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Artículos originales

The *Albizia julibrissin* and *Caesalpinia decapetala* extracts induces potential enzymes and cell growth inhibition via anti-acetylcholinesterase, anti-lipase, anti-glycation and cytotoxicity activity

Los extractos de *Albizia julibrissin* y *Caesalpinia decapetala* inducen la inhibición potencial de enzimas y el crecimiento celular a través de la actividad antiacetilcolinesterasa, antilipasa, antiglicación y citotoxicidad

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Conflict of interests

The authors declare no conflict of interest.

Resumen

Introducción: Las plantas medicinales son una fuente dinámica de salud humana debido a su potencial terapéutico en el tratamiento de diversas dolencias. Este estudio tuvo como objetivo determinar las actividades antiacetilcolinesterasa, antilipasa, antiglicación y anticancerígena de *Albizia julibrissin* y *Caesalpinia decapetala*. (family Fabaceae).

Métodos: Los extractos de hojas se prepararon para investigar sus capacidades inhibitorias contra la acetilcolinesterasa, la lipasa y los productos de glicación. El potencial anticancerígeno se evaluó contra las líneas celulares HeLa, PC3 y 3T3 utilizando el ensayo MTT y se realizó un análisis HPLC para cuantificar seis compuestos.

Resultados: Los resultados indicaron la mayor actividad anti-acetilcolinesterasa (IC50 2,391 µg/ml) en el extracto metanólico de *A. julibrissin*, mientras que la mayor actividad anti-lipasa (114,9 µg/ml) y anti-glicación (43,69 µg/ml) se registró en el extracto metanólico de *C. decapetala*. El mayor potencial citotóxico también se observó en *C. decapetala* contra las líneas celulares PC3, 3T3 y HeLa. (144,3 ppm, 201,0 ppm and 236,0 ppm). Al final, la HPLC mostró que *A. julibrissin* exhibe la mayor concentración de ácido clorogénico (56,06 ppm) y dihidrato de quercetina (15,71 ppm), mientras que los extractos de hojas de *C. decapetala* poseen la mayor concentración de ácido gálico. (73,55 ppm).

Conclusiones: Los resultados sugieren que estos extractos inhiben significativamente las actividades enzimáticas, los productos de glicación y el crecimiento de células citotóxicas y, por lo tanto, pueden ser utilizados como nuevos compuestos farmacológicos para tratar diversas enfermedades.

Palabras clave: Cáncer; Enzimas; lipasa; Glucosilación; Cromatografía líquida de alta resolución; Productos naturales

Abstract

Introduction: Medicinal plants are dynamic source of human health because of their therapeutic potential in treating various ailments. This study aimed to determine the anti-acetylcholinesterase, anti-lipase, anti-glycation and anti-cancer activities of *Albizia julibrissin* and *Caesalpinia decapetala* (family Fabaceae).

Methods: The leaf extracts were prepared to investigate their inhibitory capacities against acetylcholinesterase, lipase and glycation products. The anti-cancer potential was evaluated against HeLa, PC3 and 3T3 cell lines using MTT assay and HPLC analysis was done to quantify six compounds.

Results: Results indicated highest anti-acetylcholinesterase (IC_{50} 2.391 µg/ml) activity in *A. julibrissin* methanolic extract while highest anti-lipase (114.9 µg/ml) and anti-glycation (43.69 µg/ml) activity was recorded in *C. decapetala* methanolic extract. Highest cytotoxic potential was also observed in *C. decapetala* against PC3, 3T3 and HeLa cell lines (144.3 ppm, 201.0 ppm and 236.0 ppm). In the end, HPLC showed that *A. julibrissin* exhibit the highest concentration of chlorogenic acid (56.06 ppm) and quercetin dihydrate (15.71 ppm) while *C. decapetala* leaves extracts possess highest concentration of gallic acid (73.55 ppm).

Conclusions: The results suggest that these extracts significantly inhibit the enzymatic activities, glycation products and the growth of cytotoxic cells and thus, can be used as novel pharmacological leads to treat various diseases.

Keywords: Cancer; Enzymes; lipase; High-performance Liquid Chromatography; Medicinal Plants

Highlights

• Medicinal plants, including *Albizia julibrissin* and *Caesalpinia decapetala* from the Fabaceae family, have significant therapeutic potential in managing various health conditions. This study aimed to investigate their ability to inhibit acetylcholinesterase, lipase, glycation, and cancer cell growth.

