

Evaluation of Phytochemical Screening, ‘Antibacterial, Antioxidant, Anti-Inflammatory, and Antidiabetic Activities’ of Sequentially Extracted *Ixora duffii* Leaves Extract

Chong Kim Thien Duc¹, Duy Toan Pham¹, Huynh Bich Lieu Nguyen² and Chi Linh Tran^{2,*}

¹Department of Health Sciences, College of Natural Sciences, Can Tho University, Can Tho 94000, Vietnam

²Faculty of Medicine, Nam Can Tho University, Can Tho 94000, Vietnam

(*Corresponding author’s e-mail: tc Linh@nctu.edu.vn)

Received: 22 February 2025, Revised: 3 March 2025, Accepted: 10 March 2025, Published: 20 May 2025

Abstract

Identifying its bioactive compounds is essential for understanding its therapeutic potential. The increasing prevalence of antibiotic resistance, inflammation-related diseases, and diabetes underscores the need for effective natural alternatives. *Ixora duffii*, a medicinal plant from the Rubiaceae family commonly used in traditional Vietnamese medicine, has not been evaluated its biological activities. Therefore, this study was conducted to investigate the antibacterial, antioxidant, anti-inflammatory, and antidiabetic activity of the ethanol, *n*-hexane, dichloromethane, ethyl acetate, and water extracts of *I. duffii* leaves. The antioxidant activity was assessed by methods of ABTS^{•+}, DPPH, RP, FRAP, and TAC. Anti-inflammatory effect was assessed by assays of protecting red blood cell membranes (RBCs), inhibiting denaturation of bovine serum albumin (BSA), inhibiting nitric oxide (NO[•]). Antidiabetic efficacy was assessed by the inhibitory assays on 2 enzymes of α -amylase, α -glucosidase. Antibacterial activity was determined based on the diameter of the inhibition zone, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) against 4 strains of Gram-negative, Gram-positive bacteria (*Listeria innocua*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*). The results showed that *I. duffii* leaf extracts had good activity in all tests. The ethyl acetate fraction (EF) exhibited the lowest IC₅₀ values in antioxidant, antidiabetic, and anti-inflammatory tests, ranging from 5.15 ± 0.06 to 66.22 ± 0.40 μ g/mL. EF inhibited Gram-positive bacteria more effectively than Gram-negative bacteria, with antibacterial zone diameters ranging from 9.13 ± 0.31 to 26.83 ± 0.76 mm, MIC and MBC values ranging from 8 to 32 and 16 to 64 μ g/mL. The *I. duffii* leaves extracts’ effects were linked to their polyphenol, flavonoid, alkaloid, tannin contents. These findings point to the possible use of *I. duffii* leaves as a natural antioxidant, antidiabetic, anti-inflammatory, and antibacterial agents.

Keywords: Antibacterial, Antioxidant, Anti-inflammatory, Antidiabetic, *Ixora duffii*, MIC, MBC

Introduction

Diabetes is one of the most widespread metabolic disorders in the world, which is identified by abnormally elevated blood glucose levels. According to a 2019 World Health Organization (WHO) report, this is the ninth most common cause of death worldwide. Among the best methods to lower postprandial hyperglycemia is to block the enzymes that hydrolyze carbohydrates, such as α -amylase and α -glucosidase, in order to lessen the absorption of glucose [1,2]. Enzyme (α -amylase and α -

glucosidase) inhibitor compounds originated from medicinal plants are becoming more and more popular due to their low side effects and relative effectiveness. In particular, positive outcomes were found in a report by Tran *et al.* [3] about the antidiabetic potential of the polyphenol-rich optimum extract from *Curcuma zedoaria* species and the extract shows the ability to effectively inhibit enzymes α -amylase and α -glucosidase.

In addition to metabolic disorders, diabetes is also closely related to oxidative stress and inflammation. Long-term abnormally high blood sugar levels cause an excess of free radicals, resulting in oxidative stress, which leads to insulin resistance and makes glucose absorption simpler for cells. Furthermore, oxidative stress has a wide range of detrimental consequences on organs in the body, including the kidneys, which alter kidney function and contribute to diabetic kidney disease. Oxidative stress boosts the synthesis of inflammatory mediators, whereas inflammation causes an increase in reactive oxygen species. Many studies determined that chronic inflammation can also lead to insulin resistance, which raises blood sugar. Apart from the aforementioned issues, infections in individuals with diabetes are a reason for concern, since a compromised immune system makes most diabetic patients vulnerable to infections. Medicinal herbs have a lot of bioactive compounds that have strong biological properties, including polyphenols, flavonoids, alkaloids, tannins, and polysaccharides, among others, and they have few side effects [4-10]. Therefore, natural therapeutic agents are needed that have strong antioxidant, anti-inflammatory, and antibacterial properties.

Vietnamese traditional medicines frequently use a plant species called *Ixora duffii*, which is a member of the Rubiaceae family. Nonetheless, there are still relatively few scientific investigations on this medicinal plant. Therefore, we have conducted research on *Ixora duffii*, in order to clearly demonstrate their use according to scientifically based evaluation methods. The majority of these studies focus on *Ixora* species that are members of the family above, such as *Ixora megalophylla*, *Ixora brachiata*, *Ixora coccinea*, *Ixora finlaysoniana*, and *Ixora brachypoda*. In particular, Sadeghi-Nejad and Saki [11] found that ethanol and methanol extracts of *I. brachiata* roots suppressed the parasite *Leishmania major* promastigotes. The *I. coccinea* roots' methanol

extract, according to Muhammad *et al.* [12] includes natural compounds with potent antibacterial and antioxidant properties. Because the peptide fraction of *I. brachypoda* has antioxidant and good inhibitory properties against bacteria, fungi, and viruses, in addition to having moderate cytotoxicity on 2 cell lines (Human breast (MCF-7) cancer cell and Rhabdomyosarcoma (RD) cancer cell), Ogbole *et al.* [13] reported that the leaves of this species have good activity. The *I. finlaysoniana* leaf extract exhibits antibacterial, anti-inflammatory, and antioxidant properties, following to a study by [14]. These investigations have shown that the species *Ixora* have therapeutic potentials. As a result, we conducted a survey on the species *I. duffii*, which has yet to be the subject of published scientific research.

Materials and methods

Herbal materials

Leaves of *I. duffii* (**Figure 1**) were gathered in Ninh Kieu district, Can Tho city (sampling coordinates: 10°0'36"N, 105°27'0"E). Under the code CT_Idu202304010011, the samples were processed and kept in the Animal and Plant Laboratory of the Department of Biology, College of Natural Sciences, Can Tho University. *I. duffii* was identified by Associate Professor Dr. Dang Minh Quan (Department of Biology Education, School of Education, Can Tho University). After processing, 1500 g of fresh *I. duffii* leaves were obtained and dried at 55 °C before being crushed into powder. Particles of medicine powder measuring 60-mesh were chosen for the investigation using a specialised powder sieve. Then, the YOKE DSH-10A moisture analyzer (YOKE, China) was used to determine the moisture content of *I. duffii* leaves powder and gave a result of 6.77 ± 0.35 %. The powder was then managed at 4 °C for subsequent experiments.



Figure 1 *I. duffii* leaves.

***I. duffii* leaves extraction**

The *I. duffii* leaf powder (950 g) was enclosed in a cotton bag and the study used the maceration method (for 24 h in 1000 mL of 99.5 % ethanol at ambient temperature). To produce the *I. duffii* leaf's ethanol extract (199.5 g), the soaking solution was filtered through a filter paper, and the solvent was evaporated. *n*-Hexane, ethyl acetate, dichloromethane, and water were the solvents of increasing polarity that were utilized in liquid-liquid extraction with the *I. duffii* leaf's ethanol extract (10 g). By using a rotating vacuum evaporator, extracts of *n*-hexane (2.66 g), ethyl acetate (1.48 g), dichloromethane (1.05 g), and water (3.69 g) were produced, in that order. To use in further studies, all *I. duffii* leaves extracts were kept in a refrigerator at 4 °C.

