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Artículos originales

Therapeutic Potential of Fabaceae Species: A Phytochemical and Bioactivity Investigation

Potencial terapéutico de las especies de Fabaceae: Una investigación fitoquímica y de bioactividad

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Conflict of interests

The authors declare no conflict of interest

Resumen

Introducción: La creciente prevalencia de enfermedades crónicas y dolencias infecciosas requiere la exploración de nuevos agentes terapéuticos. Este estudio tiene como objetivo elucidar la composición fitoquímica y las actividades biológicas de especies seleccionadas de Fabaceae.

Métodos: El potencial antioxidante se evaluó con el ensayo DPPH, mientras que la protección del ADN y la actividad antidiabética se probaron mediante ensayos de inhibición. Los potenciales anti-leishmania y anti-dengue se evaluaron contra *Leishmania* major y *Aedes aegypti*.

Resultados: *Sophora mollis*, *Mucuna pruriens* y *Sesbania sesbans* exhibieron el mayor contenido de fitoquímicos. El poder de captura de DPPH de *M. pruriens* fue el más alto (14,09 ± 3,60 µg/ml), y la capacidad de protección del ADN de los extractos de metanol de *Pueraria tuberosa* fue la más alta. El extracto metanólico de *S. sesban* efectivamente inhibió la leishmaniasis (82,99 % de inhibición) y el dengue. (70,10 ppm LD50). Mediante GC/MS se identificó 37 compuestos, con predominancia de 9-octadecenamida (Z) y tetradecanamida.

Conclusión: Los extractos de hojas en metanol de especies seleccionadas de Fabaceae revelaron propiedades biológicas significativas en comparación con los extractos de metanol-DMSO y metanol-glicerol. Además, llevar a cabo los efectos sinérgicos de los compuestos antileishmaniales, anti-dengue, antidiabéticos y antioxidantes, puede conducir al desarrollo de estrategias terapéuticas más efectivas y completas.

Palabras clave: Fabaceae; Extractos de Plantas; Antioxidantes; Agentes Antiparasitarios

Abstract

Introduction: The increasing prevalence of chronic diseases and infectious ailments necessitates the exploration of novel therapeutic agents. This study aims to elucidate selected Fabaceae species' phytochemical composition and biological activities.

Methods: Antioxidant potential was assessed with the DPPH assay, while DNA protection and antidiabetic activity were tested via inhibition assays. Anti-leishmanial and anti-dengue potentials were evaluated against *Leishmania major* and *Aedes aegypti*.

Results: *Sophora mollis*, *Mucuna pruriens*, and *Sesbania sesbans* exhibited the highest phytochemical content. The DPPH-scavenging power of *M. pruriens* was highest (14.09 ± 3.60 µg/ml), and the DNA protection ability of *P. tuberosa* methanol extracts was highest. The methanol extract of *S. sesbans* effectively inhibited leishmaniasis (82.99 % inhibition) and dengue (70.10 ppm LD50). GC/MS identified 37 compounds, with 9-octadecenamide (Z) and tetradecanamide predominating.

Conclusion: The methanol leaves extracts of selected Fabaceae species revealed significant biological properties compared to the methanol-DMSO and methanol-glycerol extracts. Furthermore, carrying out the synergistic effects of antileishmanial, anti-dengue, antidiabetic, and antioxidant compounds, may lead to the development of more effective and comprehensive therapeutic strategies.

Keywords: Fabaceae; Plant Extracts; Antioxidants; Antiparasitic Agents

Highlight

This study evaluates the phytochemical composition and biological activities of selected Fabaceae species, including antioxidant, DNA protection, antidiabetic, anti-leishmanial, and anti-dengue properties.

Sophora mollis, *Mucuna pruriens*, and *Sesbania sesbans* exhibited significant bioactivity, with *M. pruriens* showing the highest antioxidant activity and *S. sesbans* demonstrating strong anti-leishmanial (82.99 %) and anti-dengue (70.10 ppm LD50) effects.