• The methanolic extract of *A. julibrissin* exhibited the strongest acetylcholinesterase inhibition with an IC50 of 2.391 µg/ml.The methanolic extract of *C. decapetala* showed the most potent lipase inhibition (114.9 µg/ml) and anti-glycation activity (43.69 µg/ml). *C. decapetala* extract demonstrated significant cytotoxic effects on PC3 (144.3 ppm), 3T3 (201.0 ppm), and HeLa (236.0 ppm) cell lines.

• HPLC analysis revealed that *A. julibrissin* contained high levels of chlorogenic acid (56.06 ppm) and quercetin dihydrate (15.71 ppm), while *C. decapetala* was rich in gallic acid (73.55 ppm).

Introduction

Oxidative stress produces excessive free radicals that damage cell components *viz.* lipids, proteins, carbohydrates and nucleic acid; resulting in neurodegenerative and metabolic disorders such as Alzheimer disease (AD), cancer and diabetes[1]. According to an estimate, about 55 million people were diagnosed with AD in 2020 and this number may increase to 78 million in 2030[2]. One of the most useful approaches to treat AD includes increasing acetylcholine (ACh) levels in the brain[3]. AChE is the major enzyme in the pathogenesis of Alzheimer's disease and its suppression reduces the Alzheimer's disease[4]. The synthetic drugs adverse reactions have prompted researchers to screen alternative natural plant-based products to treat AD[5,9]. Similarly, obesity and inactive lifestyles have caused diabetes in many individuals by increasing blood glucose levels in the body. The International Diabetes Federation (IDF) observed 537 million diabetic individuals in 2021[6]. Due to the increased insulin resistance, hyperglycemic condition leads to the protein glycation of proteins that produces advanced glycation end products (AGEs)[7-8]. Moreover, cancer is another critical life-threatening condition and the cervical cancer accounts for 15.9 % of the total cancer with 300,000 million cases every year[10].

Previously, some studies have explored the biological properties of Fabaceae species. For instance, *Albizia julibrissin* is traditionally used to treat ulcers, fractures, anxiety, bruises and hemorrhoids[11-12]. *Caesalpinia decapetala* is used as antiseptic, astringent, analgesic, anti-pyretic and to treat burns[13-14]. Due to the multiple health benefits of these species, present studies were designed to investigate the acetylcholinesterase, porcine pancreatic lipase, non-enzymatic glycation and cytotoxic inhibitory potential of *A. julibrissin* and *C. decapetala*. Additionally, the concentration of six compounds was evaluated using HPLC.

Methods

Extracts preparation

Fresh leaves of *A. julibrissin* subsp. Julibrissin and *C. decapetala* (Roth) Alston were collected and their accession numbers were obtained from the Herbarium of National Agriculture Research Center (NARC), Islamabad. The weight of extracts was measured to ascertain extract yield as shown in Table 1.

Table 1. List of selected species, their accession numbers and extract yield.

Plant species	Accession Numbers	Extracts	Extract weight (g)	Extract yield (%)
A. julibrissin	RAW101502	Methanol	5.32	26.60
		Meth-DMSO	5.80	29.00
C. decapetala	RAW101504	Methanol	7.53	37.65
		Meth-DMSO	8.07	40.35

*Meth: Methanol; DMSO: Dimethyl sulfoxide; the weight of extract was taken in grams (g) and the extract yield was measured in percentage (%).

Acetylcholinesterase inhibitory assay

The acetylcholinesterase inhibition potential was assessed using 140 µL of 0.1 M sodium phosphate buffer (pH 8) was added in 20 µL of plant extract, 15 µL of AChE enzyme solution (0.2 units/ml) and 10 µL of 15 mM DTNB followed by the incubation for 10 min at room temperature. The absorbance of the final product was measured at 412 nm and galantamine (100, 500 and 1000 µg/ml) was measured as a positive control. The enzyme inhibition was determined as

$$Enzyme\ Inhibition\ \% = 100 - percent\ enzyme\ activity$$
$$Percent\ Enzyme\ Activity\ \% = 100\ x\ V/Vmax$$

Where V_{max} is an enzyme activity without inhibitor.

