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PHYTOCHEMICAL COMPOSITION OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS*) AND INDIAN JUJUBE (*ZIZIPHUS MAURITIANA*) LEAVES EXTRACTED USING DIFFERENT ETHANOL CONCENTRATIONS

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Abstract: Consumers rely on medical plants for their health benefits. Jackfruit leaves and Indian jujube leaves are commonly used in traditional remedies to address conditions such as ulcers and gout. This study was carried out to determine the phytochemical composition of *Artocarpus heterophyllus* and *Ziziphus mauritiana* leaves by conducting alkaloid and coumarin tests, pigments analysis, and phenolic compound profiling. Antioxidant activity was assessed using DPPH radical scavenging assay on extracts prepared with 50% and 100% ethanol as solvents. The colour (L* value) of each leaf showed significant variation, with the topside being considerably lighter and the underside being noticeably darker. Coumarins were present in the 50% ethanolic leave extracts. *A. heterophyllus* leaves extracted in 100% ethanol had the highest amount of total chlorophyll content (85.95 ± 0.24 mg/g), followed by *A. heterophyllus* leaves extracted in 50% ethanol (50.91 ± 0.30 mg/g), *Z. mauritiana* leaves extracted in 100% ethanol (42.89 ± 1.44 mg/g) and *Z. mauritiana* leaves extracted in 50% ethanol (18.38 ± 2.14 mg/g). Additionally, *Z. mauritiana* leaves contained a higher concentration of caffeic acid (1.49 mg/mL) compared with *A. heterophyllus* leaves (0.15 mg/mL) when extracted with 50% ethanol. Finally, the 50% ethanolic extract of *A. heterophyllus* leaves exhibited higher radical scavenging activity than the 100% ethanolic leaves extract.

Keywords: Sustainability, jackfruit leaf, jujube leaf, health.

Introduction

A medicinal plant is defined as any plant whose components in one or more of its organs can be utilised to synthesise beneficial medications and provide a valuable basis for contemporary drug design. The bioactive components of medicinal plants can be found in various parts, including leaves, flowers, roots, stems, bark or wood (Ai *et al*., 2024; Ralte *et al*., 2024; Taleghani *et al*., 2024; Zemede *et al*., 2024). These components, collectively referred to as phytochemicals, include terpenes, alkaloids, flavonoids, bioflavonoids, benzophenones, xanthenes and certain metabolites, such as tannins, saponins, cyanates, oxalate and anthraquinones. Many plants containing these secondary metabolites exhibit biological potential that includes antimicrobial, antioxidant and other properties (Mwangi *et al*., 2024; Taleghani *et al*., 2024; Zhang *et al*., 2024). Research indicates that medicinal plants, which are a rich source of bioactive compounds, offer significant therapeutic applications against pathogens, including bacteria, fungi and viruses that affect both humans and animals (Daniel *et al*., 2020). Traditional remedies that have been used in modern pharmacotherapy and ancient folk medicine for millennia have become subjects of modern research due to their proven efficacy. These native medicinal plants are also used as food and spices, and are sometimes incorporated into diets for expectant and nursing mothers for medicinal purposes. Traditional medicine, which

is primarily plant-based, has been instrumental in disease prevention throughout human history. Although the exact origins of these medications are unclear, the World Health Organisation (WHO) supports, recommends and encourages the use of traditional remedies despite the significant advances in modern medicine and synthetic drugs (WHO, 2019). Today, traditional remedies are commonly referred to as herbal pharmaceuticals or herbal treatments. Traditional remedies are often preferred due to their fewer side effects (Jeevitha *et al*., 2021).