GC/MS analysis identified 37 bioactive compounds, with 9-octadecenamide (Z) and tetradecanamide predominating. These findings underscore the therapeutic potential of these species, offering a foundation for integrated antiviral, antidiabetic, and antioxidant strategies.

Introduction

Oxygen is an essential component of life. However, excessive production of reactive oxygen species (ROS), such as hydroxyl radical (OH-) and nitric oxide (NO), along with alterations in DNA and protein, may cause oxidative stress [1]. If affected cells fail to repair completely, then it may cause chronic diseases like diabetes, Alzheimer's disease and ageing. The plants can scavenge ROS due to the compounds and antioxidant molecules, including polyphenols that can scavenge free radicals, chelating free metals, thereby protecting the integrity of cell membranes and free-radical mediated oxidative stress[2].

Besides these, investigations on the antidiabetic activities of wild plants have also gained considerable attention due to their strong potential as natural antidiabetic agents. [1] As the mechanisms of these diseases are complex, the rate of response of synthetic drugs is low and more likely to cause adverse effects. Hence, it is necessary to explore novel plants for treating such diseases in which immune responses are vital for disease development[3].

The Fabaceae family is second to Graminae, with approximately 750 genera and includes various economically and medicinally important flowering plants. In this context, some studies have highlighted the biological significance of Fabaceae species. For instance[4] , proposed antimicrobial, hypoglycemic, anti-tumour, antioxidant and anti-Parkinson properties of *Mucuna pruriens* seed extract. Similarly, *Lablab purpureus* leaves and flowers treat cholera, diarrhea, nausea, inflammations and uterus inflammation[5,6], *Pueraria tuberosa* and *Sesbania sesbans* are used in traditional medicines to cure blood and urinary diseases and as cardiotonic, demulcent, anthelmintic, diuretic and galactagogue[7,8]. Anti-mutagenic effects of *P. tuberosa* have been described, and [9] flavonoids' presence has been attributed to them. Furthermore, the authors [10] revealed the antibacterial, cytotoxic, antipyretic, analgesic and anti-tumour potential of different compounds extracted from *Sophora mollis*.

Hence, the objectives of the current study were to prepare the methanol, methanol-dimethyl sulfoxide (DMSO) and methanol-glycerol extracts of fresh leaves of five Fabaceae species commonly grown in Pakistan. The five species were: *M. pruriens*, *S. sesbans, S. mollis*, *P. tuberosa* and *L. purpureus*. Subsequently, the phytochemical content, antioxidant, DNA damage protection, anti-diabetic, anti-leishmanial and anti-dengue potential of the selected leaf extracts was determined using standard bioassays. Lastly, the concentration of different hydrocarbons, fatty acids and esters was evaluated using GC/MS method.

Methods

Extracts preparation

Fresh leaves of five selected species were collected and their accession numbers were assigned from the Herbarium of the National Agriculture Research Centre (NARC), Islamabad. The extracts were weighed to determine the extract yield (Table 1).

Table 1. Selected Fabaceae species, their accession numbers, extract yield and acronyms.

Plant species	Accession numbers	Methanol extracts		Methanol-DMSO extracts		Methanol-Glycerol extracts	
		Yield	Acronym	Yield	Acronym	Yield	Acronym
***Mucuna pruriens* (Linn.) DC.**	RAW101497	24.25	MPM	27.10	MPMD	50.50	MPMG
***Sesbania sesbans* (L.) Merrill**	RAW101498	72.35	SSM	43.65	SSMD	97.40	SSMG
***Sophora mollis* (Royle) Baker**	RAW101499	29.75	SMM	33.00	SMMD	72.50	SMMG

Plant species	Accession numbers	Methanol extracts		Methanol-DMSO extracts		Methanol-Glycerol extracts	
		Yield	Acronym	Yield	Acronym	Yield	Acronym
***Pueraria tuberosa* (Roxb. ex Willd.) DC.**	RAW101500	27.60	PTM	39.00	PTMD	92.00	PTMG
***Lablab purpureus* (L.) Sweet**	RAW101501	31.50	LPM	39.75	LPMD	72.25	LPMG

*DMSO: Dimethyl sulfoxide; The extract yield was measured in percentage (%).

