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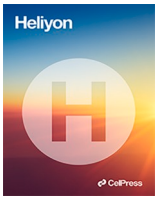

Research article

Green solvent-based extraction of three Fabaceae species: A potential antioxidant, anti-diabetic, and anti-leishmanial agents

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ABSTRACT

The Fabaceae is renowned for its diverse range of chemical compounds with significant biological activities, making it a valuable subject for pharmacological studies. The chemical composition and biological activities of three Fabaceae species were investigated using methanol separately and in combination with dimethyl sulfoxide (DMSO) and glycerol for extraction. The results revealed the highest phenolic (49.59 ± 0.38 mg gallic acid equivalent/g), flavonoid (29.16 ± 0.39 mg rutin equivalent/g), and alkaloid (14.23 ± 0.54 mg atropine equivalent/g) contents in the *Caesalpinia decapetala* methanol extracts. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and DNA protection activity were the highest (0.88 ± 0.43 μg/mL IC_{50} and 2149.26 band intensity) in *Albizia julibrissin* methanol extracts. The α-amylase activity was highest in all methanol extracts (<15 μg/mL IC_{50} values), while the α-glucosidase inhibition potential was highest (<1 μg/mL IC_{50} value) in the methanol–glycerol and methanol–DMSO extracts. Pearson coefficient analysis showed a strong positive correlation between the DPPH and α-amylase assays and phytochemicals. Anti-leishmanial activity was observed in decreasing order: *A. julibrissin* (74.75 %) > *C. decapetala* (70.86 %) > *Indigofera atropurpurea* (65.34 %). Gas chromatography-mass spectrometry revealed 33 volatile compounds and, aamong these (Z)-9-octadecenamide was detected in the highest concentration ranging from 21.85 to 38.61 %. Only the methanol extracts of the examined species could be assessed for *in vivo* studies for immediate applications.

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1. Introduction

Different factors, such as reactive oxygen species (ROS), toxins, drugs, and other pollutants, increase the concentration of free radicals in the body, which causes oxidative stress that destroys proteins, DNA, and other living cells [1]. Among these, DNA is chiefly damaged by ROS. In addition, other exogenous factors, such as UV light, ionizing radiations, alkylating agents, aflatoxins, smoking, and electrophilic reactant metabolites, threaten the integrity of the entire genome [2]. A specific interconnected network of cellular and molecular events, *i.e.*, DNA damage response (DDR), is triggered by the affected cells to maintain genomic integrity [3]. The DDR eliminates the critical conditions of the cell by sensing DNA damage and deciding the fate of the damaged cells [4]. If affected cells fail to repair completely, it can lead to mutations and other chronic diseases, such as cancer, diabetes mellitus (DM), atherosclerosis, pulmonary dysfunction, cataracts, and radiation damage [5].

Oxidative stress and other factors, such as obesity and sedentary lifestyles, may also result in the development of DM, which is a metabolic disorder caused by insulin secretion and the degradation of secreted insulin [6]. The International Diabetes Federation (IFD) reported 366 million adult diabetic cases globally in 2011, which is expected to increase to 552 million by 2030 [7]. Various synthetic anti-diabetic agents, such as acarbose, metformin, and miglitol, are available on the market. Despite this, their effectiveness is limited by side effects, including flatulence, diarrhea, heart failure, and abdominal pain [8].

Moreover, leishmaniasis has also increased the mortality rate in approximately 98 countries, putting 1.7 billion people at high risk [9]. The parasites of the genus *Leishmania* (family Trypanosomatidae) are considered the primary causal agent of leishmaniasis. This infection can be cutaneous, visceral, and muco-cutaneous. On the other hand, the mechanism of this infection is complex. Hence, the response rate of synthetic drugs is low and associated with adverse effects. Therefore, it is necessary to investigate novel plants for treating various diseases [10].

Some researchers have highlighted the biological properties of Fabaceae species that are directly associated with multifarious bioactive compounds [11–13]. *Caesalpinia decapetala* is used as an astringent and antiseptic to prevent colds and treat burns and biliousness [11]. Ochora et al. [12] and Zhang et al. [14] reported anti-plasmodial and anti-viral activities of *C. decapetala*. *Indigofera atropurpurea* flowers are used for diarrhea and dysentery [15]. Similarly, *Albizia julibrissin* is used in Chinese medicine to treat ulcers, boils, bruises, fractures, insomnia, anxiety, and hemorrhoids [13,16]. The reported pharmacological properties of *A. julibrissin* include antitumor, antidepressant, antioxidant, and immunomodulatory activities [17].

The first step in exploiting wild plants is the selection of appropriate solvents for extraction. The choice of solvent chiefly depends on worker safety (toxicity, carcinogenicity, and mutagenicity), process safety (volatility, explosiveness, and flammability), and the sustainability of the process. Hazardous organic solvents (hexane, benzene, chloroform, acetone, and petroleum ether) have been minimized recently because of their toxicity and undesirable effects on the environment and the quality and safety of food and other products [18]. Accordingly, green solvents should be non-toxic, have low volatility, and be chemically stable, easy to use, and recyclable [19].

Considering the ethno-medicinal importance of selected species and the diverse characteristics of green solvents, the present study examined the biological potential of *A. julibrissin, I. atropurpurea*, and *C. decapetala* (family Fabaceae) using methanol separately and in combination with glycerol and dimethyl sulfoxide (DMSO) for extraction. Therefore, they can be exploited at the industrial level. Subsequently, the concentration of different hydrocarbons, fatty acids, and esters was determined using gas chromatography-mass spectrometry (GC-MS).

2. Materials and methods

2.1. Extracts preparation

A. julibrissin, *I. atropurpurea*, and *C. decapetala* species were collected from Islamabad, and their voucher numbers were obtained from the Herbarium of the National Agricultural Research Center (NARC), Islamabad. Their fresh leaves were isolated, washed thoroughly, and dried in the shade at room temperature for two to three days. The leaves were powdered using a grinder (Grindomix; Retsch Technology GMbH, Germany) and extracted using a combination of different solvents, including 80 % methanol, methanol–DMSO (80:10), and methanol–glycerol (80:10). Methanol was used as a solvent to extract the hydrophilic and hydrophobic

Table 1
Accession numbers, weight of extract, and extract yield of examined species.

Plant species	Accession number	Extraction solvent	Weight of extract (g)	Extract yield (%)
Albizia julibrissin subsp. Julibrissin	RAW101502	Methanol	5.32	26.60
		Methanol–DMSO	5.80	29.00
		Methanol–glycerol	15.70	78.50
Indigofera atropurpurea Buch.-Ham.ex Horn.	RAW101503	Methanol	6.30	31.50
		Methanol–DMSO	7.95	39.75
		Methanol–glycerol	14.45	72.25
Caesalpinia decapetala (Roth) Alston	RAW101504	Methanol	7.53	37.65
		Methanol–DMSO	8.07	40.35
		Methanol–glycerol	17.72	88.70

*DMSO: Dimethyl sulfoxide.

compounds. DMSO is a polar aprotic solvent suitable for extracting compounds used for medical applications [20]. On the other hand, glycerol is used as a green extraction solvent to obtain polyphenolic-rich extracts because it can change the water polarity and extract the maximum number of active compounds [21].

The mixtures (20 gm in 200 mL each) were placed in an incubator shaker for 48 h and filtered (Whatman filter paper; Merck, Germany). Subsequently, the methanolic filtrates were placed in a hot water bath (Treviglio, Italy) at 50 °C with a temperature deviation of ±2 °C to evaporate the solvent from the methanol–DMSO and methanol–glycerol extracts under vacuum using a rotary evaporator (BUCHI Rotavapor R-220). The obtained crude extracts were weighed to determine the extract yield (Table 1). All chemicals and reagents used in the study were purchased from Sigma–Aldrich and were of analytical grade.

2.2. Phytochemical analysis

2.2.1. Total phenolic contents (TPC)

Briefly, sample (100 μL) was dissolved in 500 μL of Folin–Ciocalteu reagent, 1 mL of distilled water (dH_2O), and 1.5 mL of 20 % sodium carbonate (Na_2CO_3). The resulting solution was then incubated for 30 min at 40 °C. The absorbance was noted at 760 nm using a spectrophotometer (Shimadzu UV-1800, Japan), and the gallic acid (standard) was used to derive the calibration curve ($y = 0.0185x + 0.0293$, $R^2 = 0.998$). The results were interpreted as mg gallic acid equivalent per gram (mg GAE/g) of dry samples [22].

2.2.2. Total flavonoid contents (TFC)

For the TFC, the method reported by Park et al. [23] was followed with minor modifications. Each extract (250 μL) was added to 1.25 mL of dH_2O, 75 μL of 5 % sodium nitrite ($NaNO_2$), and 150 μL of 10 % aluminum chloride ($AlCl_3$). After 5 min, 500 μL of 1 M sodium hydroxide (NaOH) was added, and the absorbance at 510 nm was recorded. The standard curve was prepared using rutin, and the regression equation ($y = 0.0018x + 0.059$; $R^2 = 0.994$) was used to derive results in terms of mg RE/g of dried sample.

2.2.3. Total alkaloid contents (TAC)

Bromocresol green (BCG) was prepared by heating 13.96 mg of BCG with 0.6 mL NaOH and 1 mL of dH_2O and then diluted to 200 mL using dH_2O. Subsequently, BCG (5 mL) was added to 5 mL of phosphate buffer and 4 mL of chloroform, and volume was made up to 10 mL using chloroform. The absorbance was taken at 470 nm. Similarly, atropine (standard; 1 mg dissolved in 10 mL of dH_2O) was measured at different concentrations (0.4 mL–1.2 mL) taken from 1 mg/mL of plant extract to obtain the linear equation, $y = 0013x – 0.0065$ ($R^2 = 0.983$) [24].