Jackfruit (*Artocarpus heterophyllus*), a member of the Moraceae family, is native to Southeast Asia. It is extensively cultivated in tropical countries such as Bangladesh, India, Burma, the Philippines, Pakistan, Sri Lanka, Malaysia, and Thailand, as well as in regions of Brazil, Queensland, Africa, and various Australian and American states. Jackfruit contains phytonutrients with anticancer, antiulcer, antihypertensive and anti-aging properties, including lignans, flavones and saponins (Bonsakhteh and Rustaiyan, 2014; Sarkar *et al*., 2023). In Asia, it is used in traditional medicine for its antibacterial, anti-diabetic, antioxidant, anti-inflammatory, and anti-helminthic qualities (Khan *et al*., 2021). According to Khan *et al*. (2021), the *Atrocrapus* plant has been used in traditional medicine for various purposes. The leaves are commonly used to treat ulcers and may aid in managing diabetes due to their hypoglycaemic and hypolipidemic properties. The leaves and stems contain compounds such as sapogenins, cyclooctenone, cycloartenol, β-sitosterol, and tannins, which exhibit estrogenic activity. Additionally, in Indonesia, *Artocarpus* has traditionally been used to treat a range of ailments, including burns, diarrhoea, diabetes, hypertension, and asthma (Fitrya *et al*., 2023).

Jujube (*Ziziphus mauritiana* Lam.), a fruit from the genus Ziziphus and a member of the Rhamnaceae family (Rashwan *et al*., 2020; Seri *et al*., 2020), is known for its delicious taste and numerous health benefits. This family also includes buckthorn (Sareen *et al*., 2020). Jujube is widely cultivated in tropical and subtropical areas, particularly in East Asia, China, India, North Africa and Middle Eastern countries. Globally, there are about 40 species of jujube. It has a long history of use as a traditional remedy (Nairfana *et al*., 2022) and as a vital food source. Various parts of the jujube plant, including its roots, stem, leaves, flowers, and fruits, are used as pharmacological agents (Rashwan *et al*., 2020). According to Seri *et al*. (2020), the thorny shrub *Z. mauritiana*, which grows in the savannah and central and northern regions of Ivory Coast, is used in traditional medicine to treat nervous diseases, anaemia, hypertonia, and other conditions. It is also used to treat various forms of inflammation after it was introduced to the Ivory Coast's forests. The leaves are used to manage liver disorders, fever, asthma (Javed *et al*., 2022), diabetes, high blood pressure, gonorrhoea, and diarrhoea. Additionally, *Ziziphus* leaves are effective in facial and neck bleaching and promoting hair growth (Yahia *et al*., 2020).

Materials and Methods

Sample Collection

A. heterophyllus leaves were obtained from a local farm in Puncak Jalil, Seri Kembangan, Selangor, while *Z. mauritiana* leaves were obtained from a local farm in Darulaman, Jitra, Kedah. Fully developed, green leaves were harvested, avoiding the top and base of the branches. The samples were then placed in zip-lock plastic bags, labelled, and transported to the Food Science laboratory at the Faculty of Applied Sciences, UiTM Shah Alam, Selangor, Malaysia.

Preparation of Leaves Solvent Extraction

Firstly, the mature leaves of *A. heterophyllus* were washed to remove dirt and debris. The samples were then dried in a cabinet dryer at 31.5°C for several days until completely moisture-free. The dried samples were ground

into a coarse powder using a high-speed grinder. Extraction was performed using the maceration method (Javed *et al*., 2022; Nairfana *et al*., 2022; Rosa *et al*., 2022). Subsequently, 10 grammes of *A. heterophyllus* leaf powder were macerated by soaking in 100 mL of solvents—50% ethanol and 100% ethanol—for 24 hours, with stirring every six hours to ensure complete extraction. After 24 hours, the ethanol extract was filtered through Whatman No. 41 filter paper. The obtained extract was then evaporated using a rotary evaporator and stored in the dark at 4°C in the refrigerator until further analysis. The steps were repeated for the *Z. mauritiana* leaves.