Determination of phytochemicals

For total phenolic contents (TPC), total flavonoid contents (TFC) and total alkaloid contents (TAC), the procedure was the same as the previously reported method(11,12, 13).

Antioxidant assay

In this assay, 0.1 mM DPPH solution was made by adding 3.94 mg of DPPH in 100 mL of methanol, and the absorbance (0.98 ± 0.02) was set at 517 nm. The procedure was followed as per the previously reported method[14].

DNA damage protection assay

Briefly, 14 mg of iron sulphate heptahydrate (FeSO4.7H2O) was added to 25 ml of distilled water (dH2O), and 0.2 µg/µl pBR322 plasmid was diluted using 50 mM of phosphate buffer saline (PBS). Then, 3 µl of pBR322 plasmid DNA was added in Fenton reagent and plant extracts, and the volume was made up to 15 µl using dH2O. The reaction mixture was incubated (30 min) at 37 °C, and then 1 % agarose gel was prepared. Subsequently, 5 µl of ethidium bromide (EtBr) was added to the gel and transferred to the gel tank, using 1x Tris-borate EDTA (TBE) as a running buffer. The reaction mixtures (10 µl) were loaded on the gel, and 2 µL of loading dye and electrophoresis were conducted. After 20-30 min, DNA bands were observed under UV light[15].

Antidiabetic assays

α-amylase inhibition activity and α-glucosidase inhibition activity

The α-amylase inhibition activity and α-glucosidase inhibition activity were performed per the reported method[16,17].

Anti-leishmanial assay

For parasites culture, *L. major* isolates were obtained from the Department of Zoology, University of Peshawar (Pakistan) and grown in RPMI-1640 culture medium with 10 % heat-inactivated fetal calf serum (HIFCS) in the presence of penicillin and streptomycin solution (100 µg/mL each) at 23 °C. The MTT (3-(4,5-dimethylthiazole-2yl)-2,5-diphenyltetrazolium bromide) assay was done per the previously reported protocol[18].

Anti-dengue assay

A. aegypti larvae were collected with an aquatic net and then transferred to the laboratory. The larvae were fed dog biscuits and yeast powder (3:1) and maintained at 28 ± 2 °C. Afterwards, the 4th instar larvae were visually detected by a relatively larger size, and larvicidal activity was performed following the WHO protocol[19]. Finally, % mortality and LD50 values were determined.

Gas chromatography-mass spectrometry (GCMS) analysis

For GC/MS, 70 µL of plant oil was accumulated by adding 10 grams of powdered plant material in 100 mL of ultrapure dH2O and then performing microwave-assisted hydro-distillation for 40 min. GCMS instrument (Agilent technologies – GC7890B and MS5977A) was equipped with DB-5MS fused capillary column. Various compounds were recognized with the help of NIST and WILEY database[20,21].

Statistical analysis

All assays were performed twice and mean ±SD were calculated. The least significant difference (LSD) was observed using Statistix 8.1, and a *P*-value (< 0.05) was used to establish statistical significance.

Results and Discussion

Plant extraction

The polarity of extraction solvents plays a critical role in plants' biological activities[22]. In this study, DMSO and glycerol were chosen as green solvents in combination with the strongly polar solvent (methanol) to determine the phytochemicals and biological activities of five species.

Total phenolics, flavonoids and alkaloids content

Our study showed a large variation in TPC, TFC and TAC among selected extracts. TPC and TFC were observed highest in *S. mollis* (32.35 ± 0.19 mg gallic acid equivalent/g and 15.00 ± 0.78 mg rutin equivalent/g) and *M. pruriens* (31.62 ± 0.22 mg GAE/g and 16.66 ± 0.78 mg RE/g) methanol extracts while lowest in the methanol-glycerol extracts of *P. tuberosa* (7.75 ± 0.26 mg GAE/g and 3.33 ± 0.78 mg RE/g) and *M. pruriens* (8.05 ± 0.07 mg GAE/g and 3.05 ± 0.39 mg RE/g) (Figure 1).