2.3. Antioxidant assay

The DPPH scavenging method reported by Wu et al. [25] was performed to evaluate the antioxidant potential of selected plant extracts. Briefly, a 0.1 mM DPPH solution was prepared by adding 3.94 mg of DPPH to 100 mL of pure methanol to achieve an absorbance of 0.98 ± 0.02 at 517 nm. Subsequently, 1.5 mL of each extract (100, 500, and 1000 μg/mL) was added to 1.5 mL of a DPPH solution and incubated (30 min) in the dark. The absorbance was taken at 517 nm, and the activity was measured using the percentage inhibition formula. The IC_{50} values were then calculated using GraphPad Prism 5 software.

$$DPPH\ scavenging\ activity\ (\%) = Abscontrol - Abssample/Abscontrol\ x\ 100$$

2.4. DNA damage protection assay

Initially, about 14 mg of iron sulfate heptahydrate ($FeSO_4.7H_2O_2$) was added to 25 mL of dH_2O, and 0.2 μg/μL *pBR322* plasmid was diluted using 50 mM of PBS (pH 7.4). Subsequently, 3 μL of the *pBR322* plasmid was dissolved in the Fenton reagent and plant extracts and made up to the final volume of 15 μL using dH_2O. The mixture was incubated for 30 min, and 1 % agarose gel (0.3 g agarose powder was dissolved in 30 mL of 1x Tris-borate-EDTA (TBE) buffer and heated for 1 min) was then prepared. After a few minutes, 5 μL of ethidium bromide (EtBr) was added to the gel and transferred to the tank in which 1x TBE was poured as a running buffer. The reaction mixtures (10 μL) were loaded on the gel along with 2 μL of loading dye, and electrophoresis was performed using a gel electrophoresis tank (Thermo Fisher Scientific Inc. USA). Ascorbic acid was used as the standard, while *pBR322* was used as the control without a plant sample. After 20–30 min, the DNA bands were visualized under UV light, and the band intensity was determined [26].

2.5. Anti-diabetic assays

2.5.1. α-amylase inhibition activity

Initially, phosphate buffer (50 μL; 100 mM), α-amylase (10 μL; 2 U/mL), and 20 μL of each (100, 500 and 1000 μg/mL) plant extracts were incubated for 20 min at 37 °C. Subsequently, 20 μL of 1 % starch was added as a substrate and incubated for the next 30 min. Subsequently, 100 μL of 3,5-dinitrosalicylic acid (DNS) reagent (12 g sodium potassium tartrate tetrahydrate in 20 mL of 96 mM of DNS and 8 mL of 2 M NaOH) was added and placed in a water bath for 10 min at 85–90 °C. The mixture was cooled, and the absorbance (540 nm) was measured using a microplate (Bio-Rad Laboratories, Inc., CA, USA). Acarbose was used as a standard and performed similarly, replacing the plant extract. The results are expressed as % inhibition using the above equation, and then IC_{50} values were determined by plotting the % inhibition values against extract concentrations [27].

2.5.2. α-glucosidase inhibition activity

About 50 μL of phosphate buffer (100 mM) was added to 10 μL of α-glucosidase (1 U/mL) and 20 μL of plant extracts (100, 500, and 1000 μg/mL) and incubated (37 °C) for 15 min. Subsequently, 20 μL of 4-nitrophenyl β-D-glucopyranoside (*p*-NPG; 5 mM) was added and incubated for 20 min. Subsequently, Na_2CO_3 (50 μL) was added, and the absorbance was assessed at 405 nm. Acarbose was measured as the standard. The control was measured by performing a similar reaction without a plant sample. Finally, % inhibition and IC_{50} values were determined [28].

2.6. Anti-leishmanial assay

Leishmania major parasites were taken from the Department of Zoology, University of Peshawar, Pakistan, and grown in RPMI-1640 culture medium with 10 % heat-inactivated fetal calf serum (HIFCS) and 100 μg/mL of each penicillin and streptomycin at 23 °C. In the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, *L. major* promastigotes were seeded in microtiter plates in RPMI-1640 and 10 % HIFCS and incubated for 72 h along with different concentrations of plant extracts at 25 ± 1 °C. After the incubation period, 100 μL of a 5 mg/mL MTT solution was added to each well and incubated for 4 h at 25 ± 1 °C. Subsequently, 100 μL of 50 % isopropanol and 10 % sodium dodecyl sulfate (SDS) were added, and the absorbance was noted at 570 nm [29].

2.7. Gas chromatography-mass spectrometry (GC-MS) analysis

Ten (10) g of powdered plant material was added to 100 mL of ultrapure dH_2O and subjected to microwave-assisted hydrodistillation for 30 min. The GC-MS instrument (Agilent Technologies -GC7890B and MS5977A) had a DB-5MS fused capillary column with 30 × 0.25 mm ID dimensions and 0.25 μm film thickness. The initial temperature was 50 °C, which was increased to 250 °C at 5 °C/min. The helium gas flow rate was 1.22 mL/min, and 1 μL sample was injected into the column with an 82.7 kPa pressure. The injector temperature was adjusted at 250 °C while the ion-source temperature was set at 280 °C. The detector was adjusted at 35–500 mass scan (*m/z*) with a 1666 scanning speed and a 42 min total GC running time. The compounds were identified using NIST and WILEY databases [30,31].

2.8. Statistical analyses

All experiments were performed twice, and the mean ± SD was calculated. The data were analyzed using Statistix 8.1 software and compared using the least significant difference (LSD). The IC_{50} values were calculated using GraphPad Prism 5, while the intensity of DNA bands was measured using ImageJ software. Pearson correlation coefficient (PCC) analysis was conducted to observe the correlation between phytochemicals (TPC, TFC, and TAC) and different bioassays.

3. Results and discussion

3.1. Total phenolic, flavonoid, and alkaloid contents

The bioactivities of plants depend on their phytochemical richness, which affects their biological activities. Phenolic compounds exhibit cytotoxicity, allelopathy, and free-radical scavenging activities [32]. The phytochemical contents in plants also depend on the polarity of solvents used for the extraction and play a critical role in the biological activities of plants [33]. In the present study, a rational approach to identifying the active compounds from Fabaceae species was based on using methanol (strongly polar solvent) separately and in combination with DMSO and glycerol, and *in vitro* experimental studies were conducted. DMSO was selected because

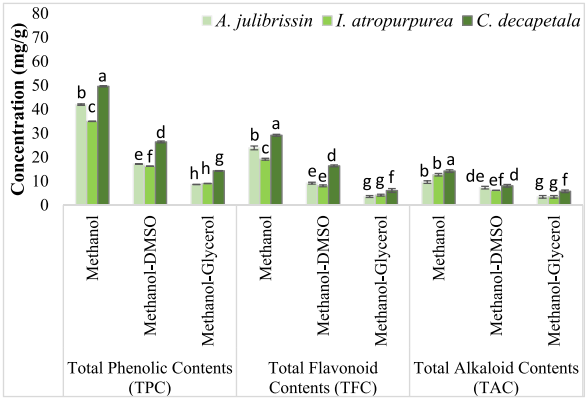


Fig. 1. Quantitative phytochemical analysis of *A. julibrissin, I. atropurpurea*, and *C. decapetala* leaves extracts. The data indicate mean ± SD and alphabetical values (a–h) are not significantly different by$P < 0.05$, as determined by LSD.

it can dissolve specific hydrophilic and hydrophobic molecules and is exploited in various pharmaceutical drugs. Similarly, glycerol is a low-cost, biocompatible, non-toxic, and water-soluble solvent [33,34].

The results revealed the highest TPC and TFC in the *C. decapetala* (49.59 ± 0.38 mg GAE/g and 29.16 ± 0.39 mg RE/g) and *A. julibrissin* (41.94 ± 0.03 mg GAE/g and 23.88 ± 0.78 mg RE/g) methanol extracts. In contrast, they were lowest in the methanol–glycerol extracts of *A. julibrissin* (8.62 ± 0.03 mg GAE/g and 3.61 ± 0.39 mg RE/g) and *I. atropurpurea* (9.02 ± 0.07 mg GAE/g and 4.16 ± 0.39 mg RE/g). Similarly, TAC was also detected in higher concentrations in the *C. decapetala* methanol extracts (14.23 ± 0.54 mg AE/g) while lowest in *A. julibrissin* and *I. atropurpurea* (3.46 ± 0.54 mg AE/g) methanol–glycerol extracts (Table S1). The phytochemicals of the abstracts were recorded in descending order: methanol extracts > methanol–DMSO extracts > methanol–glycerol extracts (Fig. 1).

The present study corroborates earlier data [35], showing a polyphenol content of 63.8 mg GAE/g in the leaves of *C. decapetala*. Similarly, Liu et al. [36] also observed the phenolic contents in the methanol extracts of *C. decapetala*, which was confirmed in the present study. On the other hand, the present study is in disagreement with the results reported by Zengin et al. [37], who determined 105.49 ± 1.24 mg GAE/g TPC and 3.33 ± 0.16 mg RE/g TFC in the methanol leaves extracts of *C. decapetala* grown in West Africa. On the other hand, the quantitative phytochemical analysis of *A. julibrissin* and *I. atropurpurea* leaves is reported for the first time. The differences in the distribution of phenolic and flavonoids were attributed to biological factors, e.g., the vegetative stage of the plant and technical factors, such as the extraction method, solvents used and their concentration. Moreover, the phenolic compounds also show variations depending on the geographical localities and harvest season [38].