Colour Analysis

The colour quality of the samples was assessed using a Hunter Lab colourimeter, following the method described by Shivangi *et al*. (2021). The CIE LAB scale values for colour were determined with the Hunter colourimeter. The L* value, which ranges from 0.0 (black) to 100.0 (white), denotes lightness. Redness (+a* value) to greenness (-a* value) and yellowness (+b* value) to blueness (-b* value) are represented by the other two coordinates, a* and b*, respectively.

Moisture Analysis

About 5g of dried leaf sample were placed in the A&D MX-50 Moisture Analyser and heated at 140°C. The moisture content of the samples was displayed on the screen of the moisture analyser, and the reading was recorded as a percentage.

Alkaloid Test

The alkaloid test was conducted according to the method described by Daniel *et al*. (2020). About 1 mL of the crude ethanolic extract was combined with 8.5 mL of 1% hydrochloric acid. The mixture was warmed to 40°C and then filtered. The filtrate was divided into two equal portions, and treated with the standard solution of Dragendorff's and Mayer's reagents in separate test tubes. The formation of precipitates and any colour change were observed.

Coumarin Test

The coumarin test was performed following the procedure outlined by Daniel *et al*. (2020). To detect the presence of coumarin, 1.5 mL of 10% sodium hydroxide was added to the plant extract, and any resulting colour change was observed.

Pigment Analysis

The photosynthetic pigment chlorophyll was measured by spectrophotometry, following the method described by Wang *et al*. (2020), with some modifications. The absorbance of the leaf extract was measured at wavelengths of 663 nm and 645 nm using a 1700-UV spectrophotometer. The concentrations of chlorophyll-a and chlorophyll-b were then calculated using the following equations:

Chlorophyll a (mg/g)

$$= (12.7 \times A663) - (2.59 \times A645) \quad (1)$$

Chlorophyll b (mg/g)

$$= (22.9 \times A645) - (4.7 \times A663) \quad (2)$$

Chlorophyll total (mg/g)

$$= (8.2 \times A663) + (20.2 \times A645) \quad (3)$$

Phenolic Compound Profiling

Phenolic extracts were separated using High-Performance Liquid Chromatography (HPLC) as described by Dhibi *et al*. (2022). The analysis was conducted using an Agilent 1200 Series system, equipped with a C18 Technochrom Eurosphere 100 analytical column (250 mm x 8 mm) and a UV detector (280 nm). A linear gradient was used to pump the mobile phase, composed of acetonitrile and sulfuric acid/water (2:98 v/v), with a ratio of 80:20%, at a flow rate of 0.5 mL/min. After each extract was passed through a 0.45m membrane filter in 3 mL, 20 L of the mixture was injected directly into the HPLC system. The column temperature was maintained at 25 ± 1°C. Data were processed and stored using the HPLC Chemstation software. Phenolic compounds, specifically caffeic acid, were identified based on their retention times and quantified using an external standard calibration curve.

Antioxidant Activities

The antioxidant activities of the leaf extracts were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay, as described by Murali *et al*. (2021), with some adjustments. Crude extract solutions were prepared at various concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. Then, 2 mL of each concentration was combined with 1 mL of freshly prepared DPPH solution (0.1 mM = 3.943 mg/100 mL in methanol). Ascorbic acid solutions, prepared using the same solvent at concentrations ranging from 0.2 to 1.0 mg/mL, were used as a positive control in the DPPH test. The mixtures were then kept in the dark for 30 minutes. The UV-VIS absorbance was measured at 517 nm, with methanol serving as the blank. For the control, methanol was added to the reaction mixture in place of sample to measure the absorbance. The results were expressed as the percentage reduction in absorbance of the reacting species relative to the control. A decrease in the reaction mixture's absorbance suggests a higher radical-scavenging capacity of the crude methanolic extract. The DPPH free radical scavenging activity was calculated using the following formula:

$$\text{DPPH radicals scavenged} = (A_0 - A_1) / A_0 \times 100\% \quad (4)$$

where
A_0: absorbance of the control solution
A_1: absorbance of the sample

Statistical Analysis

Data were analysed using analysis of variance (ANOVA) and presented as "mean ± standard deviation". Duncan's multiple range test ($p < 0.05$) was applied to validate the procedure and determine the significance of differences. The statistical software SPSS version 29 was used to conduct ANOVA analyses.