Similarly, the highest TAC was also recorded in SMM (9.61 ± 0.54 mg AE/g) and MPM (9.61 ± 0.54 mg AE/g) and lowest in MPMG (2.69 ± 0.54 mg AE/g) and PTMG (3.07 ± 0.00 mg AE/g). However, all phytochemical contents were found in descending order of methanol extracts > methanol-DMSO extracts > methanol-glycerol extracts.

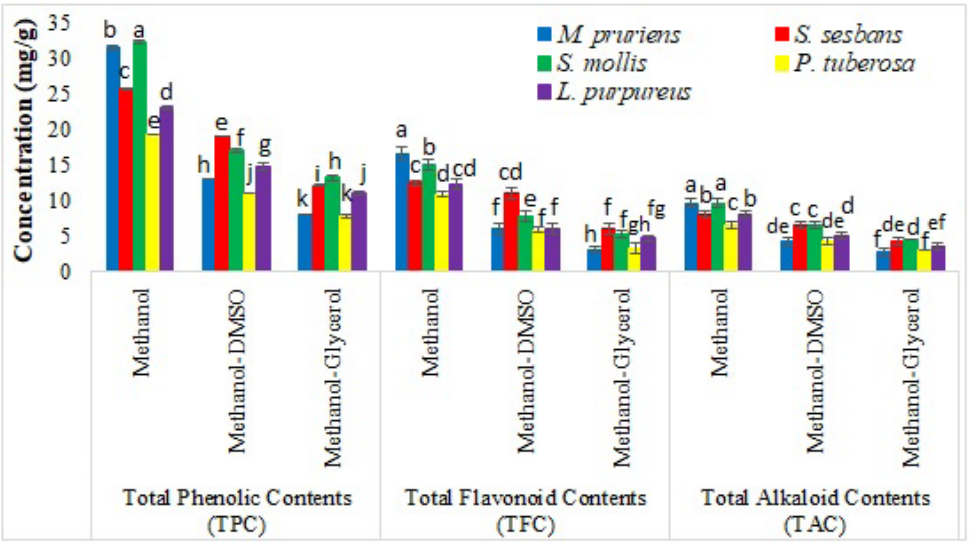

Figure 1. Phytochemicals observed in five Fabaceae extracts. Data represents mean ± SD and alphabetical values (a-k) are significantly different by $P < 0.05$.

Antioxidant activity

The antioxidant activity revealed DPPH activity in descending order of methanol extracts > methanol-DMSO extracts > methanol-glycerol extracts. The lowest IC50 value was recorded in MPM, *i.e.* 14.09 ± 3.60 µg/mL, indicating the highest scavenging activity, while the highest IC50 value was found in PTMG, *i.e.* 1772.66 ± 10.01 µg/mL showing the lowest antioxidant capacity (Figure 2a). The correlation test showed that DPPH activity was strongly correlated with TPC (r = 0.845), TFC (r = 0.842) and TAC (r = 0.842) (Figure 2b). It can be suggested that the DPPH scavenging activity of leaf extracts is directly associated with the concentration of phenolic, flavonoid and alkaloid content in leaves. Similarly, our results are comparable to the other studies that TPC and DPPH activity were 10.96 ± 0.21 mg GAE/g and 61.51 ± 0.33 % in *S. sesbans*, 2.89 ± 0.01 mg GAE/g and 28.21 ± 1.29 % in *L. purpureus* and 39.96 ± 0.00 mg GAE/g and 40-60 % in *M. pruriens* grown in Thailand and India(23, 6).

