.34 and 339.35 µg/mL respectively. While the plant extract exhibited a higher IC_{50} compared to the standard, indicating lower potency, it still demonstrated measurable anti-diabetic activity. A higher IC_{50} does not imply a complete lack of potential, as many plant-derived compounds exhibit moderate activity and can serve as leads for further optimisation.

The investigation focused on assessing the inhibitory impacts of *P. sphenoptera* ethanolic extract and acarbose on the enzyme activity of α-glucosidase. The results showed considerable inhibition by both Acarbose and the ethanolic extract of *P. sphenoptera*, with IC_{50} values of 27.26 µg/mL and 35.35 µg/mL, respectively. The comparison revealed no significant difference between the two, indicating that the plant extract holds promising anti-diabetic potential similar to that of acarbose.

Conclusion

The study on *P. sphenoptera* Fresen. encompassed a wide range of analyses, including preliminary phytochemical screening and evaluations of its potential benefits. Initial screenings revealed the presence of various bioactive substances, suggesting its potential for producing pharmacologically active compounds. Literature suggests that phenols present in the plant are known to possess properties like anti-oxidant and anti-diabetic activities, which may be

due to a common mechanism like nitric oxide inhibition. The plant showed promising anti-microbial properties in laboratory tests, indicating it could be useful in fighting against microbial infections. Additionally, its anti-oxidant effects suggest it may help counteract oxidative stress-related conditions by scavenging free radicals. The extract also displayed promising anti-diabetic effects, potentially affecting glucose metabolism. Further research, including activity-guided isolation of phytochemicals and mechanism of action for positive pharmacological activities, may be undertaken.

Conflict of interest

The authors declare no conflict of interest in relation to the present research paper.

References

1 Debbie S, Graeme L, Pierre D, Elizabeth W and Kelvin C, Pharmacovigilance of herbal medicine, *J Ethnopharmacol*, 2012, **140**(3), 513–518, doi: 10.9734/jocamr/2020/v12i130198.

2 Hlatshwayo S, Thembane N, Krishna S B N, Gqaleni N and Ngcobo M, Extraction and processing of bioactive phytoconstituents from widely used South African medicinal plants for the preparation of effective traditional herbal medicine products: A narrative review, *Plants*, 2025, **14**, 206, doi: 10.3390/plants14020206.

3 Tran N, Pham B and Le L, Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery, *Biology*, 2020, **9**(9), 252, doi: 10.3390/biology9090252.

4 Sofowora A, Ogunbodede E and Onayade A, The role and place of medicinal plants in the strategies for disease prevention, *Afr J Tradit Complement Altern Med*, 2013, **10**(5), 210-29, doi: 10.4314/ajtcam.v10i5.2.

5 Li S, Hou Z, Ye T, Song X, Hu X, *et al.*, Saponin components in *Polygala tenuifolia* as potential candidate drugs for treating dementia, *Front Pharmacol*, 2024, **15**, 1431894, doi: 10.3389/fphar.2024.1431894.

6 Coates A, Hu Y, Bax R and Page C, The future challenges facing the development of new anti-microbial drugs, *Nat Rev Drug Discov*, 2002, **1**(11), 895-910, doi: 10.1038/nrd940.

7 Lushchak V I, Free radicals, reactive oxygen species, oxidative stress and its classification, *Chem Biol Interact*, 2014, **224**, 164-175, doi: 10.1016/j.cbi.2014.10.016.

8 Junsathian P, Nakamura S, Katayama S and Rawdkuen S, Anti-oxidant and anti-microbial activities of Thai edible plant extracts prepared using different extraction techniques, *Molecules,* 2022, **27**(19), 6489, doi:10.3390/molecules27196489.

9 Salam M A, Al-Amin M Y, Salam M T, Pawar J S, Akhter N, *et al.*, Anti-microbial resistance: A growing serious threat for global public health, *Healthcare*, 2023, **11**(13), 1946, doi: 10.3390/healthcare11131946.

10 Kariuki S, Kering K, Wairimu C, Onsare R and Mbae C, Anti-microbial resistance rates and surveillance in sub-Saharan Africa: Where are we now?, *Infect Drug Resist*, 2022, 3589-609, doi: 10.2147/IDR.S342753.

11 Vaou N, Stavropoulou E, Voidarou C, Tsigalou C and Bezirtzoglou E, Towards advances in medicinal plant anti-microbial activity: A review study on challenges and future perspectives, *Microorganisms*, 2021, **9**(10), 2041, doi: 10.3390/microorganisms9102041.

12 Dungan K M, Braithwaite S S and Preiser J C, Stress hyperglycaemia, *The Lancet*, 2009, **373**(9677), 1798-1807, doi: 10.1016/S0140-6736(09)60553-5.

13 Jang H N and Oh T J, Pharmacological and nonpharmacological treatments for painful diabetic peripheral neuropathy, *Diabetes Metab J*, 2023, **47**(6), 743, doi: 10.4093/dmj.2023.0018.

14 Galli F, Piroddi M, Annetti C, Aisa C, Floridi E, *et al.*, Oxidative stress and reactive oxygen species, In *Cardiovascular disorders in hemodialysis*, vol 149, 2005, 240-60, doi: 10.1159/000085686.

15 Polygala_senega @ en.wikipedia.org. Available from: https://en.wikipedia.org/wiki/Polygala_senega, accessed on 29/08/2024.

16 Zhang L, Yong Y Y, Deng L, Wang J, Law B Y, *et al.*, Therapeutic potential of Polygala saponins in neurological diseases, *Phytomedicine*, 2023, **108**, 154483, doi: 10.1016/j.phymed.2022.154483.