Results and Discussion

This study aims to determine the phytochemical composition of *A. heterophyllus* and *Z. mauritiana* leaf extracts through alkaloid and coumarin tests, pigment analysis, phenolic compound profiling, and antioxidant radical scavenging assay of the extracted compounds using 50% and 100% ethanol as solvents.

In this analysis, leaves of similar size were used. After cleaning and drying, the moisture content of the leaves was measured to ensure it was below 10%. Leaves that did not meet this moisture requirement were dried further. The final moisture content was 8.55% for *A. heterophyllus* leaves and 7.78% for *Z. mauritiana* leaves.

Colour Analysis

Table 1 presents the colour scales for both leaves, expressed as L*, a* and b* values. L*, which ranges from 0.0 (black) to 100.0 (white), denotes lightness. Redness (+a* value) to greenness (-a* value) and yellowness (+b* value) to blueness (-b* value) are represented by the other two coordinates, a* and b*, respectively. Based on Table 1, the L* value of the upper side of the leaf is lower than the underside in both *A. heterophyllus* and *Z. mauritiana* leaves. This indicates that the colour is darker, as the scale values of 40.31 for the jackfruit leaf and 38.15 for the jujube leaf are closer to black. Next, the a* value of the jackfruit leaf shows minimal difference between the upper and lower sides, with both sides leaning towards greenness. In contrast, the upper side of the jujube leaf is considerably greener than the lower side. Lastly, the yellowness of the leaf is indicated by the higher +b* values. Both leaves are more yellowish on the lower side, with the jujube leaf having a more pronounced colour difference between the upper and lower sides. The colour description of the leaves is illustrated in Figure 1. According to Manuela and Xu (2020), leaves are adapted to absorb as much sunlight as possible while losing minimising water loss, which contributes to their colour appearance.

Table 1: The colour quality of samples leaf

Samples	View	L*	a*	b*
Jackfruit leaf	Top	40.31 ± 5.04[b]	-5.69 ± 1.18[b]	6.49 ± 1.24[c]
	Bottom	55.09 ± 1.76[a]	-8.37 ± 0.43[c]	13.64 ± 0.91[a]
Jujube leaf	Top	38.15 ± 1.08[b]	-6.53 ± 0.68[b]	9.63 ± 0.62[b]
	Bottom	58.74 ± 0.67[a]	-0.88 ± 0.33[a]	14.66 ± 0.96[a]

*Result for each leaf is presented as Mean ± SD. Mean value in the same column with different letter are significantly difference ($p < 0.05$).

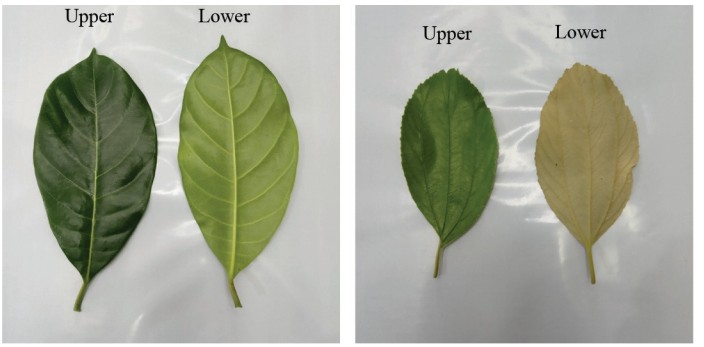


Figure 1: The view of jackfruit leaf (left) and jujube leaf (right)

Phytochemical Screening

Table 2 presents the results of the phytochemical screening of the samples. All extracts tested negative for alkaloids, as no precipitate was observed.