Phytochemical screening

Following the standard methods, stated by Sharma *et al.* [15], the amounts of total polyphenols, flavonoids, alkaloids, and tannins in *I. duffii* leaves extracts were calculated. In which, the methods used for quantification are: Spectrophotometric method (polyphenols and flavonoids), gravimetric method (alkaloids), titrimetric method (tannins). Using the standard curve equation, the total of polyphenols, flavonoids, alkaloids, and tannins content in the extracts of *I. duffii* leaf were calculated in respective with gallic acid (mg GAE/g extract), quercetin (mg QE/g extract), atropine (mg AE/g extract), and catechins (mg CE/g extract), correspondingly. Particularly, CE ($y = 0.0026x + 0.0124$; $R^2 = 0.9968$), AE ($y = 0.002x - 0.0008$; $R^2 = 0.9964$), QE ($y = 0.0096x - 0.019$; $R^2 = 0.9955$), and GAE ($y = 0.0087x + 0.0215$; $R^2 = 0.9953$) are the standard curves for the aforementioned chemicals.

Examining the antioxidant capacities *in vitro*

The methods reported by Duc *et al.* [16] were modified to assess the *in vitro* antioxidant activity of *I. duffii* leaves extracts. These methods included 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺), reducing power (RP), ferric reducing-antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and total antioxidant activity (TAC) [18]. In all 5 previously stated antioxidant methods, ascorbic acid was utilized as the positive control. The half-maximal inhibitory concentration (IC₅₀) and antioxidant effect were assessed based on the study conducted by [17].

Examining the antidiabetic effects *in vitro*

The *in vitro* antidiabetic efficacy of *I. duffii* leaves extract was assessed by its activity to inhibit the activities of α -amylase and α -glucosidase enzymes. The inhibition of α -amylase enzyme by *I. duffii* leaf extract was assessed using the approach described by [18]. Besides, according to Kifle *et al.* [19], the α -glucosidase enzyme inhibitory effect of *I. duffii* leaf extract was ascertained. In each of the aforementioned operations, acarbose served as the positive control. According to research by Wickramaratne *et al.* [18], Kifle *et al.* [19], the inhibitory concentration of 50 % value (IC₅₀, the concentration at which the extract or acarbose inhibits 50 % of the activity of the 2 enzymes) will be used to assess how well α -amylase and α -glucosidase are inhibited.

Examining the anti-inflammatory efficacy *in vitro*

The *in vitro* anti-inflammatory activity of *I. duffii* leaves extracts is determined by its ability to protect red blood cell membranes (RBCs), inhibit denaturation of bovine serum albumin (BSA), and inhibit nitric oxide

(NO[•]) as described by Tran *et al.* [3], Shah *et al.* [20], with some modifications. In this investigation, diclofenac and ascorbic acid served as positive controls, while dimethyl sulfoxide (DMSO) 10 % was used as a negative control.

Examining the antibacterial activity

The antibacterial efficacy of *I. duffii* leaves extracts will be assessed using 3 methodologies including agar well diffusion, microdilution, and droplet counting, as delineated by [21]. The study will document the resistance ring diameter, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) for each extract. Penicillin served as the positive control, whereas DMSO 10 % functioned as the negative control in the assessment of antibacterial activity. Bacterial strains, including *Listeria innocua* ATCC33090, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27855, and *Escherichia coli* ATCC 25922TM, were supplied by the Department of Biology, College of Natural Sciences, Can Tho University.

Analytical statistics

Data in the study are presented as mean $\pm$ standard error and were evaluated by one-way ANOVA

(Tukey's) test using Minitab 16 software for Windows. Significant differences were defined as p -values < 0.05 .

Results and discussion

Polyphenol, flavonoid, alkaloid and tannin content of *I. duffii* leaves extracts

Numerous published studies have demonstrated the significant biological benefits of polyphenols, flavonoids, alkaloids, and tannins found in medicinal plants, including their antiviral, antibacterial, antidiabetic, anti-inflammatory, and antioxidant qualities [22-24]. From there, this study quantified their content and evaluate their potential biological activities from the leaves of *I. duffii*. As shown in **Table 1**, the total polyphenol content (TPC) ranged from 48.18 ± 2.02 to 218.49 ± 5.18 mg GAE/g extract, while the total flavonoid content (TFC) varied between 38.19 ± 1.31 and 167.88 ± 4.70 mg QE/g extract. Besides, between 88.67 ± 9.46 and 239.50 ± 9.01 mg AE/g extract, the total alkaloid content (TAKC) changed, whereas the highest the total tannin content (TTC) is reached at 272.95 ± 9.09 mg CE/g extract and the lowest at 86.41 ± 4.00 mg CE/g extract.

Table 1 Results of surveying TPC, TFC, TAKC, TTC of *I. duffii* leaves extracts.

Contents	EE	HF	DF	EF	WF
TPC (mg GAE/g extract)	$184.00^b \pm 2.39$	$48.18^c \pm 2.02$	$64.27^d \pm 5.21$	$218.49^a \pm 5.18$	$87.26^e \pm 2.72$
TFC (mg QE/g extract)	$116.67^b \pm 2.08$	$38.19^c \pm 1.31$	$57.29^d \pm 3.25$	$167.88^a \pm 4.70$	$74.83^e \pm 1.50$
TAKC (mg AE/g extract)	$162.83^b \pm 5.77$	$88.67^d \pm 9.46$	$110.33^c \pm 3.82$	$239.50^a \pm 9.01$	$145.33^b \pm 3.82$
TTC (mg CE/g extract)	$188.33^b \pm 12.36$	$86.41^e \pm 4.00$	$109.49^d \pm 4.84$	$272.95^a \pm 9.09$	$140.90^c \pm 5.88$

Note: TPC stands for total polyphenol content, TFC (total flavonoid content), TAKC (total alkaloid content), TTC (total tannin content), EE (ethanol extract), HF (n-hexane fraction), DF (dichloromethane fraction), EF (ethyl acetate fraction), and WF (water fraction). Values followed by different letters (a, b, c, d) in the same column are statistically different ($p < 0.05$).

Furthermore, the data indicate that the ethyl acetate fraction (EF) exhibited superior values in TPC, TFC, TAKC, and TTC, compared to other extracts, specifically TPC (218.49 ± 5.18 mg GAE/g extract), TFC (167.88 ± 4.70 mg QE/g extract), TAKC (239.50 ± 9.01 mg AE/g extract), and TTC (272.95 ± 9.09 mg CE/g extract). Conversely, the values in *n*-hexane fraction (HF) were the lowest, with 4.53, 4.39, 2.70, and

3.16 times lower than those in EF, respectively. This is elucidated based on the polarity of polyphenols, flavonoids, alkaloids, and tannins. The majority of these compounds have medium to high polarity, and because ethyl acetate is an organic solvent with the similar polarity, it is excellent for extracting the matching groups of natural compounds with medium to high polarity.

Antioxidant activity of extracts from *I. duffii* leaves (*in vitro*)

Table 2 and **Figure 2** illustrates the antioxidant capacities of *I. duffii* leaves extracts and ascorbic acid (AA). In this experiment, the concentration ($\mu\text{g/mL}$) at which 50 % of free radicals were neutralized or decreased by each sample or positive control ($\text{Abs}_{0.5}$ or IC_{50}) was employed. Duc *et al.* [16] stated that samples with IC_{50} values less than 50 $\mu\text{g/mL}$ were very strong antioxidants, strong antioxidants were those with IC_{50} values between 50 and 100 $\mu\text{g/mL}$, moderate antioxidants were those with IC_{50} values between 101 and 150 $\mu\text{g/mL}$, and weak antioxidants were those with IC_{50} values more than 150 $\mu\text{g/mL}$ [16]. In all 5 methods

such as ABTS^{++} , DPPH, RP, FRAP, and TAC, the extracts from *I. duffii* leaves demonstrated strong to very strong antioxidant activity, according to the study's findings. In all 5 methods, the extracts' IC_{50} values for antioxidant activity varied from 8.91 ± 0.16 to 66.22 ± 0.40 $\mu\text{g/mL}$. EF outperformed the other extracts in all 5 methods. Specifically, the IC_{50} values of EF were 10.30 ± 0.08 $\mu\text{g/mL}$ (ABTS^{++} method), 8.91 ± 0.16 $\mu\text{g/mL}$ (DPPH method), 14.94 ± 0.16 $\mu\text{g/mL}$ (RP method), 13.02 ± 0.08 $\mu\text{g/mL}$ (FRAP method), and 16.11 ± 0.20 $\mu\text{g/mL}$ (TAC method). In the FRAP method in particular, the IC_{50} value of EF was roughly 6.3 times higher than AA ($\text{IC}_{50} = 81.49 \pm 3.97$ $\mu\text{g/mL}$).