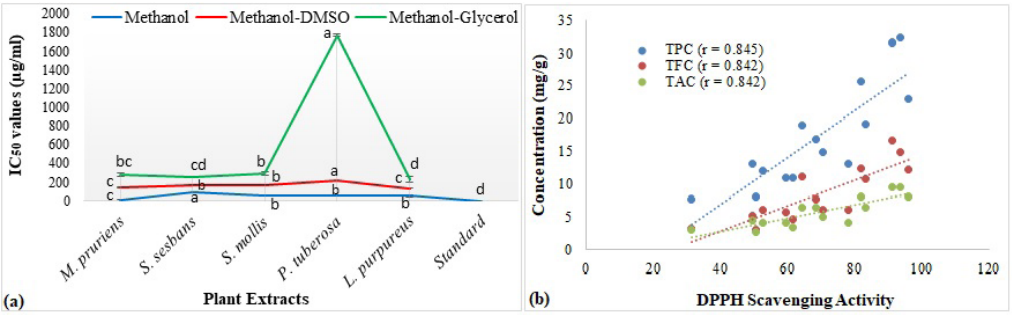

Figure 2. Antioxidant activity of plant extracts assessed at different concentrations and its correlation with phytochemicals (a) IC50 values determined in DPPH assay (b) Correlation between DPPH activity and phytochemicals.

DNA damage protection assay

In this assay, untreated plasmid expressed two bands while strand scission was observed on treating DNA with Fenton reagent. The gel photographs showed that PTM (3631.88) and SSMD (2050.85) exhibited highest protection, whereas SSM (1283.59), MPM (1088.60), SMM (1044.59), PTMD (886.41) and SSMG (211) possesses lowest protection against oxidative DNA damage, as corroborated by densitometric analysis (Figure 3b). However, all other extracts were found to be ineffective in protecting DNA from damage (Figure 3a). In the current study, methanol extracts protected DNA by scavenging the oxidation products that damage the DNA. It can be inferred that the abundance of TPC, TFC and TAC present in leaves acted directly on the oxidative agents and prevented the chain reaction of oxidative stress which alleviated oxidative damage in the methanol extracts.

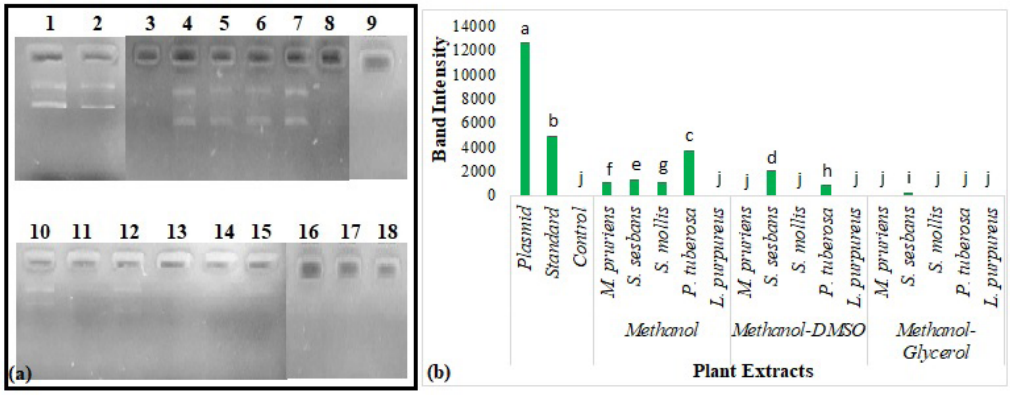

Figure 3. Effects of Fabaceae extracts showing DNA damage protection ability (a) Gel photograph displaying DNA bands (Lane 1: plasmid; Lane 2: plasmid + reagent + standard; Lane 3: plasmid + reagent; Lane 4: plasmid + reagent + MPM; Lane 5: plasmid + reagent + SSM; Lane 6: plasmid + reagent + SMM; Lane 7: plasmid + reagent + PTM; Lane 8: plasmid + reagent + LPM; Lane 9: plasmid + reagent + MPMD; Lane 10: plasmid + reagent + SSMD; Lane 11: plasmid + reagent + SMMD; Lane 12: plasmid + reagent + PTMD; Lane 13: plasmid + reagent + LPMD; Lane 14: plasmid + reagent + MPMG; Lane 15: plasmid + reagent + SSMG; Lane 16: plasmid + reagent + SMMG; Lane 17: plasmid + reagent + PTMG; Lane 18: plasmid + reagent + LPMG) (b) Densitometry calculation of DNA bands.