Figure 2 (a) shows the samples prior to the addition of reagents for the detection of alkaloids. The extract solution produced an immiscible layer after the reagents were added, as seen in Figure 2 (b).

Secondary metabolites are a broad class of naturally occurring compounds produced by plants. They play a significant role by helping plants defend themselves against microorganisms and herbivores, as well as attracting pollinators. Additionally. secondary metabolites are used by humans for various purposes, including flavouring agents, aromatisation, and medication production (Sharifi-Rad *et al*., 2021).

Table 2: The summary of the occurrence of phytochemicals of leaf extract

Phytochemicals	Test	Observation			
		Jk 50%	Jk 100%	Jb 50%	Jb 100%
Alkaloids	Dragendorff's test	–	–	–	–
	Mayer's test	–	–	–	–
Coumarins		+	–	+	–

Where (+) Present, (–) Absent. Jk50 and Jk100 represent jackfruit leaves extracted in 50% and 100% ethanol accordingly. Jb50 and Jb100 indicates jujube leaves extracted in 50% ethanol and 100% ethanol, respectively.

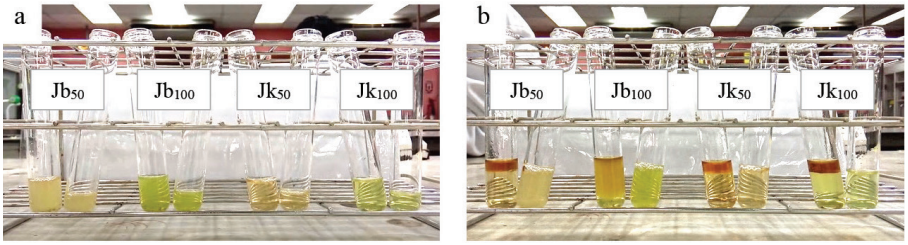


Figure 2: Samples extract before (a) and after (b) the addition of the Dragendorff's (left test tube) and Mayer's reagent (right test tube)

In the research by Nilakandhi *et al*. (2023), alkaloids were absent in the Dragendorff's test, while in Mayer's test showed a positive result, even when jackfruit leaves were extracted using ethanol. Conversely, Thakur *et al.* (2020) found that jackfruit leaves were devoid of alkaloids after extraction with absolute methanol. However, Tambunan *et al*. (2023) reported the presence of alkaloids in the Dragendorff's test using samples from Medan, Indonesia, despite the use of ethanol for extraction. This discrepancy may be attributed to the geographic location where the plant is grown, which could influence the presence of alkaloids (Li *et al*., 2020).

A study by Nairfana *et al*. (2022) revealed that alkaloid compounds were not found in most samples when jujube leaves were extracted using ethanol. On the other hand, the phytochemical screening of jujube leaf extracts by Febriza *et al.* (2022) indicated a positive alkaloid test. However, this result appears to be a misinterpretation, as it was stated that no brown sediment was present, which indicates the absence of alkaloids. Additionally, Egbe *et al*. (2022) found that leaf extracts obtained using different solvents (hexane, methanol, ethyl acetate, chloroform, and aqueous) contained alkaloids. It is important to note that the choice of solvent and the concentration of secondary metabolites can influence the presence or absence of specific compounds in plant extracts.

The colour shift from green [Figure 3(a)] to yellow [Figure 3(b)] indicates the presence of coumarins in the samples extracted using 50% ethanol.