Table 2 IC_{50} value ($\mu\text{g/mL}$) from anti-inflammatory, antidiabetic, and antioxidant *in vitro* assays.

Methods	The IC_{50} ($\mu\text{g/mL}$) values					
	EE	HF	DF	EF	WF	PC
ABTS⁺⁺	$12.10^{\text{d}} \pm 0.04$	$39.55^{\text{a}} \pm 0.24$	$34.33^{\text{b}} \pm 0.80$	$10.30^{\text{e}} \pm 0.08$	$26.32^{\text{c}} \pm 0.49$	$7.00^{\text{f}} \pm 0.17$
DPPH	$11.87^{\text{d}} \pm 0.12$	$37.38^{\text{a}} \pm 0.08$	$30.26^{\text{b}} \pm 0.36$	$8.91^{\text{e}} \pm 0.16$	$24.60^{\text{c}} \pm 0.16$	$6.12^{\text{f}} \pm 0.41$
RP	$15.98^{\text{d}} \pm 0.12$	$51.06^{\text{a}} \pm 0.79$	$66.22^{\text{b}} \pm 0.40$	$14.94^{\text{e}} \pm 0.16$	$33.98^{\text{c}} \pm 0.12$	$5.04^{\text{f}} \pm 0.25$
FRAP	$15.73^{\text{c}} \pm 0.09$	$45.72^{\text{b}} \pm 0.31$	$41.35^{\text{c}} \pm 0.56$	$13.02^{\text{e}} \pm 0.08$	$30.93^{\text{d}} \pm 1.20$	$81.49^{\text{a}} \pm 3.97$
TAC	$18.59^{\text{d}} \pm 0.07$	$57.05^{\text{a}} \pm 0.46$	$81.93^{\text{b}} \pm 0.34$	$16.11^{\text{e}} \pm 0.20$	$37.47^{\text{c}} \pm 0.13$	$13.67^{\text{f}} \pm 0.28$
α-Amylase	$8.28^{\text{d}} \pm 0.16$	$26.01^{\text{a}} \pm 0.48$	$24.03^{\text{b}} \pm 0.50$	$5.97^{\text{e}} \pm 0.11$	$19.14^{\text{c}} \pm 0.47$	$5.99^{\text{e}} \pm 0.03$
α-Glucosidase	$5.15^{\text{d}} \pm 0.06$	$22.3^{\text{a}} \pm 0.14$	$18.44^{\text{b}} \pm 0.18$	$3.09^{\text{e}} \pm 0.02$	$15.51^{\text{c}} \pm 0.04$	$5.38^{\text{d}} \pm 0.56$
BSA	$7.07^{\text{e}} \pm 0.04$	$27.71^{\text{b}} \pm 0.95$	$25.53^{\text{c}} \pm 0.21$	$5.27^{\text{f}} \pm 0.01$	$21.95^{\text{d}} \pm 0.04$	$54.27^{\text{a}} \pm 0.84$
RBCs	$9.29^{\text{e}} \pm 0.10$	$34.39^{\text{b}} \pm 0.21$	$29.66^{\text{c}} \pm 0.95$	$6.73^{\text{f}} \pm 0.08$	$27.08^{\text{d}} \pm 0.40$	$57.32^{\text{a}} \pm 0.89$
NO[•]	$14.12^{\text{d}} \pm 0.13$	$43.25^{\text{a}} \pm 0.70$	$36.04^{\text{b}} \pm 0.31$	$11.41^{\text{f}} \pm 0.07$	$28.07^{\text{d}} \pm 0.66$	$63.46^{\text{a}} \pm 0.02$

Note: The Tukey test was performed to compare mean and standard deviation. EE (ethanol extract), HF (n-hexane fraction), DF (dichloromethane fraction), EF (ethyl acetate fraction), WF (water fraction), PC (positive control). Values followed by different letters (a, b, c, d) in the same column are statistically different ($p < 0.05$).

To explain the superiority of EF, we can relate it to the polarity of the solvent (ethyl acetate) and the types of compounds commonly isolated from EF in other studies. Since ethyl acetate is a medium-polarity

solvent, it may dissolve the majority of polar and non-polar substances, particularly secondary. This completely coincides with our quantitative results, because TPC, TFC, TAKC, TTC in EF are all higher

than the remaining extracts. More than that, polyphenols, flavonoids, alkaloids, and tannins are frequently in charge of biological effects including antidiabetic, antimicrobial, anti-inflammatory, and antioxidant effects. For instance, polyphenols and flavonoids possess significant antioxidant effects due to their unique chemical structure, particularly the presence of many hydroxyl groups (-OH) connected to benzene rings. These hydroxyl groups can transfer electrons or hydrogen, which helps to neutralize free radicals and prevent oxidation in the body without creating dangerous new free radicals. The structure of flavonoids, with 3 carbon rings (C6-C3-C6) and hydroxyl groups (-OH) distributed in various places, forms a powerful defensive network. As a result, polyphenols and flavonoids not only act as antioxidants, but also aid to minimize cell damage and protect the body from oxidative illnesses [25]. The study conducted

by Gontijo *et al.* [26] isolated 4 polyphenol and flavonoid compounds from the ethyl acetate fraction of the fruit of *Garcinia brasiliensis* and conducted a survey of antioxidant activity using the DPPH and RP methods. The results showed that all 4 compounds had antioxidant activity in both test methods. Furthermore, from the ethyl acetate fraction of *Ehretia asperula* leaves, Duc *et al.* [16] identified 7 chemicals, 5 of which were polyphenols and flavonoids. They reported that all compounds, including 5 compounds from 7 compounds above, had strong to very strong antioxidant activity after evaluating the antioxidant activity of all isolated compounds using 5 methods similar to our study. Specifically, caffeic acid had IC_{50} values of 5.70 ± 0.01 ; 5.57 ± 0.02 ; 6.79 ± 0.03 ; 12.72 ± 0.06 ; and 8.43 ± 0.15 $\mu\text{g/mL}$ in all 5 methods, ABTS⁺⁺, DPPH, RP, FRAP, and TAC, respectively.