Lipase inhibitory assay

Briefly, 5 mg/ml lipase from porcine pancreatic type II (Sigma) was added to 50 mM Tris-HCl buffer (pH 8.0) and then centrifuged for 5 min at 5500 rpm. Then, 100 µL of lipase solution was added to 50 µL of plant extract (100, 500 and 1000 µg/ml prepared in DMSO) and left for incubation on ice and the absorbance was noted at 405 nm. Orlistat was measured as a standard and the inhibition potential was calculated [15].

$$Inhibition\ \% = \left(1 - \frac{Abssample}{Abscontrol}\right) x\ 100$$

Anti-glycation assay

The AGE inhibition in plant extracts was determined using the method[16]. The fluorescence was measured at the emission wavelength of 440 nm and the percentage inhibition of AGE formation was calculated as:

$$\%\ Anti - glycation = Abscontrol - Abssample\ /\ Abscontrol\ x\ 100$$

Cytotoxicity assay against various cell lines

MTT assay

The cytotoxic potential of selected extracts was evaluated using standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay[17-18]. The absorbance was measured at 490 nm and the percentage inhibition and IC_{50} values were calculated.

HPLC analysis

The plant extracts (1 mg) were dissolved in 5 ml of 10 % methanol and then filtered using membrane filters of 0.45 nm size. An Agilent 1260 HPLC system having a quaternary pump, auto-sampler and C18 column was run at 30 °C and the mobile phase was degassed before injecting into the HPLC. The separation was achieved using 0.2 % H3PO4, methanol and acetonitrile and flow rate was 1 ml/min. The mobile phase was increased from 0 to 15 %, 50 %, 70 %, 100 % and then kept isocratic for additional 5 min. The injected volume was 10 µL and the wavelength was set at 210 nm. The standards were measured at 10, 20, 30, 40, 50 and 70 ppm concentrations to derive the calibration equation. Total four phenolic compounds and two flavonoid compounds were identified based on their retention times and UV spectras'. The concentration of compounds was determined using peak area of samples versus the analyte concentration obtained from the calibration curve [19].

Statistics

All experiments were conducted in duplicate. Results were interpreted with mean ±SD and Least Significant Difference (LSD) was evaluated using Statistix 8.1. Moreover, the IC_{50} values in all biological assays were determined using GraphPad Prism 5 software.

Results and Discussion

Anti-acetylcholinesterase assay

Plants are the potential source of compounds that can prevent or treat neurodegenerative diseases by inhibiting acetylcholinesterase[20]. The present study revealed highest acetylcholinesterase inhibitory activity in *A. julibrissin* methanolic extract (IC_{50} 2.391 µg/ml) followed by *A. julibrissin* methanol-DMSO extract (IC_{50} 10.16 µg/ml) and *C. decapetala* methanolic extract (IC_{50} 22.79 µg/ml). However, *C. decapetala* methanol-DMSO extract showed lowest anti-acetylcholinesterase activity by displaying IC_{50} value of 30.24 µg/ml (Table 2).

Table 2: Anti-acetylcholinesterase activity of extracts

Plant species	Extracts	Percentage Inhibition at Different Doses (µg/ml)			IC_{50} values
		100	500	1000	
A. julibrissin	Methanol	71.4	74.4	83	2.391
	Methanol-DMSO	75	78.7	94.6	10.16
C. decapetala	Methanol	63.5	70.0	82	22.79
	Methanol-DMSO	65.3	69	90.86	30.24
Standard*		78.0	84.5	93	5.99

*Galantamine was used as a standard and IC_{50} values indicates half maximal inhibitory concentration and is measured in µg/ml

The AChE inhibition potential of these extracts could be attributed to the existing phenolic and flavonoid compounds[21]. A previous report [22] indicated anti-acetylcholinesterase activity in the ethanolic leaves extracts of *Albizia lucidor* (IC_{50} 24.89 ± 1.60 µg/ml) and *Albizia procera* (IC_{50} 43.50 ± 2.10 µg/ml). In another study, [23] demonstrated highest inhibition potential (10.20 mg galantamine equivalent/g) of *C. decapetala* leaves extracts against butyrylcholinesterase. However, the acetylcholinesterase inhibitory activity of selected species has been examined for the first time. It can be inferred that the variations within the same plant species could be due to the different phyto-constituents of the plants that are grown in various geographical areas in different seasons of the year. The phyto-constituents may vary depending on the soil, water, stage of plant and the time of collection[24]. It can be suggested that *A. julibrissin* and *C. decapetala* leaves may serve as an ideal candidate for designing new AChE inhibitors to treat neurodegenerative disorders. However, detailed *in vivo* tests are needed to validate the efficiency of these species for medicinal use.