α-Amylase and α-glucosidase inhibition assays

In current study, α-amylase assay revealed lowest IC50 value in SMM (10.62 ± 7.71 µg/mL) and SSM (17.01 ± 11.94 µg/mL) indicating highest α-amylase inhibition potential (Figure 4a). In α-glucosidase assay, highest activity was recorded in the methanol-glycerol and methanol-DMSO extracts of *S. sesbans* (*i.e.* 0.02 ± 0.01 µg/mL and 0.03 ± 0.03 µg/mL IC50 values). In contrast, lowest α-glucosidase inhibitory potential was found in all extracts of *P. tuberosa* (IC50 values 13.82 to 26.36 µg/mL) (Figure 4c). Comparatively, α-amylase activity was significantly correlated with TPC (r = 0.490) and TFC (r = 0.470) as compared to the TAC (r = 0.394) (Figure 4b). Likewise, α-glucosidase inhibitory activity also depicted moderately positive correlation with TPC (r = 0.497), TFC (r = 0.446) and TAC (r = 0.483) (Figure 4d). The r value of 0.4 indicates a moderately positive correlation but not a stronger one. The results further corroborate previous studies[22] that elucidated > 80 % α-glucosidase inhibitory activity in *S. sesbans* and *L. purpureus* extracts. Similarly, Gulati et al. [4] documented significantly lower IC50 values for both α-amylase (< 25 µg/mL) and α-glucosidase (< 5 µg/mL) inhibitory activities in *M. pruriens* grown in Ethiopia.

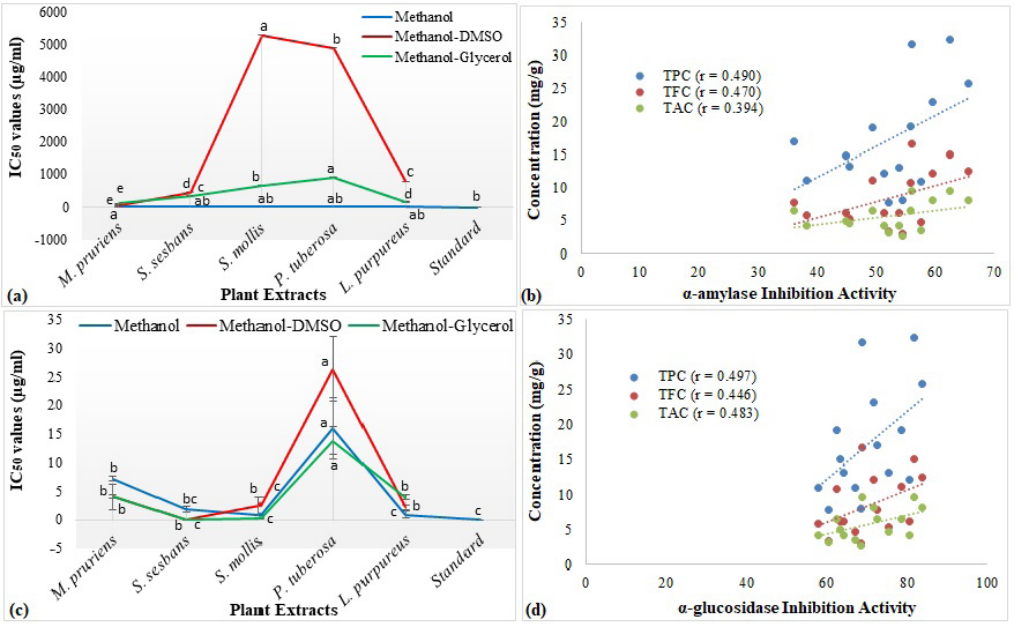

Figure 4. Antidiabetic activity of plant extracts and its correlation with phytochemicals (a) α- amylase inhibition assay (b) Correlation of α- amylase inhibition activity with phytochemicals (c) α- glucosidase inhibition assay (d) Correlation of α- glucosidase inhibition activity with the phytochemicals. * r is correlation coefficient and IC50 stands for half-maximal inhibitory concentration. * Acarbose was used as a standard.