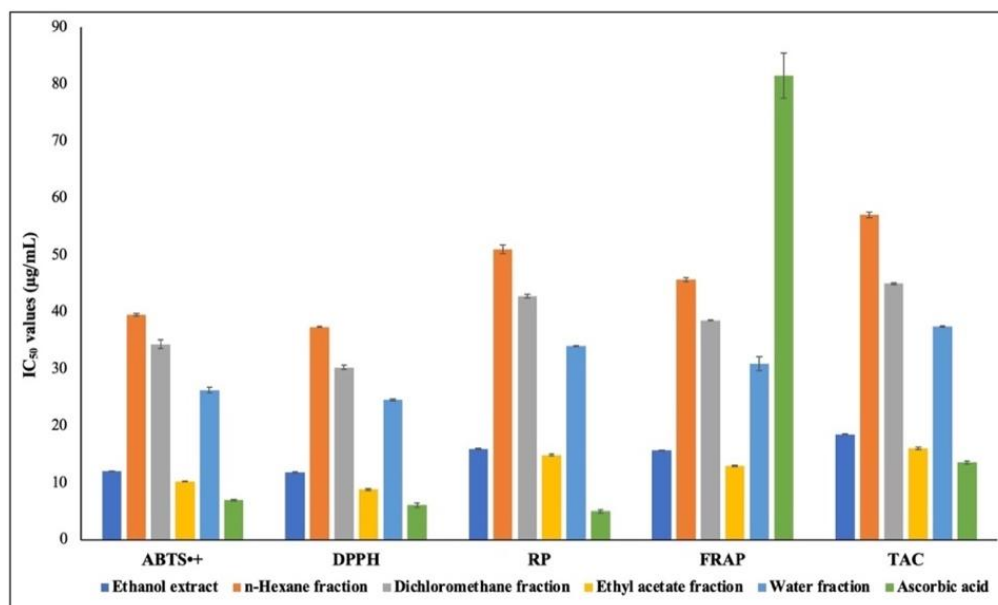


Figure 2 The IC_{50} values of 5 antioxidant methods.

***In vitro* antidiabetic activity of extracts from *I. duffii* leaves**

The results shown in **Table 2** and **Figure 3** indicate that EF exhibits the most favorable IC_{50} values. The α -amylase and α -glucosidase enzyme inhibitory activities for EF have $IC_{50} = 5.97 \pm 0.11$ and $IC_{50} = 3.09 \pm 0.02$ $\mu\text{g/mL}$, respectively. Compared to the IC_{50} values of AC (IC_{50} , α -amylase = 5.99 ± 0.03 $\mu\text{g/mL}$; IC_{50} , α -glucosidase = 5.38 ± 0.56 $\mu\text{g/mL}$), these 2 results were comparatively superior. The α -amylase as well as α -

glucosidase enzymes had IC_{50} values of 26.01 ± 0.48 and 22.3 ± 0.14 $\mu\text{g/mL}$, respectively, making HF the sample with the lowest findings. Furthermore, the polarity of the solvent and the compounds that frequently surfaced in EF extracts in other studies, which were covered in the section on assessing the antioxidant activity of *I. duffii* leaves extracts, may help to explain why EF had such superior IC_{50} values in antidiabetic testing. In fact, many studies have demonstrated that secondary metabolites such as

polyphenols, flavonoids, alkaloids or tannins are related to antidiabetic activity [27-29], our study quantified the extracts from *I. duffii* leaves and found that EF is rich in these compounds. A number of published research can be consulted to have a better understanding of secondary metabolites' antidiabetic potential. According to a study conducted by Bouyahya *et al.* [28], flavonoids primarily assist regulate hyperglycemia via binding to the glucose transporter receptor and the peroxisome proliferator-activated receptor gamma. Human lipid metabolism, glucose uptake, insulin utilization, and glucose tolerance will all be improved by this interaction. Furthermore, alkaloids are very promising compounds for the treatment of diabetes [28]. According to Mechanick *et al.* [30], berberine, a quinoline alkaloid, has the activity to deactivate di-saccharides in cells, which makes it an intriguing component of alkaloid compounds' antidiabetic effect. Furthermore, diabetes and oxidative stress are inextricably linked, since several studies have found that oxidative stress plays an indispensable role in the adverse progression of diabetes. Diabetes,

particularly type-2 diabetes, is linked to high levels of oxidative stress because elevated blood sugar levels produce reactive oxygen species (ROS), which damage cells, proteins, and DNA. This oxidative stress leads to insulin resistance, cellular malfunction, and diabetes consequences (neuropathy and nephropathy). Antioxidants can boost insulin sensitivity by lowering oxidative stress in organs including the liver, muscle, and adipose tissue. This enhances cells' activity to react to insulin and absorb glucose effectively. Many natural compounds, including polyphenols, flavonoids, alkaloids, and tannins have antioxidant and antidiabetic activities. They not only inhibit oxidation, but also regulate glucose metabolism and insulin action [31]. Therefore, it can be inferred that EF has good antioxidant qualities due to its secondary metabolites, which can reduce oxidative stress and diabetes more effectively than other extracts. This is also the first time extracts from *I. duffii* have been tested for *in vitro* antidiabetic efficacy utilizing 2 different techniques of blocking the aforementioned 2 enzymes.

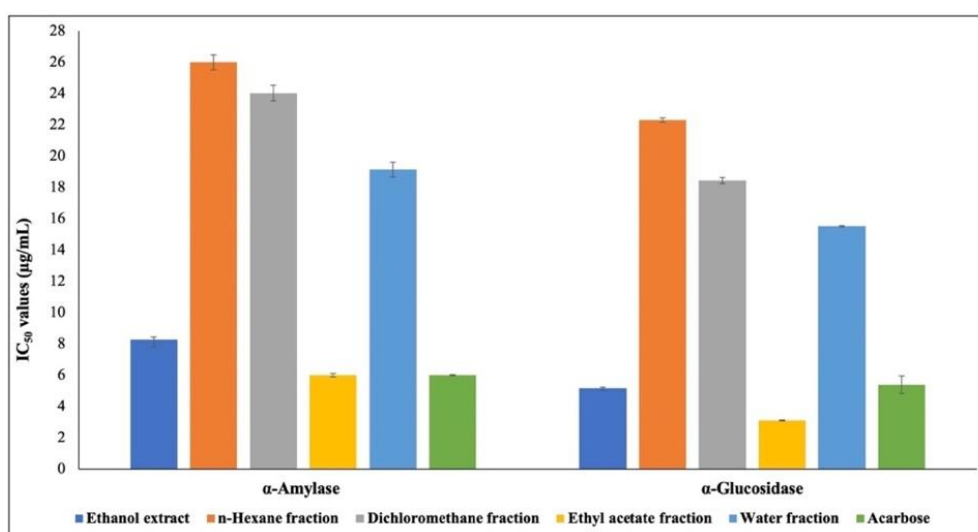


Figure 3 The IC₅₀ values of 2 antidiabetic methods.

***In vitro* anti-inflammatory activity of extracts from *I. duffii* leaves**

Table 2 and **Figure 4** show that *I. duffii* leaves extracts outperformed the positive control (PC) with IC₅₀ values in anti-inflammatory testing ranging from 5.27 ± 0.01 to 43.2 ± 0.70 µg/mL. EF had the highest IC₅₀ values compared to the other extracts in all 3 techniques (BSA, RBCs, NO[•]), whereas HF produced the lowest findings, 5.26, 5.11, and 3.80 times lower

than those of EF, respectively. Once again, EF was the most significant subject according to the IC₅₀ values obtained from analyzing the extracts' anti-inflammatory activity. It also demonstrated that its anti-inflammatory activity was highly prominent, in addition to its strong antioxidant and antidiabetic capabilities. This is completely consistent with published studies on the role of compounds such as polyphenols, flavonoids, alkaloids, tannins in the antioxidant process, reducing

oxidative stress, thereby controlling inflammation as well as diabetes. Prolonged inflammation oxidizes macromolecules such as proteins, DNA, and RNA, resulting in the generation of many new damaging free radicals [8]. Besides, Kalaskar *et al.* [32] extracted and investigated anti-inflammatory activity of components from the ethyl acetate fraction of *Ficus microcarpa* stem bark, including catechin, *p*-hydroxycinnamic acid, and oleanolic acid. Catechin, a flavonoid compound, has the strongest COX (cyclooxygenase) inhibitory activity, with $IC_{50} = 9.02 \mu M$ for COX-1 and $50.38 \mu M$ (COX-2) [32]. In 2024, Krishna *et al.* [33] extracted 2 flavonoid

compounds from the water fraction (the fraction with the strongest antioxidant activity in that study) and investigated their anti-inflammatory potential by measuring $NO^{\bullet}$ generation inhibition in RAW 264.7 cells. The study found that diosmetin-7-O- β -D-glucuronide, a flavonoid compound, effectively inhibited the enzyme with an IC_{50} value of $16.72 \pm 1.01 \mu M$ [33]. Therefore, by combining these articles and our data on the content of TPC, TFC, TAKC, and TTC in EF, we can thoroughly explain how these compounds contribute to EF's anti-inflammatory, antioxidant, and antidiabetic activities.