The coumarin content of jackfruit leaves is consistent with Thapa *et al*. (2016), who reported the presence of coumarins in the leaves, highlighting their anti-tumour, anti-fungicidal, and blood-thinning properties. This finding is supported by Moke Emmanuel *et al*. (2019), who also extracted coumarins from jackfruit leaves. Additionally, da Costa *et al*. (2023) found that coumarins, derived from the metabolism of phenylalanine, are widely distributed in plants. Angiosperms are the most common sources of coumarins, which are known for their ultraviolet absorption, distinctive odour, and use as flavourings in food, cosmetics, and cleaning products. The pharmacological properties of coumarins include anticoagulant, hypotensive,

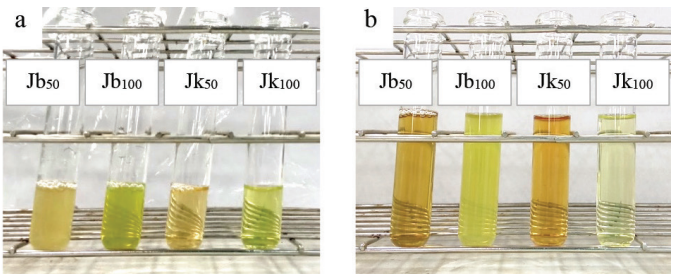


Figure 3: Leaves extract before (a) and after (b) the addition of NaOH

immunosuppressive and hypolipidemic effects, which may be associated with the traditional medicinal uses of *A. heterophyllus* (Costa *et al*., 2023).

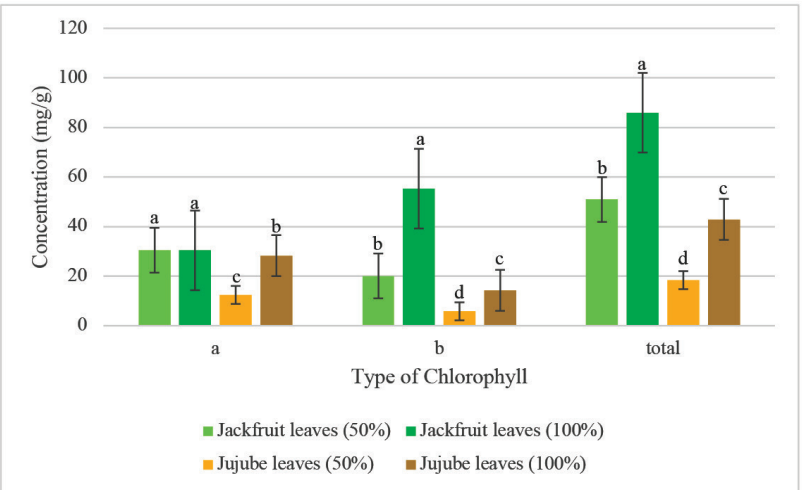
A study by Al Ghasham *et al*. (2017) revealed that jujube leaves contain coumarins, which are significant for their healthcare applications. Additionally, Pansambal *et al*. (2017) confirmed the presence of coumarins in jujube leaves through phytochemical analysis. Thus, the presence of coumarins in both jackfruit and jujube leaves is supported by these studies.

Moreover, over the past few decades, coumarins have been the subject of much phytochemical and pharmacological investigations. More than 400 coumarins have been documented in scientific journals in the last three years. The concentration of coumarins varies among plants. These natural bioactive compounds are known for their pharmacological effects, including anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antituberculous, anticonvulsant, antiadipogenic and antihyperglycemic properties, as well as neuroprotective and antioxidant activities (Sharifi-Rad *et al*., 2021).

According to Akay *et al*. (2021), coumarin, known for its sweet, herbaceous scent is an active component found in many plants. Coumarin and its derivatives are utilised across various industries, including food, cosmetics, and pharmaceuticals. They serve multiple functions, such as optical brightening agents, fluorescent probes, laser dyes, sensitisers in dye-sensitised solar cells, and additives in food and cosmetics. Additionally, coumarins are renowned for their diverse biological and anti-therapeutic activities, including anti-diabetic, anti-coagulant, anti-cancer, anti-viral, anti-microbial, antioxidant, anti-parasitic and anti-inflammatory properties. These attributes contribute to their frequent use as clinical drugs.