3.2. Antioxidant assay

Free radicals are produced in the human body and have been implicated in various pathological manifestations. The antioxidants combat these radicals and protect people from numerous diseases. The human body neutralizes oxidative stress by producing natural antioxidants such as catalase and glutathione peroxidase. These enzymes can be taken externally through foods and supplements [39]. On the other hand, antioxidant defense mechanisms do not meet demand when ROS production is exceeded in many diseases. In this regard, the evolution of plant-based drugs has intensified because of chemical compounds such as phenolic, flavonoids, peptides, and organic acids [25,26].

The DPPH assay is one of the most common methods offering an evaluation of the antioxidant capacity of plants. The antioxidant compounds reduced the purple chromogen DPPH radical to the pale yellow hydrazine [40]. In the current study, the results showed that the methanol extracts of *A. julibrissin* had the lowest IC_{50} value (0.88 ± 0.43 μg/mL), followed by *C. decapetala* (2.19 ± 1.09 μg/mL), indicating the highest scavenging potential (Table S2). On the other hand, the methanol–glycerol extracts of *A. julibrissin* and *C. decapetala* expressed the lowest scavenging activity by displaying the highest IC_{50} values, *i.e.,* 827.13 ± 26.12 μg/mL and 358.30 ± 3.74 μg/mL. The methanol extracts of all species showed the highest activity compared to the methanol–DMSO and methanol–glycerol extracts (Fig. 2a).

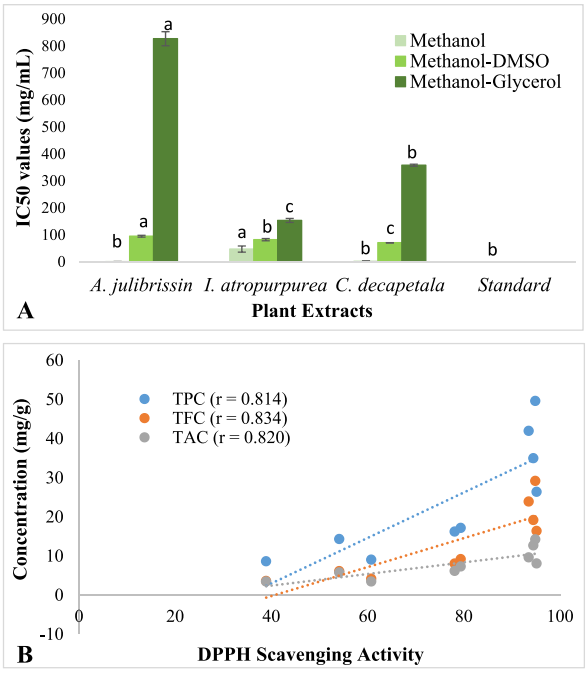


Fig. 2. DPPH scavenging activity observed in the selected species and its correlation with phytochemicals (A) IC_{50} values recorded in the DPPH scavenging activity. (B) Correlation of the DPPH activity with phytochemicals (TPC, TFC, and TAC).

This study concurs with Zengin et al. [37], who revealed a DPPH scavenging activity of 399.15 ± 0.5 μg/mL TAE/g in the *C. decapetala* leaves extract. Wei et al. [40] and Parveen et al. [41] reported the antioxidant potential in various compounds, such as quercetin and ascorbic acid, isolated from *C. decapetala*. The output of this study further corroborates previous reports [11,36,37], elucidating significant DPPH scavenging activity in *C. decapetala* and *A. julibrissin* grown in different countries. Nawinda [42] and Tamokou et al. [43] also observed DPPH scavenging activity in different species (*A. adianthifolia* and *A. anthelmintica*) of the genus *Albizia*. On the other hand, the DPPH activity of *I. atropurpurea* has been evaluated for the first time. Hence, the active compounds existing in these species contain phenolic hydroxyl groups, leading to their high DPPH scavenging abilities.

A PCC test was performed in the current study, which displayed a strong positive correlation between the DPPH activity with the phenolic (r = 0.814), flavonoid (r = 0.834) and alkaloid (r = 0.820) contents, suggesting that the phytochemicals play a vital role in antioxidant property (Fig. 2b). Studies carried out on different plant extracts [36–39] also indicated the antioxidant activities positively correlated with their phyto-constituents. The antioxidant activity in the methanol–DMSO and methanol–glycerol extracts was lower than that recorded in the methanol extract (Fig. 2a). Hence, methanol can dissolve the compounds involved in the scavenging activity. Moreover, their high antioxidant potential can be attributed to the presence of flavonoids in polar solvents because these are hydrogen donors and inhibit the production of free radicals, ultimately increasing the antioxidant activity. Therefore, the *A. julibrissin* and *C. decapetala* methanol extracts can be used as potent antioxidants to treat diseases, such as neurodegenerative disorders, inflammation, gastric ulcers, and viral infections.

* IC_{50} stands for half-maximal inhibitory concentration, and r is the correlation coefficient indicating the statistical significance *P*-values <0.5 when DPPH activity was correlated with TPC, TFC, and TAC.

3.3. DNA damage protection assay

Hydroxyl (OH^-) radicals, generally produced by the photolysis of H_2O_2, damage cellular DNA and proteins. OH^- binding to DNA modifies the nitrogenous bases and strand breakage that opens up the DNA circular structure [44]. The Fenton reagent is one of the primary physiological sources of OH^- produced near DNA molecules in the presence of copper, iron, and other metals. The oxidative DNA damage caused by these radicals may progress towards cancer. Studies have shown that polyphenol-rich diets decrease the risk of diseases by alleviating oxidative stress. The Fenton reagent is prevented by OH^- scavenging polyphenolic acids, such as chlorogenic acid, ferulic acid, caffeic acid, and flavonoids [45].

Herein, the potential of selected plant extracts to protect DNA from damage was evaluated using *pBR322* plasmid DNA. The untreated *pBR322* plasmid was in circular form (lane 1), and it was nicked to relaxed and linear DNA upon treatment with the Fenton reagent (lane 3). Nicked DNA regained its native form after treating with an ascorbic acid treatment, as shown in lane 2 (Fig. 3a). Among the selected plant extracts, the *A. julibrissin* methanol extract (2149.26), *A. julibrissin* methanol–DMSO extract (2035.26), *C. decapetala* methanol extract (1698.35), and *I. atropurpurea* methanol extract (1164.48) displayed significant DNA protection from the hydroxyl radical (*i.e.*, Fenton reagent) induced oxidative damage; this was further corroborated by densitometric analysis (Fig. 3b). On the other hand, the remaining extracts were not effective in mitigating oxidative stress because they did not have protective effects (Fig. 3a).

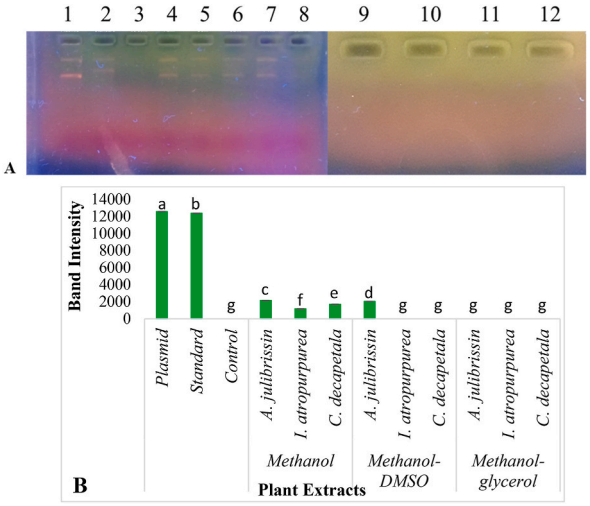


Fig. 3. DNA damage protection activity observed in selected extracts using *pBR322* plasmid DNA (A) Gel images of DNA bands visualized after applying various plant extracts (Lane 1: Control plasmid; Lane 2: plasmid + reagent + Ascorbic acid; Lane 3: plasmid + reagent; Lane 4: plasmid + reagent + *A. julibrissin* methanol; Lane 5: plasmid + reagent + *I. atropurpurea* methanol; Lane 6: plasmid + reagent + *C. decapetala* methanol; Lane 7: plasmid + reagent + *A. julibrissin* methanol–DMSO; Lane 8: plasmid + reagent + *I. atropurpurea* methanol–DMSO; Lane 9: plasmid + reagent + *C. decapetala* methanol–DMSO; Lane 10: plasmid + reagent + *A. julibrissin* methanol–glycerol; Lane 11: plasmid + reagent + *I. atropurpurea* methanol–glycerol; Lane 12: plasmid + reagent + *C. decapetala* methanol–glycerol) (B) Measurement of DNA band intensities.