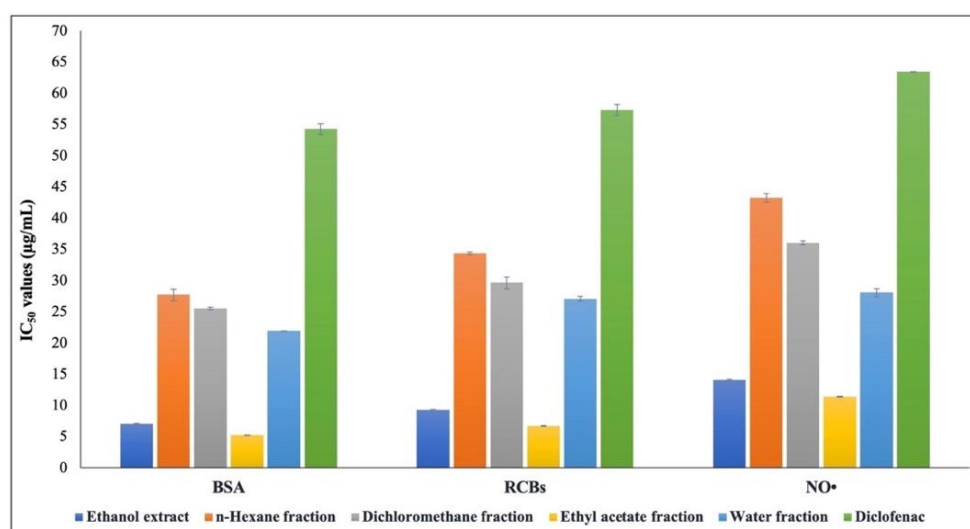


Figure 4 The IC_{50} values of 3 anti-inflammatory methods.

***In vitro* antibacterial activity of extracts from *I. duffii* leaves**

Diabetes and infections are strongly associated because high blood glucose levels can impair the immune system and allow bacterial invasion. Foot infections are one of the most devastating conditions among patients with severe diabetes. Many studies have demonstrated that diabetes and infections frequently interact, with some conditions causing insulin resistance to worsen [34,35]. The extracts of *I. duffii* leaves were investigated for antibacterial activity using 3 methods: Agar well diffusion, microdilution, and drop count. These are typical approaches for screening novel antibacterial compounds, and this is the first time they have been employed with *I. duffii*. The study investigated the antibacterial activity of *I. duffii* leaf extracts using 4 types of Gram-negative (*P. aeruginosa*, *E. coli*), Gram-positive (*L. innocua*, *S. aureus*) bacteria,

which can grow well and safely under basic laboratory conditions. The 2 kinds of Gram-negative bacteria are briefly described as follows: *P. aeruginosa* is frequently connected with infections caused by a weakened immune system, whereas *E. Coli* is frequently associated with pathogens in the intestinal, urinary, or sepsis. Both kinds of bacteria have an outer layer that renders them resistant to antibacterial agents and medicines. As a result, their selection will aid in the complete evaluation of the antibacterial activity of extracts from *I. duffii* leaves against difficult-to-treat microorganisms. The remaining 2 bacteria are then preliminary characterized, *L. innocua* is a Gram-positive bacterium and a non-pathogenic relative of *L. monocytogenes*. Therefore, *L. innocua* was chosen for the survey because it has physiological properties with *Listeria* species but is safer because it is not pathogenic [36]. Finally, *S. aureus* (Gram-positive bacterium),

which is usually linked with pneumonia or skin infections, has a high resistance to methicillin (a penicillin-derived semisynthetic antibiotic) [37]. Both of these Gram-positive bacteria lack an outer membrane, therefore extracts from *I. duffii* leaves were tested against basic bacterial cell structures to determine their antibacterial activity. Finally, by selecting these 4 bacterial strains for the antibacterial activity of *I. duffii* leaves, the study will be able to determine if this medicinal plant may be a viable alternative or complement to prevent antibiotic resistance.

Table 3 compares the results of antibacterial diameter of *I. duffii* leaves extracts through the agar well diffusion method. Extracts from *I. duffii* leaves have antibacterial zone diameters ranging from 0 to 26.83 ± 0.76 mm, whereas penicillin (PN) has an antibacterial zone diameter of 0 to 29.40 ± 0.70 mm. Specifically, in the survey on Gram-negative bacteria (*P. aeruginosa*), the inhibitory concentrations of the extracts and PN ranged from 32 to 128 $\mu\text{g/mL}$. Of which, EF had the best antibacterial zone diameter compared to the remaining extracts with antibacterial zone diameter values of 10.47 ± 0.71 mm at 32 $\mu\text{g/mL}$, 13.83 ± 0.45 mm at 64 $\mu\text{g/mL}$, and 16.03 ± 0.72 mm at 128 $\mu\text{g/mL}$, respectively. These values were 1.21, 1.09, and 1.15 times lower than penicillin at the corresponding concentrations. In addition, HF and DF were not able to inhibit *P. aeruginosa* at all tested concentrations. In the results of antibacterial zone diameter evaluation for *E. coli*, the

antibacterial zone diameter of the extracts ranged from 9.13 ± 0.31 to 16.57 ± 0.21 mm, corresponding to the concentration level from 16 to 128 $\mu\text{g/mL}$. Notably, at a concentration of 16 $\mu\text{g/mL}$, EF showed an antibacterial zone diameter of 9.13 ± 0.31 mm, while the remaining extracts and PN had no inhibitory ability. For the *L. innocua*, the inhibitory concentrations of the extracts (8 to 128 $\mu\text{g/mL}$) and the most prominent extract in terms of antibacterial zone diameter results was still EF, this extract showed antibacterial zone diameter results in all 5 concentrations tested including 8, 16, 32, 64, 128 $\mu\text{g/mL}$. At 8 $\mu\text{g/mL}$, EF had an antibacterial zone diameter of 9.30 ± 0.40 mm, while PN, EE, HF, DF, WF had no results. In addition, when compared with other extracts of *I. duffii* leaves at a concentration of 128 $\mu\text{g/mL}$, the antibacterial zone diameter of EF was the largest (23.80 ± 0.66 mm), larger than EE, HF, DF, WF by 1.47, 2.18, 1.79, 2.03 times, respectively. Finally, the data from *S. aureus*, EF was still the extract with the best antibacterial zone diameter, with the antibacterial zone diameter value at a concentration of 8 $\mu\text{g/mL}$ being 13.77 ± 0.40 mm, while other extracts and penicillin did not give any results. It is noteworthy that at 16 $\mu\text{g/mL}$, EF had an antibacterial zone diameter of 17.07 ± 0.38 mm, while EE was 11.53 ± 0.31 mm, PN was 15.30 ± 0.46 mm, HF and DF and WF did not show any results. Conclusively, EF outperformed the other extracts in its ability to inhibit all the bacteria tested (*P. aeruginosa*, *E. coli*, *L. innocua*, *S. aureus*) at most of the tested concentrations.

Table 3 Antibacterial zone diameter (mm) of *I. duffii* leaves' extracts and penicillin (PN).