Anti-lipase and anti-glycation assays

The inhibition of pancreatic lipase (a lipolytic enzyme) and glycation products is a highly effective method that protects against fat absorption in individuals with obesity and hyperglycemia[25]. In this study, pancreatic anti-lipase and anti-glycation potential of two species have been investigated and results are presented in Figure 1a and b. The highest lipase and glycation inhibitory potential was observed in *C. decapetala* methanolic extract (IC_{50} 114.9 µg/ml and 43.69 µg/ml) followed by the methanol-DMSO extract of *A. julibrissin* (IC_{50} 138.7 µg/ml and 74.06 µg/ml). However, weak anti-lipase and anti-glycation activity was recorded in *A. julibrissin* methanolic extract (IC_{50} 155.2 µg/ml and 182.5 µg/ml) and *C. decapetala* methanol-DMSO extract (IC_{50} 168.4 µg/ml and 247.3 µg/ml) respectively (Table 2). The remarkable inhibitory activity of selected extracts indicates their potential use as a powerful source of anti-obesity and anti-glycation agents.

Previously, researchers have investigated biological potential of different species of *Albizia* and *Ceasalpinia* grown in different regions of world. For instance, [26] demonstrated pancreatic lipase inhibition potential in *Caesalpinia sappan* commonly grown in Thailand. Contrarily, [27] observed no lipase inhibitory potential in *Albizia lebbeck* that was grown in Thailand. It can be inferred that species belonging to the same family, irrespective to different climatic conditions, exhibit potent anti-lipase and anti-glycation activities. The relative bio-efficacy can be ascribed to the presence of phenolic and flavonoid compounds as suggested in previous literature [28-29]. However, the plant extract is a mixture of bioactive compounds that contribute to the bioactivities altogether. This suggests that the composition of active compounds, structural features and other potential factors in plant extract may have also affected pancreatic lipase and hyperglycemic conditions. Therefore, the exact relationship between the active compounds and biological activities needs to be further investigated.

Table 2: Percentage inhibition and IC_{50} values observed in anti-lipase and anti-glycation assays.

Plant species	Extracts	Anti-lipase assay (µg/ml)				Anti-glycation assay (µg/ml)			
		Percentage Inhibition			IC_{50} values	Percentage inhibition			IC_{50} values
		100	500	1000		100	500	1000	
A. julibrissin	Methanol	45.89 ± 3.35	60.64 ± 1.06	67.85 ± 4.87	155.2	43.90 ± 4.10	54.25 ± 1.76	82.95 ± 1.34	182.5
C. decapetala	Meth-DMSO	46.60 ± 4.12	62.90 ± 1.95	77.49 ± 3.81	138.7	56.00 ± 2.12	70.60 ± 5.65	89.10 ± 1.41	74.06
	Methanol	48.30 ± 5.72	68.40 ± 0.43	77.50 ± 1.85	114.9	58.50 ± 4.94	69.50 ± 0.70	78.85 ± 3.04	43.69
	Meth-DMSO	41.52 ± 2.83	67.10 ± 3.45	76.56 ± 3.53	168.4	43.00 ± 2.82	53.95 ± 0.07	63.05 ± 1.34	247.3
Control		49.00 ± 1.41	59.00 ± 1.41	81.00 ± 1.41	127.5	69.00 ± 2.82	77.70 ± 0.84	88.50 ± 1.27	17.33

Meth. stands for methanol and DMSO stands for dimethyl sulfoxide; Values in tables are presented as mean ± SD (n=3); IC_{50} value indicates half maximal inhibitory concentration.

Orlistat was used as a control in anti-lipase assay and rutin was used as a control in anti-glycation assay.