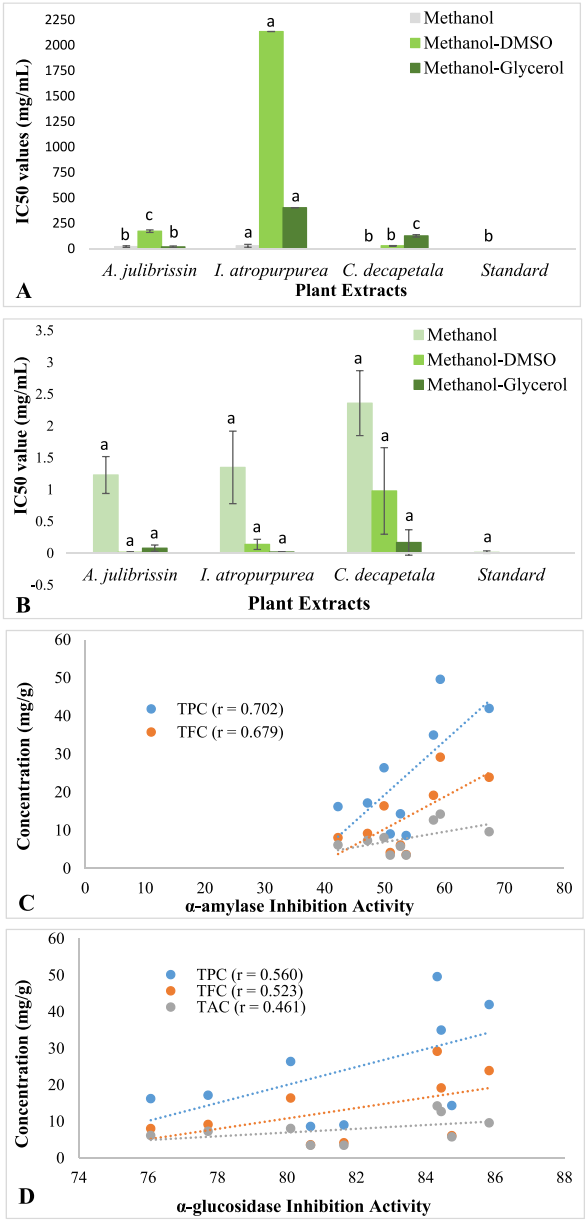


Fig. 4. Determination of the anti-diabetic potential in three Fabaceae species and its correlation with phytochemicals (A) α-amylase inhibition assay (B) α-glucosidase inhibition assay (C) Correlation of the α-amylase inhibition activity with phytochemicals (D) Correlation of the α-glucosidase inhibition activity with phytochemicals.

These results align with previous studies conducted on the DNA protective efficacy of various plant extracts [44–46]. Lin et al. [47] showed that the herbaceous plants, e.g., *Mentha arvensis, Centella asiatica*, and *Bidens alba* protected DNA from damage in human lymphocytes. Similarly, *Leonurus sibiricus* can protect and repair the DNA of Chinese hamster ovary cells exposed to H_2O_2 [48]. In the present study, all selected methanol extracts and *A. julibrissin* methanol–DMSO extract had similar effects, which can be attributed to antioxidant phytochemicals that neutralize free radical agents. The intercalation of different phytochemicals in DNA can also alter its structural integrity, disrupt the production of oxidizing species, and protect against free radical-mediated DNA damage. Hence, these extracts could be recommended for treating cancer and other oxidative stress-related diseases.

Few studies have examined the DNA damage assay of the selected species; hence, these results could be assumed to be the first report.

Table 2
Anti-leishmanial activity against *L. major* promastigotes was observed after exposure to the methanol extracts.

Plant extracts	Percentage inhibition observed at different concentrations			IC_{50} values (μg/mL)	R^2 value
	250 μg/mL	500 μg/mL	1000 μg/mL		
A. julibrissin	25.37	44.16	74.75	535.50	0.985
I. atropurpurea	27.88	45.55	65.34	578.30	0.999
C. decapetala	25.23	58.17	70.86	469.30	0.943
Standard (Miltefosine)	56.37	82.21	98.60	222.90	0.986

*IC_{50}: Half-maximal inhibitory concentration; R^2 is the coefficient of determination presenting the statistical measures of variables in a regression model.

3.4. Anti-diabetic assays

Wild plants have the potential to control hyperglycemic conditions by insulin-mimicking activity and altering glucose utilization. One of the key approaches in treating T2DM is reducing glucose absorption by decreasing the starch digestion rate and inhibiting the carbohydrate digestive enzymes, *i.e.*, α-amylase and α-glucosidase. These will inhibit the monosaccharide production resulting from the breakdown of starch and reduce glucose absorption and postprandial hyperglycemia [49]. In laboratory approaches, the enzymatic activities are assessed using spectroscopy techniques with labeled substrates, which produce a measurable signal as a color (absorbance) upon cleavage. For example, the chromogenic substrate *p*-nitrophenyl-α-*d*-glucopyranoside (pPNG) is a colorless molecule containing a D-glucose residue linked to a *p*-nitrophenol (colored) moiety using a glycosidic bond that is hydrolyzed by α-glucosidase producing a color [50].

The excessive development of ROS causes oxidative stress and suppresses the antioxidant defense system to eliminate ROS, ultimately enhancing the progression of diabetic complications such as nephropathy. During severe diabetes, metabolic abnormalities lead to mitochondrial superoxide production in cells, and subsequently, high levels of ROS also activate the processes, resulting in DNA damage. Hence, diabetes is the sequelae of enhanced oxidative stress caused by a deprived metabolism and poor glycemic control in patients [51]. In this study, the anti-diabetic activity of the selected extracts was determined by performing *in vitro* enzyme inhibition assays. The highest α-amylase activity was revealed in *C. decapetala* methanol extract, which showed a 0.47 ± 0.31 μg/mL IC_{50} value. On the other hand, the lowest activity was observed in the methanol–DMSO extract of *I. atropurpurea* (1999 ± 0.00 μg/mL IC_{50} value). Among the extracts, the methanol extracts showed the highest enzyme inhibitory potential compared to the methanol–glycerol and

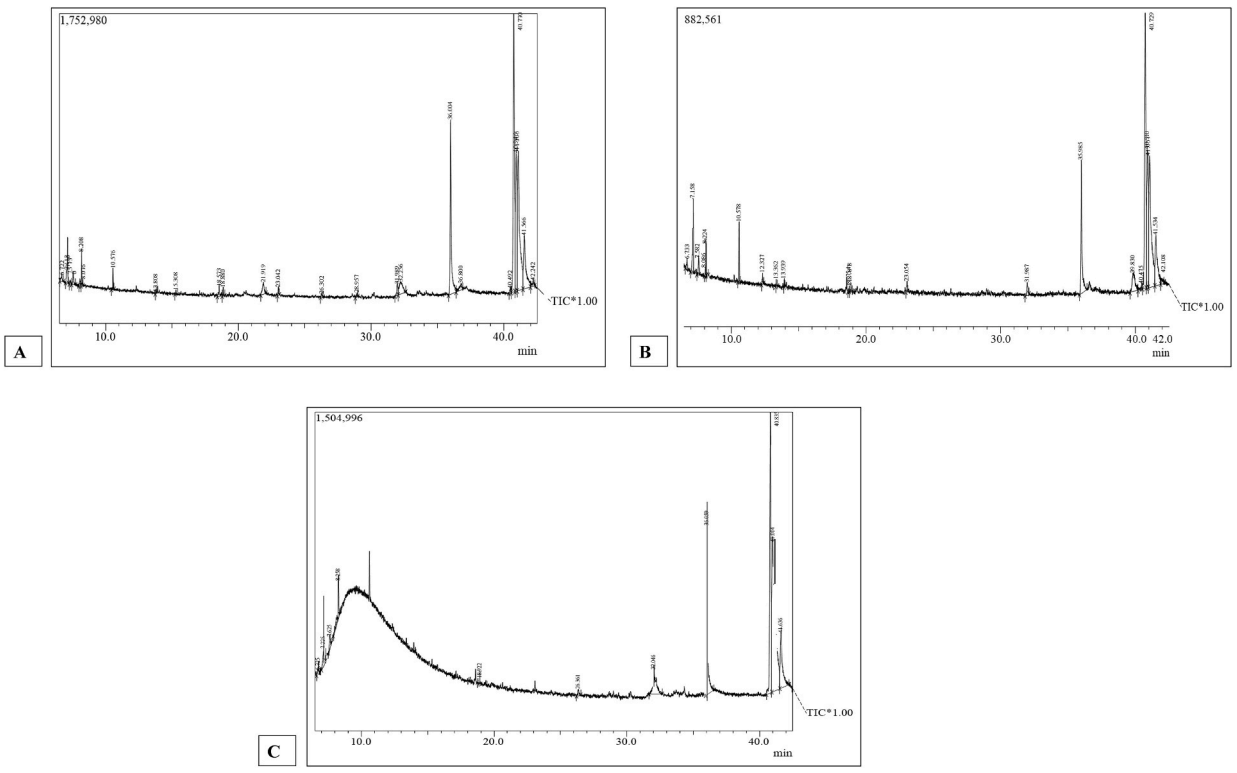
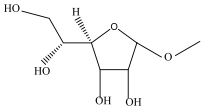
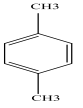
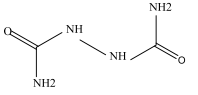
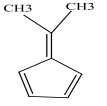
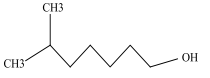
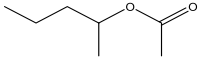
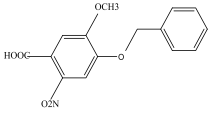
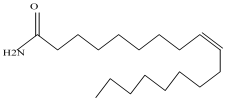
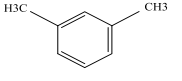


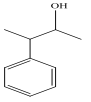
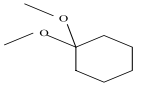
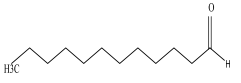
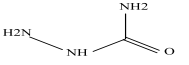
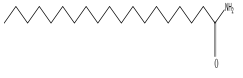
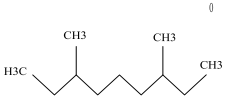
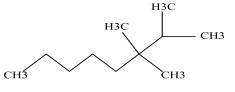
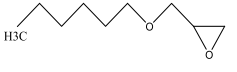
Fig. 5. GC-MS chromatograms representing the total identified compounds (TIC) in the selected species (A) *A. julibrissin*, (B) *I. atropurpurea*, and (C) *C. decapetala*.