Bacterial strains	Samples	Diameter of antibacterial zone at different concentrations ($\mu\text{g/mL}$) of extracts				
		8	16	32	64	128
<i>Pseudomonas aeruginosa</i>	EE	-	-	-	$11.07^b \pm 0.51$	$14.87^a \pm 0.40$
	HF	-	-	-	-	-
	DF	-	-	-	-	-
	EF	-	-	$10.47^c \pm 0.71$	$13.83^b \pm 0.45$	$16.03^a \pm 0.72$
	WF	-	-	-	-	$10.73^a \pm 0.40$
	PN	-	-	$12.67^c \pm 0.55$	$15.13^b \pm 0.57$	$18.43^a \pm 0.57$
<i>Escherichia coli</i>	EE	-	-	$10.50^c \pm 0.76$	$13.80^b \pm 0.46$	$16.57^a \pm 0.21$
	HF	-	-	-	-	-
	DF	-	-	-	-	-
	EF	-	$9.13^d \pm 0.31$	$11.53^c \pm 0.31$	$13.60^b \pm 0.36$	$15.70^a \pm 0.27$
	WF	-	-	-	-	$12.43^a \pm 0.15$
	PN	-	-	-	-	-

Bacterial strains	Samples	Diameter of antibacterial zone at different concentrations (µg/mL) of extracts				
		8	16	32	64	128
<i>Listeria innocua</i>	PN	-	-	14.00 ^c ± 0.46	15.93 ^b ± 1.08	19.57 ^a ± 0.40
	EE	-	9.27 ^d ± 0.32	11.37 ^c ± 0.76	13.43 ^b ± 0.65	16.23 ^a ± 0.67
	HF	-	-	-	-	10.90 ^a ± 0.53
	DF	-	-	-	-	13.30 ^a ± 0.85
	EF	9.30 ^c ± 0.40	11.83 ^d ± 0.61	14.77 ^c ± 0.50	18.97 ^b ± 0.40	23.80 ^a ± 0.66
	WF	-	-	-	9.93 ^b ± 0.59	11.73 ^a ± 0.50
	PN	-	13.00 ^d ± 0.53	16.27 ^c ± 0.84	20.37 ^b ± 0.95	25.10 ^a ± 0.79
<i>Staphylococcus aureus</i>	EE	-	11.53 ^d ± 0.31	14.13 ^c ± 0.60	17.37 ^b ± 0.55	19.90 ^a ± 0.56
	HF	-	-	-	9.23 ^b ± 0.60	12.17 ^a ± 0.59
	DF	-	-	-	10.63 ^b ± 0.67	15.37 ^a ± 0.90
	EF	13.77 ^c ± 0.40	17.07 ^d ± 0.38	19.93 ^c ± 0.35	22.90 ^b ± 0.27	26.83 ^a ± 0.76
	WF	-	-	-	12.03 ^b ± 0.50	14.27 ^a ± 0.49
	PN	-	15.30 ^d ± 0.46	19.10 ^c ± 0.44	24.17 ^b ± 0.85	29.40 ^a ± 0.70

Note: Values followed by different letters (a, b, c, d) in the same column are statistically different ($p < 0.05$). "-" indicates no observed activity. EE (ethanol extract), HF (n-hexane fraction), DF (dichloromethane fraction), EF (ethyl acetate fraction), WF (water fraction), PN (penicillin).

The 2 most often used metrics for evaluating antibacterial activities are MIC and MBC. Resazurin is utilized as a color indicator in the microdilution technique, which is used to assess the antibacterial activity of antibacterial agents on 96-well plates. The lowest concentration that inhibits MIC is the lowest concentration of an extract that may stop bacterial growth in the measured concentration range (without affecting the color of resazurin). MBC is used to test the ability to eliminate bacteria rather than only inhibit them. The droplet live count technique was used to perform MBC; no colonies developed on the LB agar plate, and the MBC value is the lowest concentration in the extracts' concentration range that can kill all bacteria

[21]. For the above reasons, MIC and MBC were studied to evaluate antibacterial activity in addition to the well diffusion method. The results of MIC and MBC are shown in **Table 4**. Depending on the MIC values, the sample was classified as possessing outstanding antibacterial activity ($\text{MIC} \leq 20 \mu\text{g/mL}$), excellent ($20 < \text{MIC} \leq 40 \mu\text{g/mL}$), very good ($40 < \text{MIC} \leq 80 \mu\text{g/mL}$), good ($80 < \text{MIC} \leq 160 \mu\text{g/mL}$), moderate ($160 < \text{MIC} \leq 320 \mu\text{g/mL}$), and weak ($\text{MIC} > 320 \mu\text{g/mL}$). In addition, if the value $(\text{MBC}/\text{MIC}) \leq 4$, the sample is considered to have bactericidal effect (killing or eliminating bacteria), $(\text{MBC}/\text{MIC}) > 4$, the sample is considered to have bacteriostatic effect [38-40].

Table 4 The results of MIC and MBC of extracts from *I. duffii* leaves.

Samples	<i>P. aeruginosa</i>		<i>E. coli</i>		<i>L. innocua</i>		<i>S. aureus</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
EE	64	128	32	64	16	32	16	32
HF	> 128	-	> 128	-	128	-	64	128
DF	> 128	-	> 128	-	128	-	64	128
EF	32	64	16	32	8	16	8	16
WF	128	-	128	-	64	128	64	128
PN	32	64	32	64	16	32	16	32

Note: “-” indicates no observed activity. EE (ethanol extract), HF (n-hexane fraction), DF (dichloromethane fraction), EF (ethyl acetate fraction), WF (water fraction), PN (penicillin).

Accordingly, the MIC values of the extracts from *I. duffii* leaves varied from 16 to > 128 µg/mL for 2 Gram-negative bacteria and from 8 to 128 µg/mL for 2 Gram-positive bacteria. Furthermore, the extracts' MBC values for Gram-positive and Gram-negative bacteria range from 8 to 128 and 32 to 128 µg/mL, respectively. The strongest of these is EF, which has MIC and MBC values of 32 and 64 µg/mL for *P. aeruginosa* and 16 and 32 µg/mL for *E. coli*. This indicates that the antibacterial activity of EF is excellent to outstanding. Furthermore, the MIC as well as MBC values of this extract against 2 Gram-positive bacteria (*L. innocua*, *S. aureus*) were outstanding as both MIC and MBC values for the 2 strains were 8 and 16 µg/mL, which were both lower than 20 µg/mL. In addition, it is interesting that the (MBC/MIC) value in all 4 tests was 2, which was lower than 4, so it can be concluded that EF has a complete bactericidal effect [38-40].

In summary, the EF is a very good antibacterial extract, especially against Gram-positive bacteria. The reason may originate from the morphological characteristics of the 2 strains of Gram-negative and Gram-positive bacteria. For Gram-positive bacteria, they have a cell wall composed mainly of peptidoglycan, while Gram-negative bacteria have a cell wall consisting of a thin layer of peptidoglycan combined with an outer membrane surrounded by lipopolysaccharide [36,37]. Therefore, EF can resist Gram-positive bacteria better because it can easily penetrate inside to contact the cell, while for Gram-negative bacteria, it is more difficult to inhibit because they have a lipopolysaccharide membrane protecting the cell. To explain why EF has such good antibacterial

properties, it is possible to relate the relationships between the polarity of the ethyl acetate solvent and the groups of compounds that often appear in the ethyl acetate extract that the study discussed. In addition, several investigations have shown that polyphenols, flavonoids, alkaloids, and tannins all have antibacterial properties [41-44]. The molecular structures of the compounds listed above contribute significantly to their antibacterial actions. Flavonoids' special structure with hydroxyl groups (-OH) gives them strong antibacterial qualities. Bacterial cell membranes can be severely impacted by these (-OH) groups, which can reduce bacterial cell integrity and disturb membrane structure. Additionally, flavonoids' ring structure can chelate, or bind, with metal ions like Fe²⁺ and Cu²⁺, deactivating the metabolic activities and preventing bacterial development [42,45]. In fact, Farooq *et al.* [46] tested 3 chalcone compounds (precursors of flavonoids) for antibacterial activity against Gram-positive bacteria (*S. aureus*), and found that all 3 derivatives were resistant to this bacterial strain [46]. According to Huang *et al.* [41], tannins are a secondary metabolite with high antibacterial activity. This study group discovered that tannins may degrade bacterial cell membranes and attach to cell wall proteins and certain bacterial enzymes, limiting their growth and deactivating their normal physiological activities. This ability comes from the chemical structure of tannins, because tannins also belong to the polyphenol group like flavonoids, so their structure also has (-OH) groups, but is more complex (the structure of tannins is a combination of many phenolic groups). Therefore, (-OH) groups in the structure of tannins have the ability