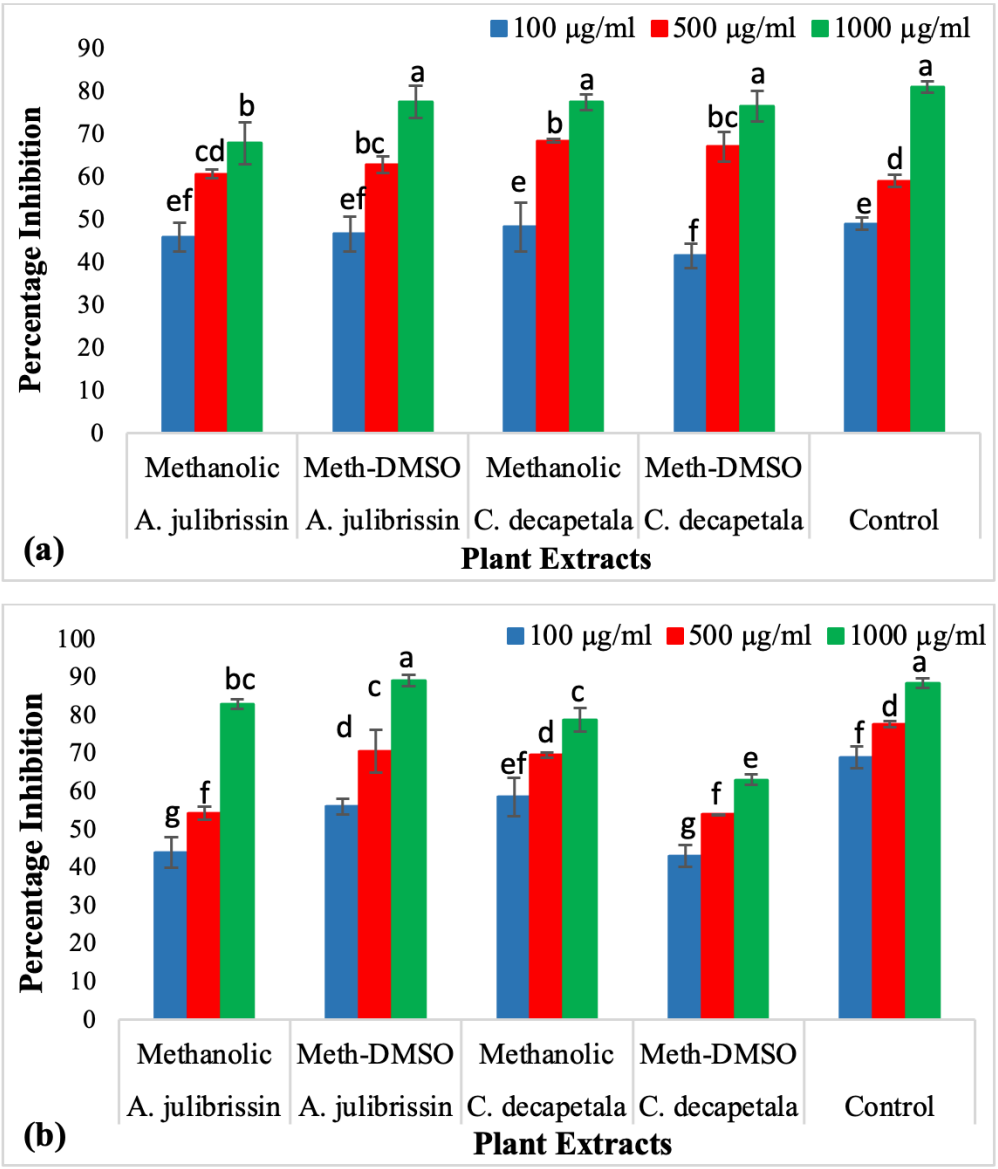

Figure 1: Anti-lipase and anti-glycation activity of leaves extracts of selected species (a) Anti-lipase assay (b) Anti-glycation assay. *Data indicates mean ± SD (3n) and each alphabetical letter (a-g) indicates a significance difference at $P < 0.05$. *Orlistat was used as a control in anti-lipase assay and rutin was used as a control in anti-glycation assay.

Cytotoxic activity

As the methanolic extracts displayed significant biological activities, hence were further tested to observe there *in vitro* cytotoxic potential against three different cell lines. Among different plant extracts, *C. decapetala* showed highest cytotoxic potential against PC3 and 3T3 cell lines by showing IC_{50} value of 144.3 ppm and 201.0 ppm. Contrarily, the lowest activity was recorded in *A. julibrissin* methanolic extract against PC3 cell lines (IC_{50} 459.8 ppm) and 3T3 cell lines (IC_{50} 392.6 ppm). However, the moderate inhibitory potential was observed in both species when tested against HeLa cell lines. Overall, *C. decapetala* leaf extract displayed the highest cytotoxic potential compared to *A. julibrissin* (Table 3).