Table 3
Different compounds observed in three Fabaceae species using the GC-MS method.

Names of Compounds	RT	CC	Structure	Concentration (%)			Biological Properties	References
				A. julibrissin	I. atropurpurea	C. decapetala		
β-D-mannofuranoside, methyl	21.91	C8		1.55	–	–	–	–
p-Xylene	7.62	C3		–	1.04	1.03	Antioxidant	[57,58]
1,2-Hydrazinedicarbox-amide	40.47	C11		–	0.83	–	–	–
1,3-Cyclopentadiene, 5-(1-methylethylidene)	8.07	C11		0.36	–	–	Antifungal	[59]
1-Heptanol, 6-methyl-	13.93	C6		–	0.22	–	–	–
2-Pentanol, acetate	7.15	C5		2.07	2.91	2.66	–	–
4-(Benzyloxy)-5-methoxy-2-nitrobenzoic acid	13.80	C3		0.11	–	–	–	–
8-Octadecenal	31.98	C9		–	0.83	–	Antibacterial, antifungal	[60]
9-Octadecenamide, (Z)	41.01	C10		21.85	22.84	38.61	Hypolipidemic, atherosclerosis	[61]
Benzene, 1,3-dimethyl-	7.57	C3		0.74	–	–	–	–

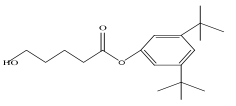
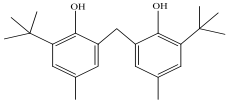
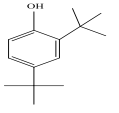
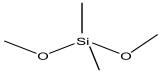
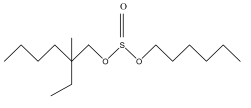
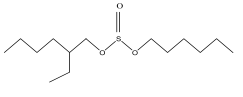
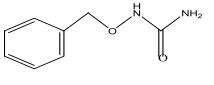
(*continued on next page*)

Table 3 (*continued*)

Names of Compounds	RT	CC	Structure	Concentration (%)			Biological Properties	References
				A. julibrissin	I. atropurpurea	C. decapetala		
Benzeneethanol, α, β-dimethyl-	8.08	C6		–	0.48	–	–	–
Cyclohexane, 1,1-dimethoxy	10.57	C2		0.60	1.69	–	–	–
Cyclohexanone	8.22	C2		1.18	1.28	2.36	Toxicological properties	[62]
Dodecanal	26.30	C9		0.29	–	–	Antimicrobial	[63]
Hydrazinecarboxamide	6.72	C11		0.56	0.55	0.61	–	–
Nonadecanamide	41.53	C10		–	8.26	–	Antimicrobial, antifungal	[64]
Nonane, 3,7-dimethyl-	12.32	C1		–	0.48	–	–	–
Octane, 2,3,3-trimethyl-	18.75	C1		–	0.28	–	Anti-inflammatory	[65]
Oxirane, hexadecyl	32.04	C7		–	–	5.55	Adhesive, antimicrobial	[66]
Oxirane, [(hexyloxy)methyl]-	42.10	C7		–	0.80	–	–	–
Oxirane, tetradecyl	26.36	C7		–	–	0.57	Antimicrobial	[67]
Pentadecanal	31.98	C9		0.95	–	–	Antimicrobial	[63]

(*continued on next page*)

Table 3 (*continued*)

Names of Compounds	RT	CC	Structure	Concentration (%)			Biological Properties	References
				A. julibrissin	I. atropurpurea	C. decapetala		
Pentanoic acid, 5-hydroxy-, 2,4-di-*t*-butylphenyl esters	18.87	C5		–	0.31	–	–	–
Phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-meth	42.24	C3		0.85	–	–	–	–
Phenol, 2,4-bis(1,1-dimethylethyl)	18.88	C3		0.36	–	0.64	Antifungal	[68]
Silane, dimethoxydimethyl-	13.36	C11		–	0.23	–	–	–
Stearic acid hydrazide	40.49	C4		0.39	–	–	Antioxidant, Antimicrobial	[69,70]
2-ethylhexyl hexyl sulfite	23.05	C5		–	0.49	–	Acidifier, arachidonic acid inhibitor	[71]
2-ethylhexyl hexyl sulfite	23.04	C5		0.41	–	–	Antibacterial	[72]
Tetradecanamide	36.00	C10		14.09	10.85	14.09	Anti-mycobacterial, anti-tubercular	[73]
Urea, (phenylmethoxy)-	7.36	C11		0.21	–	–	–	–
TIC				46.57	54.37	66.12		

*Compounds are arranged in alphabetical order. RT: Retention time; TIC: Total Identified Compounds; CC: Chemical class; C1: Linear alkanes; C2: Cycloalkanes; C3: Aromatic/heterocyclic hydrocarbons; C4: Fatty acids; C5: Esters; C6: Alcohols; C7: Cyclic ether; C8: Carbohydrates; C9: Fatty aldehyde; C10: Fatty amides; C11: Other compounds.

methanol–DMSO extracts. *I. atropurpurea* indicated the lowest activity in green-solvent extracts (Fig. 4a).

The α-glucosidase assay showed that the *A. julibrissin* methanol–DMSO extract and *I. atropurpurea* methanol–glycerol extract have the highest inhibitory potential because they showed the lowest IC_{50} values, *i.e.*, 0.01 ± 0.01 μg/mL and 0.02 ± 0.002 μg/mL (Fig. 4b). In general, all species showed the highest activity in methanol–DMSO and methanol–glycerol extracts compared to the methanol extracts (Table S3). Based on these observations, the observed inhibitory activities could be linked to the phenolic and flavonoid compounds present in the plant extracts. Therefore, the Pearson correlation test was conducted to visualize the significant relationship of phytochemicals with the anti-diabetic activity of plant extracts. Comparatively, the α-amylase activity showed strong positive correlations with TPC (r = 0.702) and TFC (r = 0.679) compared to TAC (r = 0.531) (Fig. 4c). Similarly, the α-glucosidase activity was also significantly correlated with all the phytochemicals tested (r > 0.45), as shown in Fig. 4d.

* Data indicates the mean ± SD and alphabetical values (a-c) are not significantly different by $P < 0.05$ as determined by LSD.

*r is the correlation coefficient indicating $P < 0.5$ when the α-amylase inhibition activity was compared with all phytochemicals. Similarly, $P < 0.5$ was observed between the α-glucosidase inhibitory assay and TPC and TFC, whereas the α-glucosidase inhibitory assay indicated $P > 0.05$ when analyzed with TAC.

The potent anti-diabetic activity of the selected species agrees with early reports [49,50] in which researchers examined different species (*Albizia lebbeck* and *Albizia antunesiana*) belonging to the same genus investigated in the current study. Similarly, Zengin et al. [37] reported 0.67 ± 0.01 mmol α-amylase and 1.06 ± 0.01 mmol α-glucosidase inhibition potential in the *C. decapetala* leaves extract, which was confirmed in this study. Parveen et al. [41] determined α-glucosidase activity in the flavonoid compounds isolated from *C. decapetala*. Hence, these data confirmed that the selected extracts contain significant bioactive compounds that inhibited two major carbohydrate digestive enzymes correlating to reducing the plasma glucose level. These active compounds must be isolated and identified to discover novel anti-diabetic drugs.

3.5. Anti-leishmanial assay

Leishmaniasis occurs in cutaneous, visceral, and muco-cutaneous forms. Among these, cutaneous leishmaniasis is the most common form of the disease. The methanol extracts revealed the highest antioxidant and α-amylase inhibition potential in the current study. Hence, these were further tested against *L. major* promastigotes. The anti-leishmanial activity was dose-dependent, and the optimal concentration of the extracts for reducing promastigote growth was 500 and 1000 μg/mL. These doses killed almost half of the parasites, indicating that the extract has significant activity against *L. major*. The percentage inhibition of *L. major* promastigotes was found in descending order of *A. julibrissin* (74.75 % inhibition) > *C. decapetala* (70.86 % inhibition) > *I. atropurpurea* (65.34 % inhibition). Miltefosine (standard) showed 98.60 % inhibition at 1000 μg/mL (Table 2).

These results correlate with the findings of Nigussie et al. [52], who showed that *Albizia gummifera* (family Fabaceae) aqueous and n-butanol extracts inhibited the growth of *L. donovani* amastigote. On the other hand, Andrade et al. [53] did not find anti-leishmanial potential in *Albizia inundata* (family Fabaceae) extracts. The difference in antiparasitic activity may be due to the differences in plant species and the extraction solvents associated with the biological activity. Hence, the extracts act against *L. major* by modulating the immune response and having no adverse effects on the host macrophages. Although the action mechanism of killing *Leishmania* parasites has not been investigated, a study showed that *C. decapetala* and *A. julibrissin* could have a modulatory effect on the enzymes that generate a cytotoxin associated with the inhibition of *Leishmania* parasites [54].

The survival of *Leishmania* parasites within macrophages depends on the capacity of the parasite to cope with a highly oxidative environment. In this manner, leishmaniasis is influenced directly by oxidative DNA damage. Among the various biological factors, ROS and reactive nitrogen species generated by different immune cells help eliminate intracellular pathogens such as *Leishmania* [55]. These intermediates play an essential role in the host-defense strategies of organisms, and their excessive production kills parasites and causes oxidative damage in non-infected cells. Another reason for the sensitivity of *Leishmania* sp. to oxidative stress is the lack of enzymes, *i.e.*, catalase and glutathione peroxidase, in their promastigote form. This sensitivity makes the leishmania DNA system a potential target for antiparasitic drugs [56]. This paper reported the anti-leishmanial potential of selected species for the first time. Overall, *A. julibrissin* and *C. decapetala* have significant anti-leishmanial activity that could be used in pharmaceutical industries to control leishmaniosis.