to combine with proteins and metal ions, creating insoluble complexes, inhibiting the growth of bacteria [47,48]. Furthermore, this group of chemicals not only works on bacteria but also helps to regulate plant microflora by living in symbiosis with beneficial bacteria strains [41]. Alkaloids are a fascinating collection of substances that have antibacterial properties through a variety of routes, including influencing bacterial protein production, changing cell membrane permeability, destroying bacterial cells, and inhibiting their metabolism. Furthermore, because most alkaloids' structures contain at least one nitrogen atom (typically in the form of an amine), alkaloids can form complexes with metals such as iron (an crucial component in bacterial development) or interact with bacterial enzymes, limiting growth [44]. In 2018, Chi *et al.* [49] isolated 6 alkaloids and tested their antibacterial efficacy on Gram-negative and Gram-positive bacterial strains. All 6 compounds had MIC values ranging from 0.12 to 1.32 µg/mL. Polyphenols have hydroxyl groups (-OH) in their structure, which can establish hydrogen bonds with the bacterial cell membrane, causing bacterial weakness and jeopardizing viability. Furthermore, polyphenols have the ability to deactivate several enzymes that are essential for their existence. From the above research results, combined with the results of TPC, TFC, TAKC, TTC as well as the excellent antioxidant activity of EF that the research team has conducted, it can be seen that the antibacterial ability of EF is superior to the remaining extracts is completely reliable. This is also the first time that the antibacterial activity of *I. duffii* species has been evaluated from its extracts through the 3 methods mentioned above.

Conclusions

The study and determined the content of polyphenols, flavonoids, alkaloids, tannins, antioxidant, antidiabetic, anti-inflammatory and antibacterial activities of *I. duffii* leaves extracts. The findings revealed that *I. duffii* leaf extracts have antioxidant, anti-inflammatory, antibacterial, and antidiabetic properties. *I. duffii* leaves extracts demonstrated a greater inhibitory impact on Gram-positive bacteria than Gram-negative bacteria. EF (ethyl acetate fraction) has higher antioxidant, anti-inflammatory, antidiabetic, and antibacterial activity than the other extracts. This was

determined by the amount of polyphenols, flavonoids, alkaloids, and tannins present in each extract. The aforesaid surveys on antioxidant, antidiabetic, anti-inflammatory, and antibacterial properties show that *I. duffii* is a highly beneficial plant species. The study adds to reliable scientific information about this species, enriches our understanding of useful medicinal herbs, and demonstrates the great potential of *I. duffii* leaves as a natural alternative or support for synthetic drugs in the treatment of diseases caused by oxidative stress, inflammation, diabetes, or infection.

Acknowledgements

The research team would like to sincerely thank Nam Can Tho University and Can Tho University, Vietnam for creating conditions for this research to be carried out. The research team would also like to thank Associate Professor Dr. Dang Minh Quan (Department of Biology Education, School of Education, Can Tho University, Vietnam) for assisting in identifying plant samples.

References

- [1] JH Bae, KD Han, SH Ko, YS Yang, JH Choi, KM Choi, H Kwon and KC Won. Diabetes fact sheet in Korea 2021. *Diabetes & Metabolism Journal* 2022; **46**(3), 417-426.
- [2] H Kashtoh and K Baek. New insights into the latest advancement in α -amylase inhibitors of plant origin with anti-diabetic effects. *Plants* 2023; **12**(16), 2944.
- [3] CL Tran, VM Do, VT Huynh, XN Hong and KTD Chong. Optimization of polyphenol extraction conditions from rhizomes of *curcuma zedoaria* with antioxidant, anti-inflammatory and anti-diabetic activities *in vitro*. *Trends in Sciences* 2024; **21**(9), 8544.
- [4] WAH Hegazy, AAH Rajab, ASA Lila and HA Abbas. Anti-diabetics and antimicrobials: Harmony of mutual interplay. *World Journal of Diabetes* 2021; **12**(11), 1832-1855.
- [5] M Halim and A Halim. Commentary: The effects of inflammation, aging, and oxidative stress on the pathogenesis of type II diabetes. *Journal of Health Care and Research* 2020; **1**(2), 119-124.
- [6] R Ramamoorthy, M Muthalagu, S Andra, B Ravichandran and M Narayanasamy.

- Investigation on antimicrobial, antioxidant and cytotoxicity properties of triple bark extract formulated using traditional medicinal plants. *SN Applied Sciences* 2019; **1**, 772.
- [7] FC Sasso, V Simeon, R Galiero, A Caturano, LD Nicola, P Chiodini, L Rinaldi, T Salvatore, M Lettieri, R Nevola, C Sardu, G Docimo, G Loffredo, R Marfella, LE Adinolfi, R Minutolo and NID-2 study group Investigators. The number of risk factors not at target is associated with cardiovascular risk in a type 2 diabetic population with albuminuria in primary cardiovascular prevention. Post-hoc analysis of the NID-2 trial. *Cardiovascular Diabetology* 2022; **21(1)**, 235.
- [8] AK Shrivastava, M Keshari, M Neupane, S Chaudhary, PK Dhakal, L Shrestha, A Palikhey, CK Yadav, G Lamichhane, MU Shekh and RK Yadav. Evaluation of antioxidant and anti-inflammatory activities, and metabolite profiling of selected medicinal plants of Nepal. *Journal of Tropical Medicine* 2023; **2023(1)**, 6641018.
- [9] M Wronka, J Krzeminska, E Młynarska, J Rysz and B Franczyk. The Influence of lifestyle and treatment on oxidative stress and inflammation in diabetes. *International Journal of Molecular Sciences* 2022; **23(24)**, 15743.
- [10] H Yousef, AH Khandoker, SF Feng, C Helf and HF Jelinek. Inflammation, oxidative stress and mitochondrial dysfunction in the progression of type II diabetes mellitus with coexisting hypertension. *Frontiers in Endocrinology* 2023; **14**, 1173402.
- [11] B Sadeghi-Nejad and J Saki. Effect of aqueous *allium cepa* and *ixora brachiata* root extract on leishmania major promastigotes. *Jundishapur Journal of Natural Pharmaceutical Products* 2014; **9(2)**, e15442.
- [12] H Muhammad, M Qasim, A Ikram, MA Versiani, IA Tahiri, K Yasmeen, MW Abbasi, M Azeem, ST Ali and B Gul. Antioxidant and antimicrobial activities of *Ixora coccinea* root and quantification of phenolic compounds using HPLC. *South African Journal of Botany* 2020; **135**, 71-79.
- [13] OO Ogbole, A Nkumah, TE Akinleye, FE Olisaedu and AF Attah. Evaluation of multifunctional activity of bioactive peptide fractions from the leaves of *Nauclea diderrichii* (De Wild. and T. Durand) Merrill and *Ixora brachypoda* DC. *Phytomedicine Plus* 2021; **1(1)**, 100019.
- [14] A Chatterjee, D Chatterjee, M Ghosh, P Dagur, J Kaur, NK Rangra, S Dey, A Mondal, A Ghosh and P Behera. Phytochemical screening and anti-inflammatory, antioxidant, and antimicrobial investigations on extracts of *ixora javanica*. *Matrix Science Pharma* 2023; **7(2)**, 43-51.
- [15] T Sharma, R Gamit, R Acharya and VJ Shukla. Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of *Byttneria herbacea* Roxb. *AYU* 2023; **42(3)**, 143-147.
- [16] CKT Duc, TC Linh, NQC Thanh, PQ Nhien, DTX Trang, LT Danh and NT Tuan. Isolation and evaluation of the antioxidant activity of compounds from *ehretia asperula* zoll. & moritz. *Indonesian Journal of Chemistry* 2024; **24(4)**, 1206.
- [17] I Gulcin and SH Alwasel. DPPH radical scavenging assay. *Processes* 2023; **11(8)**, 2248.
- [18] MN Wickramaratne, JC Punchihewa and DBM Wickramaratne. *In-vitro* alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. *BMC Complementary Medicine and Therapies* 2016; **16**, 466.
- [19] ZD Kifle, SG Debeb and YM Belayneh. *In vitro* α -amylase and α -glucosidase inhibitory and antioxidant activities of the crude extract and solvent fractions of *hagenia abyssinica* leaves. *BioMed Research International* 2021; **2021(1)**, 6652777.
- [20] M Shah, Z Parveen and MR Khan. Evaluation of antioxidant, anti-inflammatory, analgesic and antipyretic activities of the stem bark of *Sapindus mukorossi*. *BMC Complementary Medicine and Therapies* 2017; **17(1)**, 526.
- [21] LTM. Ngan, J Moon, J Kim, T Shibamoto and Y Anh. Growth-inhibiting effects of *Paeonia lactiflora* root steam distillate constituents and structurally related compounds on human intestinal bacteria. *World Journal of Microbiology and Biotechnology* 2012; **28(4)**, 1575-1583.
- [22] D Aryal, S Joshi, NK Thapa, P Chaudhary, S Basaula, U Joshi, D Bhandari, HM Rogers, S Bhattarai, KR Sharma, BP Regmi and N Parajuli.