Anti-leishmanial and anti-dengue assays

All examined species showed significant biological activities in their methanol extracts. Hence, the methanol extracts were further tested to examine anti-leishmanial and anti-dengue potential of these species. The anti-leishmanial activity was found in descending order of *S. sesbans* (82.99 % inhibition) > *L. purpureus* (69.31 % inhibition) > *M. pruriens* (68.85 % inhibition) > *P. tuberosa* (66.92 % inhibition) > *S. mollis* (36.27 % inhibition) (Table 2). In the anti-dengue assay, only *S. sesbans* and *L. purpureus* were found to be effective in controlling the growth of *A. aegypti* as they displayed LD50 values of 70.10 and 200.00 ppm, respectively (Table 3).

Table 2. Percentage inhibition and IC50 values of methanol leaves extracts as determined in anti-leishmanial activity.

Plant extracts	Percentage inhibition observed at different concentrations			IC_{50} values (µg/ml)
	250 µg/ml	500 µg/ml	1000 µg/ml	
M. pruriens	33.11	39.78	68.85	566.10
S. sesbans	21.59	59.10	82.99	397.70
S. mollis	6.99	17.74	36.27	1368.00

Plant extracts	Percentage inhibition observed at different concentrations			IC_{50} values (µg/ml)
	250 µg/ml	500 µg/ml	1000 µg/ml	
P. tuberosa	25.45	40.17	66.92	617.20
L. purpureus	21.56	49.61	69.31	514.70

*IC50: Half-maximal inhibitory concentration.

Table 3. Larvicidal activity examined against dengue vector *Aedes aegypti* L. after exposure to the selected methanol extracts.

Plant Extracts	Percentage mortality at different concentrations (ppm)			R-square	LD_{50}	95 % CI
	50	100	200			
M. pruriens	0	0	0	-	-	-
S. sesbans	40	60	80	0.99	70.10	38.62 - 127.24
S. mollis	0	0	0	-	-	-
P. tuberosa	0	0	0	-	-	-
L. purpureus	0	30	50	1.00	200.00	105.37 - 379.60
Standard (Permethrin)	60	80	100	1.00	37.09	20.02 - 68.71

LD50: Lethal dose; CI: Confidence interval.

Previously[24], determined anti-plasmodial flavones from the roots of *S. mollis*. Hence, the antileishmanial activity of leaves examined in the current study was ineffective in inhibiting leishmanial parasites' growth. The current study confirmed the parasite inhibitory potential of *M. pruriens* using *L. major* promastigotes.

The decreased parasite growth can be ascribed to the increased production of ROS and reduced level of arginase that can be metabolized to nitric oxide (NO), a microbicidal agent responsible for the intracellular parasite removal[25].

GC/MS analysis

A total of 37 compounds, *viz.* hydrocarbons, fatty acids, alcohols, esters and carbohydrates, were identified, and differences in their peak area were observed in the GC/MS chromatograms. Among these, 9-octadecenamide, (Z) and tetradecanamide were found to be dominant compounds ranging from 11.94 to 63.89 % in all species. Similarly, 13-Docosenamide was also present in higher concentration (20.87 %) in *L. purpureus* only. However, all other compounds were found < 12 % as shown in Table 4.

Table 4. Concentration (%) of compounds observed in five Fabaceae species using GCMS method.