Pigment Analysis

The chlorophyll a and chlorophyll b contents in leaves ranged from 12.40 ± 1.97 to 30.45 ± 0.59 mg/g and 5.81 ± 0.17 to 55.30 ± 0.20 mg/g, respectively. Jackfruit leaves extracted in 100% ethanol exhibited the highest total chlorophyll content (85.95 ± 0.24 mg/g), followed by jackfruit leaves extracted in 50% ethanol (50.91 ± 0.30 mg/g), jujube leaves extracted in 100% ethanol (42.89 ± 1.44 mg/g), and jujube leaves extracted in 50% ethanol (18.38 ± 2.14 mg/g), which had the lowest total chlorophyll content. Figure 4 shows a comparison of the various



*Mean values in same type of chlorophyll with different superscript letters (a, b, c) are significantly different ($p < 0.05$).

Figure 4: Comparison of different photosynthetic pigments (Chlorophyll a, chlorophyll b and total chlorophyll) between jackfruit leaves and jujube leaves extracted using 50% and 100% ethanol

photosynthetic pigments found in the extract samples.

According to a photosynthetic physiological analysis by Cai *et al*. (2020), jackfruit leaves contained 2.05 mg/g of total chlorophyll. Acharya *et al*. (2017) reported that jackfruit trees had the highest total chlorophyll content of 2.36 mg/g f.wt. during the summer, which was recorded at 7 am, with values decreasing to 1.53 mg/g f.wt. by the evening (7 pm.). In this study, the jackfruit leaves were collected at 5 pm and the jujube leaves at 12 pm. The findings of this study are consistent with those reported by Acharya *et al*. (2017).

According to a study by Al Mayahi (2016), jujube leaves had a chlorophyll content ranging from 2.48 to 3.07 mg/100 g. In contrast, Khan *et al.* (2019) reported a higher total chlorophyll content in jujube leaves, with a value of 0.2067 mg/g. The findings of the current study show a higher total chlorophyll amount compared to these previous reports.

Leaves extracted using pure ethanol exhibited higher total chlorophyll content, as shown in Figure 4. This is because chlorophylls are non-polar pigments, making organic solvents more effective for their extraction (Osório *et al*., 2020). Ferreira *et al*. (2021) noted that due to their extreme hydrophobicity and minimal water solubility, chlorophylls, which are highly lipophilic substances, are typically extracted from natural sources using solvents such as acetone, dimethylsulfoxide, dioxane, ethanol, and dimethylformamide.

As a complex green pigment found in plants, algae and some bacteria, chlorophyll is essential to photosynthesis as it absorbs light energy and converts it into chemical energy (Wang *et al*., 2020; Roca *et al*., 2024). Generally, chlorophylls contain magnesium as the central metal ion and a phytyl chain ($C_{20}H_{40}$) esterified with the propionic acid moiety at C17, although there are exceptions. Several types of chlorophyll exist, including chlorophyll a, b, c, d, and e. The primary pigments in plants are chlorophyll a and b, with their ratios varying according to the species, climate, and stage of ripening (Roca *et al*., 2024).

Medicinal plants are used to treat a broad range of ailments, including liver diseases, cancer, musculoskeletal disorders, respiratory system disorders, skin diseases, cardiovascular disorders, and even poisonous bites. While chlorophyll is the most well-known pigment associated with these plants, it is important to recognise that other photosynthetic pigments may also contribute to the therapeutic effects of medicinal plants, which are frequently linked to their secondary metabolites. Among the various plant parts used for ethnomedical purposes, leaves are the most commonly utilised. Their benefits include easy accessibility, simple processing, long shelf life, and medicinal qualities derived from their concentration of photosynthates and phytochemicals (Martins *et al*., 2023).