3.6. GC-MS analysis

The present study identified 33 compounds at the respective retention times, including hydrocarbons, fatty acids, esters, alcohols, and carbohydrates. Their peak area was recorded to evaluate the concentration of various compounds in these species (Fig. 5a to c). 9-Octadecenamide, (Z) was detected in the highest concentration in *C. decapetala* (38.61 %) followed by *I. atropurpurea* (22.84 %) and *A. julibrissin* (21.85 %). Similarly, tetradecanamide was also recorded in higher concentrations in all species, ranging from 10.85 to 14.09 %. On the other hand, all other compounds were found in the lowest concentration (<6 %) in the examined species (Table 3).

Kiem et al. [74] revealed diterpenoids, caesaldecan, spathulenol, squalene, quercetin, lupeol, astragalin, resveratrol, and stigmasterol in *C. decapetala* leaves. Similarly, Lv et al. [75] revealed the presence of phenols in *A. julibrissin* by GC-MS, which concurs with this study. Liu et al. [76] identified 16 volatile terpenes from *A. julibrissin* leaves and flowers, while Li and Yang [13] detected 26 compounds in the *A. julibrissin* extract. These findings are also consistent with Nehdi [77], who observed 15 volatile compounds in the essential oil of *A. julibrissin* leaves using GC-MS. On the other hand, GC-MS analysis of *I. atropurpurea* leaves is reported for the first time.

p-Xylene exhibits antioxidant activity [61,62], while 3-cyclopentadiene, 5-(1-methylethylidene) exhibits significant antifungal

properties [63]. (Z)-9-Octadecenamide is used as a hypolipidemic agent and for treating atherosclerosis [65], while tetradecanamide exhibits anti-mycobacterial and anti-tubercular activities [77]. Other compounds detected in this study, such as dodecanal [67], nonadecanamide [68], oxirane, hexadecyl [70], and 8-octadecenal [64], have antibacterial and antifungal properties. Furthermore, 2, 3,3-trimethyloctane has anti-inflammatory ability [69], while 2-ethylhexyl hexyl sulfite is used as an acidifier and an arachidonic acid inhibitor [75].

Gupta et al. [66] reported the toxicological properties of cyclohexanone present in all species at low concentrations. Similarly, pentadecanal and dodecanal also possess antimicrobial properties [67] but were detected only in *A. julibrissin* leaves (0.95 and 0.29 %, respectively). Overall, the relative order of the total identified compounds in the examined species was 46.57 (*A. julibrissin*) > 54.37 (*I. atropurpurea*) > 66.12 (*C. decapetala*), and the leading compounds detected were aromatic/heterocyclic hydrocarbons followed by esters, linear alkanes, fatty amides, aldehydes, and cyclic ether. The target-oriented compounds responsible for biological activities must be isolated and exploited in various medicinal products.

4. Conclusion

The present study revealed the highest phytochemicals, DPPH activity, and α-amylase inhibition potential in the methanol extracts followed by the methanol–DMSO extracts. On the other hand, the methanol–glycerol extracts showed the lowest phytochemicals and biological activities, suggesting that the highly polar solvents (methanol) have a remarkably high capacity to dissolve certain hydrophilic and hydrophobic compounds responsible for biological activities. TPC and TFC were present at the highest levels in the methanol extracts in decreasing order of *C. decapetala* > *A. julibrissin* > *I. atropurpurea*, while TAC was recorded in the order of *C. decapetala* > *I. atropurpurea* > *A. julibrissin* methanol extracts. Similarly, the DPPH scavenging activity was also highest in *C. decapetala* methanol extracts, followed by *A. julibrissin* and *I. atropurpurea* methanol extracts. Furthermore, the *C. decapetala* methanol extracts exhibited the highest α-amylase inhibitory and anti-leishmanial activities. Hence, this paper reports a strong correlation between the phytochemicals and biological activities of selected extracts, suggesting that the phenolic, flavonoid, and alkaloid contents affect the anti-radical and anti-diabetic activities of these extracts. GC-MS confirmed the existence of 33 compounds in the selected species, while *C. decapetala* was the leading plant exhibiting the most identified compounds (66.12). In conclusion, the *C. decapetala* methanol extracts exhibited potent biological properties and is recommended for industrial applications. Nevertheless, further *in vivo* and toxicity studies will be needed to confirm their safe use at the industrial level.

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Data availability statement

Data included in article/referenced in the article.

CRediT authorship contribution statement

Iram Fatima: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Naila Safdar:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Wasim Akhtar:** Writing – review & editing, Validation, Methodology, Investigation. **Asma Ayaz:** Writing – review & editing, Software, Resources, Project administration, Methodology, Investigation. **Sajid Ali:** Writing – review & editing, Visualization, Validation, Software, Resources. **Hosam O. Elansary:** Writing – review & editing, Investigation, Funding acquisition. **Ihab Mohamed Moussa:** Writing – review & editing, Software, Resources, Methodology. **Wajid Zaman:** Writing – review & editing, Writing – original draft, Validation, Project administration, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33668.