Names of Compounds	CC	Concentration (%)				
		M. pruriens	*S. sesbans*	*S. mollis*	*P. tuberosa*	*L. purpureus*
α-D-glucopyranoside, methyl	C9	-	-	1.68	-	10.19
o- Xylene	C4	-	-	1.48	0.96	-
p-Xylene	C4	3.46	1.46	0.78	-	3.76
1-Cyclohexene, 1-ethynyl	C3	-	0.47	-	-	-
1-Docosene	C3	-	-	-	-	0.40
1-Heptanol, 6-methyl	C7	-	-	0.51	-	0.69
2-Amino-5-methylamino-1,3,4-thiadiazole	C4	-	-	-	-	1.11
2-Cyclohexen-1-one, dimethyl-	C3	-	0.38	-	-	-
2-O-Methyl-D-mannopyranosa	C10	-	-	1.65	-	-
2-Pentanol, acetate	C6	8.92	-	8.03	2.46	-
3-Ethyl-2,6,10-trimethylundecane	C1	-	-	1.17	-	-
5-methyl-5-propyl, Nonane	C1	-	-	-	-	0.63
8-Methylnonanoic acid	C5	4.76	-	-	-	-
9-Octadecenamide, (Z)	C12	57.76	63.36	62.46	63.89	43.54
13-Docosenamide	C12	-	-	-	-	20.87
16-Hexadecanoyl hydrazide	C5	-	-	-	-	0.03
Acetic acid, hydrazide	C5	-	4.14	-	-	-
Bicyclo[2.1.1]hexan-2-ol, 2-ethenyl-	C2	10.15	-	-	-	-
Butyric acid hydrazide	C5	-	-	-	-	3.75
Carbamodithioic acid, phenyl-, methyl ester	C13	-	0.06	-	-	-
cis-11-Eicosenamide	C12	-	-	-	12.03	-
Cyclohexane	C2	-	-	-	-	1.63
Cyclohexane, 1,1-dimethoxy	C2	-	2.09	1.51	-	-
Cyclohexanone	C2	-	2.39	2.30	1.66	-
Dodecane, 2,6,10-trimethyl	C1	-	0.78	-	-	-
Glycoaldehyde dimer	C10	-	-	-	-	0.37
Heptane, 3,4-dimethyl-	C1	-	-	0.74	-	-
Hexadecanal	C11	-	-	0.86	-	-
Hexadecanoic acid, 15-methyl-, methyl ester	C5	-	0.55	-	-	-
Hydrazinecarboxamide	C13	-	-	0.71	-	-

Names of Compounds	CC	Concentration (%)				
		M. pruriens	*S. sesbans*	*S. mollis*	*P. tuberosa*	*L. purpureus*
Nonane, 3,7-dimethyl-	C1	-	-	-	0.39	-
Nonyl chloroformate	C6	-	0.38	-	-	-
Oxirane, hexadecyl	C8	-	-	-	-	0.84
Pentadecanal	C11	-	0.80	2.57	-	-
Phenol, 2,4-bis(1,1-dimethylethyl)-	C4	-	0.47	-	-	-
Sulfurous acid, hexyl octyl ester	C6	-	-	-	1.07	-
Tetradecanamide	C12	14.94	34.56	13.45	17.53	11.94

*Compounds are listed in alphabetical order; CC: Chemical class; C1: Linear alkanes; C2: Cycloalkanes; C3: Unsaturated hydrocarbons; C4: Aromatic/heterocyclic hydrocarbons; C5: Fatty acids; C6: Esters; C7: Alcohols; C8: Cyclic ether; C9: Carbohydrates; C10: Aldehyde; C11: Fatty aldehyde; C12: Fatty amides; C13: Other compounds.

The 9-octadecenamide (Z) is used as a hypolipidemic agent and for treating atherosclerosis, while tetradecanamide is considered for anti-mycobacterial and anti-tubercular activities[26]. Previously[27], proposed anti-leishmanial properties of 13-docosenamide, which have been detected significantly (20.87 %) in *L. purpureus* leaves. Cis-11-eicosenamide (12.03 %) was detected only in *P. tuberosa* and hexadecanoic acid-methyl ester was recorded in minor concentration in *S. sesbans* (0.55 %) only.

Conclusion

The obtained results confirmed that the extracts prepared with green solvents differed significantly in their chemical composition, which is directly related to their biological activities. It can be concluded that the strongly polar i.e. methanol extracts of *S. mollis, M. pruriens* and *S. sesbans* exhibited the strongest degree of biological activities due to the presence of the highest amount of phenolic, flavonoid and alkaloid contents. The methanol extract of *M. pruriens* displayed the highest antioxidant potential, *P. tuberosa* showed the highest DNA protection ability, and *S. sesbans* revealed the highest anti-leishmanial and anti-dengue potential. Thus, their methanol extracts could be promising candidates as natural bioactive agents in relevant fields, yet the antidiabetic activities in animal models need to be studied.

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