Table 3: The cytotoxicity effects observed in selected species against HeLa, PC3 and 3T3 cells.

Plant species	Cell lines	Percentage Inhibition at Different Concentrations (ppm)					IC_{50} (ppm)
		30	60	90	120	150	
A. julibrissin	HeLa	0	1.2	4.9	9.7	14.7	285.4
	PC3	0	1.2	2.7	4.3	6.1	459.8
	3T3	-2.8	0	2.8	5.1	7.3	392.6
C. decapetala	HeLa	0	1.1	5.5	11.6	19.6	236.0
	PC3	10.6	21.2	31.8	42.4	53	144.3
	3T3	1.7	3.3	6.2	7.4	23.8	201.0

IC_{50}: Half-maximal inhibitory concentration.

Previously, [30] isolated emodin, baicalein and apigenin from *C. decapetala* roots and confirmed their anti-tumor activities against human gastric carcinoma cell line MGC-803 cell line with IC_{50} values of 15.6, 16.3 and 13.2 µmol/L using MTT assay. Our studies also corroborate the earlier findings [23] who reported highest cytotoxicity (*i.e.* 46.08 µg/ml CC_{50}) for the bark methanol extract of *C. decapetala* on the HeLa cells. Moreover, previous researcher [31] isolated oleanane-type saponins, julibrosides, from the stem bark of *A. julibrissin* and examined their cytotoxic effects against HCT-116, BGC-823, HepG2 and A549 cell lines. Earlier studies [32,33] revealed anti-cancer activity of another specie of *Albizia* genus *i.e. Albizia lebbeck* using MCF-7 (human breast cancer), HeLa and A549 cell lines. However, *A. julibrissin* leaves extracts have been examined for the first time against selected cell lines. The anti-carcinogenic potential of examined species could be attributed to the active compounds such as Julibroside saponins present in *A. julibrissin*[34]. In general, leaves of selected species showed promising results as a cytotoxic agent due to the relatively high toxicity on selected cells. Hence, it can be inferred that the exact mechanism of apoptosis should be investigated using flow cytometry and microscopy techniques.

HPLC analysis

In current study, HPLC method was used to quantify six compounds present in the leaves of selected species. The obtained HPLC chromatograms are presented in Figure 2 (a to d) which indicated that *A. julibrissin* possesses highest concentration of chlorogenic acid (56.06 ppm) and quercetin dihydrate (15.71 ppm) while *C. decapetala* leaves extracts exhibit the highest concentration of gallic acid (73.55 ppm). However, all other compounds were detected in lower concentrations (*i.e.* below 12 ppm) in these species (Table 4).