17 Chen Z, Yang Y, Han Y and Wang X, Neuroprotective effects and mechanisms of senegenin, an effective compound originated from the roots of *Polygala Tenuifolia*, *Front Pharmacol*, 2022, **13**, 937333, doi: 10.3389/fphar.2022.937333.

18 Polygala @ en.wikipedia.org [Internet]. Available from: https://en.wikipedia.org/wiki/Polygala, accessed on 26/08/2024

19 Chekole G, Ethnobotanical study of medicinal plants used against human ailments in Gubalafto District, Northern Ethiopia, *J Ethnobiol Ethnomed*, 2017, **13**(1), 1–29.

20 Ekor M, The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety, *Front Pharmacol*, 2014, **4**, 177, doi: 10.3389/fphar.2013.00177.

21 Gong L, Feng D, Wang T, Ren Y, Liu Y, *et al.*, Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia, *Food Sci Nutr*, 2020, **8**(12), 6320-6337, doi: 10.1002/fsn3.1987.

22 World Health Organization, Quality control methods for medicinal plant materials, *World Health Organization*, 1998.

23 Kim J H, Shin H K and Seo C S, Optimization of the extraction process for the seven bioactive compounds in Yukmijihwang-tang, an herbal formula, using response surface methodology, *Pharmacogn Mag*, 2014, **10**(Suppl 3), S606, doi: 10.4103/0973-1296.139798.

24 Shaikh J R and Patil M K, Qualitative tests for preliminary phytochemical screening: An overview, *Int J Chem Stud*, 2020, **8**(2), 603-608, doi: 10.22271/chemi.2020.v8.i2i.8834.

25 Ahlam R, Ameerah S, Ahmed B A, Hamed A, Khalifa S M, *et al.*, Thin Layer Chromatography (TLC) and phytochemical analysis of *Moringa oleifera* methanol, ethanol, water and ethyl acetate extract, *Saudi J Med Pharm Sci*, 2019, **5**(10), 817-820, doi: 10.36348/SJMPS.2019.v05i10.002.

26 Siddiqui N, Rauf A, Latif A and Mahmood Z, Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani

drug Gul-e-Zoofa (Nepetabracteata Benth), *J Taibah Univ Med Sci*, 2017, **12**(4), 360-363, doi: 10.1016/j.jtumed.2016.11.006.

27 Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Ardekani A M, *et al.*, Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in-vitro* antiproliferative effect on hela and BT-474 Cell lines, *J Taibah Univ Med Sci*, 2014, **3**(2), 102.

28 Madaan R, Bansal G, Kumar S and Sharma A, Estimation of total phenols and flavonoids in extracts of Actaeaspicata roots and anti-oxidant activity studies, *Indian J Pharm Sci*, 2011, **73**(6), 666, doi: 10.4103/0250-474X.100242.

29 Andrews J M, Determination of minimum inhibitory concentrations, *J Antimicrob Chemother*, 2001, **48**(suppl_1), 5-16, doi: 10.1093/jac/48.suppl_1.5.

30 Salem W, Leitner D R, Zingl F G, Schratter G, Prassl R, *et al.*, Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli*, *Int J Med Microbiol*, 2015, **305**(1), 85-95, doi: 10.1016/j.ijmm.2014.11.005.

31 Balouiri M, Sadiki M and Ibnsouda S K, Methods for *in-vitro* evaluating anti-microbial activity: A review, *J Pharm Anal*, 2016, **6**(2), 71-79, doi: 10.1016/j.jpha.2015.11.005.

32 Ara I, Shinwari M M, Rashed S A and Bakir M A, Evaluation of anti-microbial properties of two different extracts of *Juglans regia* tree bark and search for their compounds using gas chromatography-mass spectrum, *Int J Biol*, 2013, **5**(2), 92, doi: 10.5539/ijb.v5n2p92.

33 Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, *et al.*, Determination of anti-oxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*, *Molecules*, 2022, **27**(4), 1326, doi: 10.3390/molecules27041326.

34 Akar Z, Küçük M and Doğan H, A new colorimetric DPPH scavenging activity method with no need for a spectrophotometer applied on synthetic and natural anti-oxidants and medicinal herbs, *J Enzyme Inhib Med Chem*, 2017, **32**(1), 640-647, doi: 10.1080/14756366.2017.1284068.

35 Jaber S A, *In vitro* alpha-amylase and alpha-glucosidase inhibitory activity and *in vivo* anti-diabetic activity of *Quercus coccifera* (Oak tree) leaves extracts, *Saudi J Biol Sci*, 2023, **30**(7), 103688, doi: 10.1016/j.sjbs.2023.103688.

36 Poovitha S and Parani M, *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.), *BMC Complement Altern Med*, 2016, **16**, 1-8.

37 Heinrich M, Mah J and Amirkia V, Alkaloids used as medicines, Structural phytochemistry meets biodiversity—An update and forward look, *Molecules*, 2021, **26**(7), 1836, doi: 10.3390/molecules26071836.

38 Ullah A, Munir S, Badshah S L, Khan N, Ghani L, *et al.*, Important flavonoids and their role as a therapeutic agent, *Molecules*, 2020, **25**(22), 5243, doi: 10.3390/molecules25225243.

39 Kilcoyne M and Joshi L, Carbohydrates in therapeutics, *Cardiovasc Hematol Agents Med Chem*, 2007, **5**(3), 186-97, doi: 10.2174/187152507781058663.

40 Chung K T, Wong T Y, Wei CI, Huang Y W, Lin Y, *et al.*, Tannins and human health: A review, *Crit Rev Food Sci Nutr*, 1998, **38**(6), 421-464, doi: 10.1080/10408699891274273.

41 Hussen E M and Endalew S A, *In vitro* anti-oxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*, *BMC Complement Med Ther*, 2023, **23**(1), 146, doi: 10.1186/s12906-023-03923-y.