- Dietary phenolic compounds as promising therapeutic agents for diabetes and its complications: A comprehensive review. *Food Science & Nutrition* 2024; **12**(5), 3025-3045.
- [23] LFMD Melo, VGQ Aquino-Martins, APD Silva, HAO Rocha and KC Scortecci. Biological and pharmacological aspects of tannins and potential biotechnological applications. *Food Chemistry* 2023; **414**, 135645.
- [24] S Parham, AZ Kharazi, HR Bakhsheshi-Rad, H Nur, AF Ismail, S Sharif, S Rama Krishna and F Berto. Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants* 2020; **9**(12), 1309.
- [25] SH Hassanpour and A Doroudi. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna Journal of Phytomedicine* 2023; **13**(4), 354-376.
- [26] VS Gontijo, TCD Souza, IA Rosa, MG Soares, MAD Silva, W Vilegas, C Viegas and MHD Santos. Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. *Food Chemistry* 2012; **132**(3), 1230-1235.
- [27] T Behl, A Gupta, M Albratty, A Najmi, AM Meraya, HA Alhazmi, MK Anwer, S Bhatia and SG Bungau. Alkaloidal phytoconstituents for diabetes management: Exploring the unrevealed potential. *Molecules* 2022; **27**(18), 5851.
- [28] A Bouyahya, A Balahbib, A Khalid, HA Makeen, HA Alhazmi, M Albratty, A Hermansyah, LC Ming, KW Goh and NE Omari. Clinical applications and mechanism insights of natural flavonoids against type 2 diabetes mellitus. *Heliyon* 2024; **10**(9), e29718.
- [29] S Singh, A Bansal, V Singh, T Chopra and J Poddar. Flavonoids, alkaloids and terpenoids: A new hope for the treatment of diabetes mellitus. *Journal of Diabetes & Metabolic Disorders* 2022; **21**(1), 941-950.
- [30] JI Mechanick, A Marchetti, R Hegazi and O Hamdy. Diabetes-specific nutrition formulas in the management of patients with diabetes and cardiometabolic risk. *Nutrients* 2020; **12**(12), 3616.
- [31] A Caturano, MD Angelo, A Mormone, V Russo, MP Mollica, T Salvatore, R Galiero, L Rinaldi, E Vetrano, R Marfella, M Monda, A Giordano and FC Sasso. Oxidative stress in type 2 diabetes: Impacts from pathogenesis to lifestyle modifications. *Current Issues in Molecular Biology* 2023; **45**(8), 6651-6666.
- [32] M Kalaskar, V Redasani, M Ayyanar, M Ghante, S Firke, K Agrawal, V Ghawate, S Surana, S Alarifi, R Chikhale and S Gurav. Isolation and characterization of anti-inflammatory compounds from *figus microcarpa* L.f. stem bark. *Plants* 2023; **12**(18), 3248.
- [33] AA Krishna, BL Abhirami, MB Bashi, R Jaice, ARM Jasim and A Kumaran. Identification and anti-inflammatory activity of flavonoids from *Cardiospermum halicacabum* in murine RAW 264.7 macrophage-A bioassay-guided approach. *Food Bioscience* 2024; **61**, 104510.
- [34] RIG Holt, CS Cockram, RCW Ma and AOY Luk. Diabetes and infection: Review of the epidemiology, mechanisms and principles of treatment. *Diabetologia* 2024; **67**(7), 1168-1180.
- [35] JR Simonsen, V Harjutsalo, A Jarvinen, J Kirveskari, C Forsblom, P Groop and M Lehto. Bacterial infections in patients with type 1 diabetes: A 14-year follow-up study. *BMJ Open Diabetes Research & Care* 2015; **3**(1), e000067.
- [36] N Heredia and S Garcia. Animals as sources of food-borne pathogens: A review. *Animal Nutrition* 2018; **4**(3), 250-255.
- [37] SYC Tong, JS Davis, E Eichenberger, TL Holland and VG Fowler. *Staphylococcus aureus* Infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews* 2015; **28**(3), 603661.
- [38] CS Makade, PR Sheno, BA Bhongade, SA Shingane, PC Ambulkar and AM Shewale. Estimation of MBC: MIC ratio of herbal extracts against common endodontic pathogens. *Journal of Pharmacy and Bioallied Sciences* 2024; **16**(2), S1414-S1416.
- [39] R Mogana, A Adhikari, MN Tzar, R Ramliza and C Wiart. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical

- isolates. *BMC Complementary Medicine and Therapies* 2020; **20**, 55.
- [40] D Poljuha, B Sladonja, I Sola, M Senica, M Uzelac, R Veberic, M Hudina, IM Famuyide, JN Eloff and M Mikulic-Petkovsek. LC–DAD–MS phenolic characterisation of six invasive plant species in croatia and determination of their antimicrobial and cytotoxic activity. *Plants* 2022; **11**(5), 596.
- [41] J Huang, M Zaynab, Y Sharif, J Khan, R Al-Yahyai, M Sadder, M Ali, SR Alarab and S Li. Tannins as antimicrobial agents: Understanding toxic effects on pathogens. *Toxicon* 2024; **247**, 107812.
- [42] NF Shamsudin, QU Ahmed, S Mahmood, SA Ali Shah, A Khatib, S Mukhtar, MA Alsharif, H Parveen and ZA Zakaria. Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. *Molecules* 2022; **27**(4), 1149.
- [43] S Sun, Z Liu, M Lin, N Gao and X Wang. Polyphenols in health and food processing: Antibacterial, anti-inflammatory, and antioxidant insights. *Frontiers in Nutrition* 2024; **11**, 1456730.
- [44] Y Yan, X Li, C Zhang, L Lv, B Gao and M Li. Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics* 2021; **10**(3), 318.
- [45] L Mira, MT Fernandez, M Santos, R Rocha, MH Florencio and KR Jennings. Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. *Free Radical Research* 2002; **36**(11), 1199-1208.
- [46] S Farooq, Z Ngaini and NA Mortadza. Microwave-assisted synthesis and molecular docking study of heteroaromatic chalcone derivatives as potential antibacterial agents. *Bulletin of the Korean Chemical Society* 2020; **41**(9), 918-924.
- [47] T Okuda and H Ito. Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. *Molecules* 2011; **16**(3), 2191-2217.
- [48] AK Farha, Q Yang, G Kim, H Li, F Zhu, H Liu, R Gan and H Corke. Tannins as an alternative to antibiotics. *Food Bioscience* 2020; **38**, 100751.
- [49] W Chi, Y Jiang, J Hu and J Pan. Cytotoxic and antibacterial aspidofractinine alkaloids from *Kopsia hainanensis*. *Fitoterapia* 2018; **130**, 259-264.