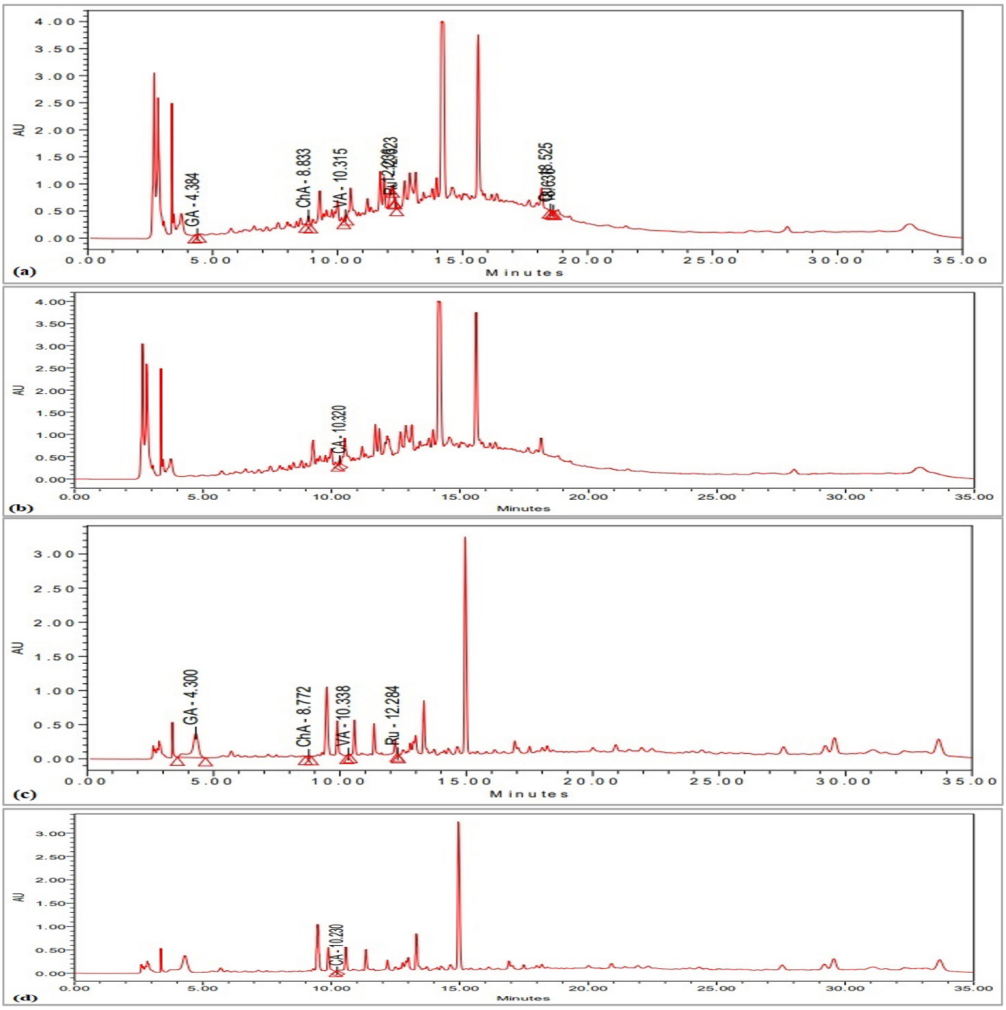

Figure 2: HPLC chromatograms indicating six phenolic and flavonoid compounds at respective retention times in selected species (a) Gallic acid, vanillic acid, chlorogenic acid, rutin trihydrate and quercetin dihydrate in *A. julibrissin* (b) Caffeic acid in *A. julibrissin* (c) Gallic acid, vanillic acid, chlorogenic acid, rutin trihydrate and quercetin dihydrate in *C. decapetala* (d) Caffeic acid in *C. decapetala*.

Table 4. Concentration of six compounds observed in selected species using HPLC.

Plant species	Concentration of compounds (ppm)					
	Gallic acid	Caffeic acid	Vanillic acid	Chlorogenic acid	Rutin trihydrate	Quercetin dihydrate
A. julibrissin	1.75	11.81	6.61	56.06	0.11	15.71
C. decapetala	73.55	0.29	4.92	8.89	1.02	ND

*ND: Not detected

Previously, scientists [13] showed the presence of gallic acid, quercetin, catechin, 4-hydroxybenoic acid and *p*-coumaric acid as the main phenolic compounds in *C. decapetala* extracts using the HPLC technique which has been confirmed in the current study. Similarly, [35] reported eight compounds in *C. decapetala* using UPLC-MS/MS. Furthermore, [36-37] indicated the presence of lignans, triterpenoids saponins and some phenolic compounds in the stem bark of *A. julibrissin*. Besides, cassane diterpenoid, caesaldecan, squalene, lupeol, resveratrol, quercetin, stigmasterol and astragalin, the main phytochemicals observed in *C. decapetala* are terpenoids, flavonoids and tannins[38]. It can be inferred that the selected species possesses a valuable reservoir of polyphenolic compounds of pharmacological significance and thus needs to be isolated and investigated for food and industrial application.

Conclusion

In the light our findings, it can be concluded that the methanolic extracts exhibited the most pronounced biological activities compared to the methanol-DSMO extracts of two selected species. *A. julibrissin* methanolic extract showed highest acetylcholinesterase inhibitory activity while *C. decapetala* methanolic extract displayed highest anti-lipase, anti-glycation and cytotoxic potential. HPLC indicated that *A. julibrissin* possesses highest concentration of chlorogenic acid and quercetin dihydrate while *C. decapetala* leaves extracts exhibited highest concentration of gallic acid. Therefore, the results of this study reinforce the potential therapeutic benefits of *A. julibrissin* and *C. decapetala* methanolic extracts. Further research and clinical trials are required to validate these findings and thereby uncover more evidence of its biological activities.

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