References

[1] A. Houldsworth, Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants, Brain Communications 6 (1) (2024) 1–12, https://doi.org/10.1093/braincomms/fcad356.
[2] K. Jomova, R. Raptova, S.Y. Alomar, S.H. Alwasel, E. Nepovimova, K. Kuca, M. Valko, Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging, Arch. Toxicol. 97 (10) (2023) 2499–2574, https://doi.org/10.1007/s00204-023-03562-9.
[3] A. Carusillo, C. Mussolino, DNA damage: from threat to treatment, Cells 9 (7) (2020) 1665, https://doi.org/10.3390/cells9071665.
[4] M. Majidinia, A. Sadeghpour, S. Mehrzadi, R.J. Reiter, N. Khatami, B. Yousefi, Melatonin: a pleiotropic molecule that modulates DNA damage response and repair pathways, J. Pineal Res. 63 (1) (2017) e12416, https://doi.org/10.1111/jpi.12416.
[5] I. Fatima, N. Safdar, W. Akhtar, A. Munir, S. Saqib, A. Ayaz, et al., Evaluation of potential inhibitory effects on acetylcholinesterase, pancreatic lipase, and cancer cell lines using raw leaves extracts of three Fabaceae species, Heliyon 9 (5) (2023) e15909, https://doi.org/10.1016/j.heliyon.2023.e15909.
[6] S. Saqib, F. Ullah, M. Naeem, M. Younas, A. Ayaz, S. Ali, W. Zaman, *Mentha*: nutritional and health attributes to treat various ailments including cardiovascular diseases, Molecules 27 (2022) 6728, https://doi.org/10.3390/molecules27196728.
[7] M. Mohiuddin, D. Arbain, A.K.M. Shafiqul-Islam, M.S. Ahmad, M.N. Ahmad, Alpha-glucosidase enzyme Biosensor for the electrochemical measurement of antidiabetic potential of medicinal plants, Nanoscale Res. Lett. 11 (2016) 95, https://doi.org/10.1186/s11671-016-1292-1.
[8] B. Lorenzati, C. Zucco, S. Miglietta, F. Lamberti, G. Bruno, Oral hypoglycemic drugs: pathophysiological basis of their mechanism of action, Pharmaceuticals 3 (2010) 3005–3020, https://doi.org/10.3390/ph3093005.
[9] L. Menghini, C. Ferrante, S. Carradori, M. D'Antonio, G. Orlando, F. Cairone, et al., Chemical and bioinformatics analyses of the anti-leishmanial and anti-oxidant activities of hemp essential oil, Biomolecules 11 (2) (2021) 272, https://doi.org/10.3390/biom11020272.
[10] W. Zaman, M. Ahmad, M. Zafar, H. Amina, S. Saqib, F. Ullah, A. Ayaz, S. Bahadur, S. Park, Diversity of medicinal plants used as male contraceptives: an initiative towards herbal contraceptives, Indian Journal of Traditional Knowledge 21 (3) (2022) 616–624.
[11] M.G. Gallego, M. Skowyra, M.H. Gordon, N.A.M. Azman, M.P. Almajano, Effect of leaves of *Caesalpinia decapetala* on oxidative stability of oil-in-water emulsions, Antioxidants 6 (1) (2017) 19, https://doi.org/10.3390/antiox6010019.
[12] D.O. Ochora, C. Murithi, R.J. Masai, F. Abdi, A. Cheruyiot, E. Katuura, et al., Ex vivo and in vitro antiplasmodial activity and toxicity of *Caesalpinia decapetala* (Roth) Alston (Fabaceae), J. Ethnopharmacol. 318 (2024) 117007, https://doi.org/10.1016/j.jep.2023.117007.
[13] W. Li, H.J. Yang, Isolation and identification of lignans and other phenolic constituents from the stem bark of *Albizia julibrissin* Durazz and evaluation of their nitric oxide inhibitory activity, Molecules 25 (9) (2020) 2065, https://doi.org/10.3390/molecules25092065.
[14] L. Zhang, J. Chen, C. Ke, H. Zhang, S. Zhang, W. Tang, et al., Ethanol extract of *Caesalpinia decapetala* inhibits influenza virus infection in vitro and in vivo, Viruses 12 (5) (2020) 557, https://doi.org/10.3390/v12050557.
[15] E. Gerometta, I. Grondin, J. Smadja, M. Frederich, A. Gauvin-Bialecki, A review of traditional uses, phytochemistry and pharmacology of the genus *Indigofera*, J. Ethnopharmacol. 253 (2020) 112608, https://doi.org/10.1016/j.jep.2020.112608.
[16] B. Huang, Y. Wu, C. Li, Q. Tang, Y. Zhang, Molecular basis and mechanism of action of *Albizia julibrissin* in depression treatment and clinical application of its formulae, Chinese Herbal Medicines 15 (2) (2023) 201–213, https://doi.org/10.1016/j.chmed.2022.10.004.
[17] A.A. Karim, A. Azlan, Fruit pod extracts as a source of nutraceuticals and pharmaceuticals, Molecules 17 (2012) 11931–11946, https://doi.org/10.3390/molecules171011931.
[18] Y.H. Choi, R. Verpoorte, Green solvents for the extraction of bioactive compounds from natural products using ionic liquids and deep eutectic solvents, Curr. Opin. Food Sci. 26 (2019) 87–93, https://doi.org/10.1016/j.cofs.2019.04.003.
[19] D. Prat, A. Wells, J. Hayler, H. Sneddon, C.R. McElroy, S. Abou-Shehada, et al., CHEM21 selection guide of classical-and less classical-solvents, Green Chem. 18 (1) (2016) 288–296, https://doi.org/10.1039/C5GC01008J.
[20] F. Al-Rimawi, H. Imtara, M. Khalid, Z. Salah, M.K. Parvez, A. Saleh, et al., Assessment of antimicrobial, anticancer, and antioxidant activity of *Verthimia iphionoides* plant extract, Processes 10 (11) (2022) 2375, https://doi.org/10.3390/pr10112375.
[21] M.W. Lim, Y. Quan Tang, M.K. Aroua, L.T. Gew, Glycerol extraction of bioactive compounds from Thanaka (*Hesperethusa crenulata*) bark through LCMS profiling and their antioxidant properties, ACS Omega 9 (12) (2024) 14388–14405, https://doi.org/10.1021/acsomega.4c00041.
[22] D.O. Kim, S.W. Jeong, C.Y. Lee, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem. 81 (3) (2003) 321–326, https://doi.org/10.1016/S0308-8146(02)00423-5.
[23] Y.S. Park, S.T. Jung, S.G. Kang, B.G. Heo, P. Arancibia-Avila, F. Toledo, et al., Antioxidants and proteins in ethylene-treated kiwifruits, Food Chem. 107 (2) (2008) 640–648, https://doi.org/10.1016/j.foodchem.2007.08.070.
[24] M. Ajanal, M.B. Gundkalle, S.U. Nayak, Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer, Ancient Sci. Life 31 (4) (2012) 198, https://doi.org/10.4103/0257-7941.107361.
[25] N. Wu, Y. Zu, Y. Fu, Y. Kong, J. Zhao, X. Li, et al., Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L, J. Agric. Food Chem. 58 (8) (2010) 4737–4743, https://doi.org/10.1021/jf904593n.
[26] J.C. Lee, H.R. Kim, J. Kim, Y.S. Jang, Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* Var. saboten, J. Agric. Food Chem. 50 (22) (2002) 6490–6499, https://doi.org/10.1021/jf020388c.
[27] T. Matsui, T. Ueda, T. Oki, K. Sugita, N. Terahara, K. Matsumoto, Alpha-glucosidase inhibitory action of natural acylated anthocyanins: survey of natural pigments with potent inhibitory activity, J. Agric. Food Chem. 49 (4) (2001) 1948–1951, https://doi.org/10.1021/jf001251u.
[28] A.O. Ademiluyi, G. Oboh, Soybean phenolic rich extracts inhibit key enzymes linked to type 2 diabetes (Alpha amylase and alpha glucosidase) and hypertension (angiotensin I converting enzyme) *in- vitro*, Exp. Toxicol. Pathol. 65 (2013) 305–309, https://doi.org/10.1016/j.etp.2011.09.005.
[29] R. Essid, F.Z. Rahali, K. Msaada, I. Sghair, M. Hammami, A. Bouratbine, et al., Antileishmanial and cytotoxic potential of essential oils from medicinal plants in Northern Tunisia, Ind. Crop. Prod. 77 (2015) 795–802, https://doi.org/10.1016/j.indcrop.2015.09.049.
[30] NIST, NIST Standard Reference Database Number, vol.69, 2011. https://webbooknistgov/chemistry/.
[31] R.P. Adams, Identification of essential oil components by gas chromatography/mass spectrometry, in: Carol Stream (IL), vol. 4, Allured Publ Corp., 2007.
[32] B. Albuquerque, S. Heleno, M. Oliveira, L. Barros, I. Ferreira, Phenolic compounds: current industrial applications, limitations and future challenges, Food and Function Journal 12 (2021) 14–29, https://doi.org/10.1039/D0FO02324H.
[33] Z. Wang, S.M. Richter, B.D. Gates, T.A. Grieme, Safety concerns in a pharmaceutical manufacturing process using dimethyl sulfoxide (DMSO) as a solvent, Org. Process Res. Dev. 16 (12) (2012) 1994–2000, https://doi.org/10.1021/op300016m.
[34] P.J.M. Lima, R.M. Da Silva, C.A.C.G. Neto, N.C. Gomes-e-Silva, J.E.D.S. Souza, Y.L. Nunes, et al., An overview on the conversion of glycerol to value-added industrial products via chemical and biochemical routes, Biotechnol. Appl. Biochem. (2021), https://doi.org/10.1002/bab.2098.
[35] M.G. Gallego, T. Rodriguez, I. Rodriguez, M.P. Almajano, Analytical characterization of polyphenols from tara and Caesalpinia decapetala as stabilizers of O/W emulsions, J. Food Sci. 81 (2016) C2676–C2685.
[36] R. Liu, R. Liu, L. Shi, Z. Zhang, T. Zhang, M. Lu, et al., Effect of refining process on physicochemical parameters, chemical compositions and *in vitro* antioxidant activities of rice bran oil, LWT 109 (2019) 26–32, https://doi.org/10.1016/j.lwt.2019.03.096.
[37] G. Zengin, M.F. Mahomoodally, M.C.N. Picot-Allain, K.I. Sinan, G. Ak, O.K. Etienne, et al., Chemical composition, biological properties and bioinformatics analysis of two *Caesalpina* species: a new light in the road from nature to pharmacy shelf, J. Pharmaceut. Biomed. Anal. 198 (2021) 114018, https://doi.org/10.1016/j.jpba.2021.114018.
[38] M. Ouerfelli, L. Bettaieb Ben Kâab, M.P. Almajano, Radical scavenging and antioxidant activity of *Anthyllis vulneraria* leaves and flowers, Molecules 23 (7) (2018) 1657, https://doi.org/10.3390/molecules23071657.
[39] Q.M. Anderson, K.R. Markham, Flavonoids: Chemistry, Biochemistry and Applications, Taylor & Francis, New York, NY, USA, 2006.
[40] X.H. Wei, S.J. Yang, N. Liang, D.Y. Hu, L.H. Jin, W. Xue, et al., Chemical constituents of *Caesalpinia decapetala* (Roth) alston, Molecules 18 (1) (2013) 1325–1336, https://doi.org/10.3390/molecules18011325.

[41] A. Parveen, M.Q. Farooqi, W.W. Kyunn, M. Arshad, Phytochemical screening and content determination of different species of genus *Caesalpinia* belonging to different origin with antidiabetic activity, Phcog. J. 9 (6) (2017) 743–749, https://doi.org/10.5530/pj.2017.6.117.

[42] T.N. Nawinda. Antibacterial, antioxidant and phytochemical investigation of *Albizia anthelmintica* leavesroots and stem bark. Master's Thesis, University of Namibia, Windhoek, Namibia, 2016.

[43] J.D.D. Tamokou, S.D.J. Mpetga, K.P. Lunga, M. Tene, P. Tane, J.R. Kuiate, Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of *Albizia adianthifolia* (Mimosoideae), BMC Compl. Alternative Med. 12 (1) (2012) 1–10, https://doi.org/10.1186/1472-6882-12-99.

[44] G. Guha, V. Rajkumar, L. Mathew, R.A. Kumar, The antioxidant and DNA protection potential of Indian tribal medicinal plants, Turkish Journal of Biology 35 (2011) 233–242, https://doi.org/10.3906/biy-0906-64.

[45] W. Kahaliw, B. Hellman, E. Engidawork, Genotoxicity study of Ethiopian medicinal plant extracts on HepG2 cells, BMC Compl. Alternative Med. 18 (1) (2018) 1–8, https://doi.org/10.1186/s12906-017-2056-x.

[46] T. Kaewkod, R. Tobe, Y. Tragoolpua, H. Mihara, Medicinal plant extracts protect epithelial cells from infection and DNA damage caused by colibactin-producing *Escherichia coli*, and inhibit the growth of bacteria, J. Appl. Microbiol. 130 (3) (2021) 769–785, https://doi.org/10.1111/jam.14817.

[47] K.H. Lin, Y.Y. Yang, C.M. Yang, M.Y. Huang, H.F. Lo, K.C. Liu, et al., Antioxidant activity of herbaceous plant extracts protect against hydrogen peroxide-induced DNA damage in human lymphocytes, BMC Res. Notes 6 (1) (2013) 1–10, https://doi.org/10.1186/1756-0500-6-490.

[48] P. Sitarek, E. Skała, H. Wysokińska, M. Wielanek, J. Szemraj, M. Toma, et al., The effect of *Leonurus sibiricus* plant extracts on stimulating repair and protective activity against oxidative DNA damage in CHO cells and content of phenolic compounds, Oxid. Med. Cell. Longev. 2016 (2016), https://doi.org/10.1155/2016/5738193.

[49] C. Lankatillake, S. Luo, M. Flavel, G.B. Lenon, H. Gill, T. Huynh, et al., Screening natural product extracts for potential enzyme inhibitors: protocols, and the standardisation of the usage of blanks in α-amylase, α-glucosidase and lipase assays, Plant Methods 17 (1) (2021) 1–19, https://doi.org/10.1186/s13007-020-00702-5.

[50] G. Granados-Guzman, R. Castro-Rios, N. Waksman-de-Torres, S.R. Aranda, Optimization and validation of a microscale in vitro method to assess alpha-glucosidase inhibition activity, Curr. Anal. Chem. 14 (5) (2018) 458–464, https://doi.org/10.2174/1573411013666170911154755.

[51] M. Abudawood, H. Tabassum, B. Almaarik, A. Aljohi, Interrelationship between oxidative stress, DNA damage and cancer risk in diabetes (Type 2) in Riyadh, KSA, Saudi J. Biol. Sci. 27 (1) (2020) 177–183, https://doi.org/10.1016/j.sjbs.2019.06.015.

[52] D. Nigussie, G. Tasew, E. Makonnen, A. Debella, B. Hurrisa, K. Urga, et al., *In-vitro* investigation of fractionated extracts of *Albizia gummifera* seed against *Leishmania donovani* amastigote stage, J. Clin. Cell. Immunol. 6 (373) (2015) 2, https://doi.org/10.4172/2155-9899.1000373.

[53] E.M. Andrade, J.S. Teixeira, D.K. Silva, T.B.D. Santos, M.G. Korn, H.N. Brandão, et al., Phytochemical analysis, multi-element composition and biological activities of extracts and lupenone from *Albizia inundata* (Mart.) Barneby & J.W. Grimes, J. Braz. Chem. Soc. 31 (2020) 1825–1837, https://doi.org/10.21577/0103-5053.20200069.

[54] M.F. Horta, B.P. Mendes, E.H. Roma, F.S.M. Noronha, J.P. Macêdo, L.S. Oliveira, et al., Reactive oxygen species and nitric oxide in cutaneous leishmaniasis, Journal of Parasitology Research (2012) 1–11, https://doi.org/10.1155/2012/203818.

[55] P. Holzmuller, D. Sereno, M. Cavaleyra, I. Mangot, S. Daulouede, P. Vincendeau, et al., Nitric oxide-mediated proteasome-dependent oligonucleosomal DNA fragmentation in *Leishmania amazonensis* amastigotes, Infect. Immun. 70 (2002) 3727–3735, https://doi.org/10.1128/IAI.70.7.3727-3735.2002.

[56] A. Mishra, M.I. Khan, P.K. Jha, A. Kumar, S. Das, P. Das, et al., Oxidative stress-mediated overexpression of uracil DNA glycosylase in *Leishmania donovani* confers tolerance against antileishmanial drugs, Oxid. Med. Cell. Longev. 2018 (2018), https://doi.org/10.1155/2018/4074357.

[57] S. Duan, S.J. Kwon, C.S. Gil, S.H. Eom, Improving the antioxidant activity and flavor of faba (*Vicia faba* L.) leaves by domestic cooking methods, Antioxidants 11 (5) (2022) 931, https://doi.org/10.3390/antiox11050931.

[58] E. Sawicka, A. Długosz, Toluene and P-xylene mixture exerts antagonistic effect on lipid peroxidation in vitro, Int. J. Occup. Med. Environ. Health 21 (3) (2008), https://doi.org/10.2478/v10001-008-0022-z.

[59] F. Anum, K. Jabeen, S. Javad, S. Iqbal, A. Tahir, Z. Javed, et al., Green synthesized silver nanoparticles as potent antifungal agent against *Aspergillus terreus* Thom, J. Nanomater. (2021) 1–10, https://doi.org/10.1155/2021/2992335.

[60] I.H. Hameed, Analysis of the secondary metabolite products of *Ammi majus* and evaluation of anti-insect activity, International Journal of Pharmacognosy and Phytochemical Research 8 (8) (2016) 1403–1411.

[61] C. Chen, X. Jin, X. Meng, C. Zheng, Y. Shen, Y. Wang, Inhibition of TNFα-induced adhesion molecule expression by (Z)-(S)-9-octadecenamide, N-(2-hydroxyethyl, 1-methyl), Eur. J. Pharmacol. 660 (2–3) (2011) 305–309, https://doi.org/10.1016/j.ejphar.2011.04.009.

[62] P.K. Gupta, W.H. Lawrence, J.E. Turner, J. Autian, Toxicological aspects of cyclohexanone, Toxicol. Appl. Pharmacol. 49 (3) (1979) 525–533, https://doi.org/10.1016/0041-008X(79)90454-X.

[63] F. Mujeeb, P. Bajpai, N. Pathak, Phytochemical evaluation, antimicrobial activity and determination of bioactive components from leaves of *Aegle marmelos*, BioMed Res. Int. (2014) 1–11, https://doi.org/10.1155/2014/497606.

[64] A.O. Olusola, A.O. Olusola, T.O. Ogidan, A.O. Elekan, O.E. Ekun, I.O. Onoagbe, GC-MS analysis of alkaloid-rich fraction of *Zanthoxylum zanthoxyloides* leaf, Int. J. Pharmaceut. Sci. Res. 5 (2020) 13–17.

[65] K. Saravanakumar, R. Chelliah, X. Hu, D.H. Oh, K. Kathiresan, M.H. Wang, Antioxidant, anti-lung cancer, and anti-bacterial activities of Toxicodendron vernicifluum, Biomolecules 9 (2019) 127, https://doi.org/10.3390/biom9040127.

[66] J. Nithyadevi, R. Sivakumar, Phytochemical screening and GC-MS, FT-IR analysis of methanolic extract leaves of *Solanum torvum* Sw, International Journal of Research Studies in Biosciences 3 (9) (2015) 61–66.

[67] N. Joshi, G.C. Sah, D. Mishra, GC-MS analysis and antimicrobial activity of essential oil of Senecio pedunculatus, IOSR J. Appl. Chem. 6 (2013) 61.

[68] N. Đukić, G. Andrić, R. Glinwood, V. Ninkovic, B. Andjelković, A. Radonjić, The effect of 1-pentadecene on Tribolium castaneum behaviour: repellent or attractant? Pest Manag. Sci. 77 (9) (2021) 4034–4039, https://doi.org/10.1002/ps.6428.

[69] V. Kontham, K.R. Ansari, K.V. Padmaja, D. Madhu, Synthesis and evaluation of stearic acid based heterocyclic Schiff bases as biolubricant additives in epoxy karanja fatty acid 2-ethyl hexyl esters base oil, Ind. Crop. Prod. 159 (2021) 113061, https://doi.org/10.1016/j.indcrop.2020.113061.

[70] H.M. Soliman, A.M. Basuny, S.M. Arafat, Utilization of stearic acid extracted from olive pomace for production of triazoles, thiadiazoles and thiadiazines derivatives of potential biological activities, J. Oleo Sci. 64 (9) (2015) 1019–1032, https://doi.org/10.5650/jos.ess14261.

[71] R. Yuvaraj, M.R.K. Rao, K. Prabhu, R.L. Sundram, S. Shil, M.S. Kumar, N. Vijayalakshmi, The gas chromatography mass spectrometry study of one medicinal plant *Stachytarpheta indica*, Indian J. Pharmaceut. Sci. 12 (8) (2019) 382–394.

[72] R.O. Silva, F.B.M. Sousa, S.R.B. Damasceno, N.S. Carvalho, V.G. Silva, F.R.M.A. Oliveira, et al., Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress, Fund. Clin. Pharmacol. 28 (4) (2014) 455–464, https://doi.org/10.1111/fcp.12049.

[73] J.K. Mali, Y.B. Sutar, A.R. Pahelkar, P.M. Verma, V.N. Telvekar, Novel fatty acid-thiadiazole derivatives as potential anti-mycobacterial agents, Chem. Biol. Drug Des. 95 (1) (2020) 174–181, https://doi.org/10.1111/cbdd.13634.

[74] P.V. Kiem, C.V. Minh, H.T. Huong, J.J. Lee, Y.H. Kim, Caesaldecan, a cassane diterpenoid from the leaves of Caesalpinia decapetala, Chemical and Pharmaceutical Bulletin. (Tokyo) 53 (2005) 428–430, https://doi.org/10.1248/cpb.53.428.

[75] J.S. Lv, L.N. Zhang, Y.Z. Song, X.F. Wang, X.Z. Chu, Biological activity exhibited by secondary metabolites of the *Albizia julibrissin* Durazz. pod, Int. Biodeterior. Biodegrad. 65 (1) (2011) 258–264, https://doi.org/10.1016/j.ibiod.2010.11.011.

[76] G. Liu, M. Yang, X. Yang, X. Ma, J. Fu, Five TPSs are responsible for volatile terpenoid biosynthesis in *Albizia julibrissin*, J. Plant Physiol. 258 (2021) 153358, https://doi.org/10.1016/j.jplph.2020.153358.

[77] I. Nehdi, Characteristics, chemical composition and utilisation of *Albizia julibrissin* seed oil, Ind. Crop. Prod. 33 (1) (2011) 30–34, https://doi.org/10.1016/j.indcrop.2010.08.004.