According to Paul *et al*. (2021), chlorophyll is associated with numerous health benefits, including support for the immune, digestive, circulatory, and detoxification systems. Chlorophyll can block the actions of carcinogenic substances and neutralise toxins. Its presence in the body increases the availability of iron in the blood, which is particularly beneficial for pregnant or nursing mothers. Additionally, chlorophyll aids in liver purification and helps regulate blood sugar levels, contributing to overall health and well-being. Chlorophyllin (E141), a green-coloured derivative of the pigment chlorophyll, is used as food additive and alternative medicine. Recent research has highlighted the promising medical and health benefits of chlorophyll.

Phenolic Compound Profiling

The chromatogram profiles are presented in Figures 5 and 6. Standard solutions of caffeic acid (0.01 to 0.08 mg/mL) were used to extrapolate the calibration curve and determine the phenolic compound's concentration in the sample extract. The caffeic acid standard curve was used to calculate the concentration of caffeic acid in the sample extract. The standard curve's regression

equation is y=48887x, where y is the peak area and x is the caffeic acid concentration. According to the calibration curve, the concentration of caffeic acid for the peak area of jackfruit leaves (7296.45 cm^2) and jujube leaves extracted in 50% ethanol (73047.9 cm^2) were 0.15 ± 0.06 mg/mL and 1.49 ± 0.00 mg/mL, respectively (Table 4). However, for both jackfruit and jujube leaves extracted in 100% ethanol, no peak was observed at the average retention time of caffeic acid (2.858 mins), as shown in Table 3.

According to Das *et al*. (2016), caffeic acid was not detected in jackfruit leaves extracted with 75% ethanol. Conversely, Vázquez-González *et al.* (2020) was found in jackfruit leaves when extracted using a combination of ethanol and water (Vázquez-González *et al.,* 2020).

According to Yahia *et al*. (2020), jujube leaves contained 4.95 ± 1.52 µg/g of caffeic acid when extracted with methanol. In contrast, Dhibi *et al*. (2022) did not detect caffeic acid in the methanolic extracts of jujube leaves. Mokhtar *et al*. (2024) conducted a liquid chromatography analysis that identified 15 flavonoids and 20 phenolic compounds in the ethanol extract of jujube leaves. This variability suggests that the extraction solvent's polarity significantly affects the yield of compounds. Polar solvents are more effective at extracting compounds compared to nonpolar solvents. As stated before, caffeic acid was only present in the 50% ethanolic

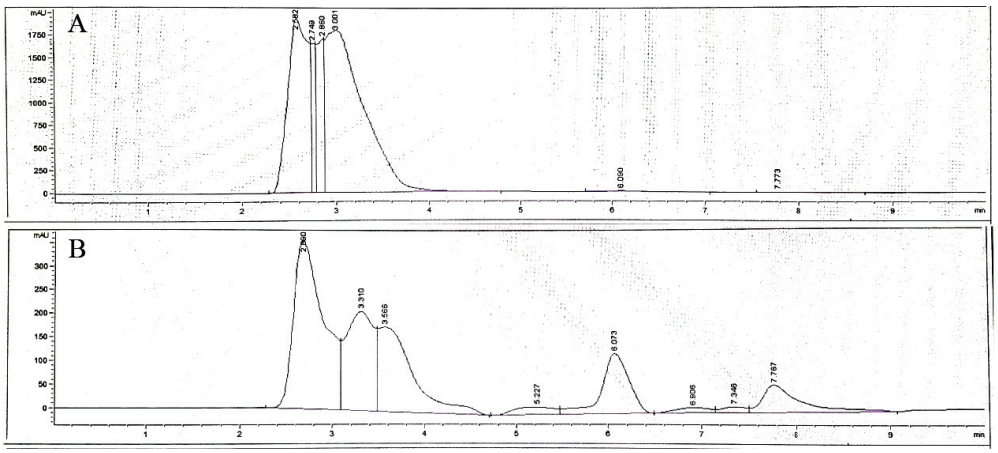


Figure 5: HPLC-DAD phenolic profile of jackfruit leaves ethanolic extract at 50% (A) and 100% (B)

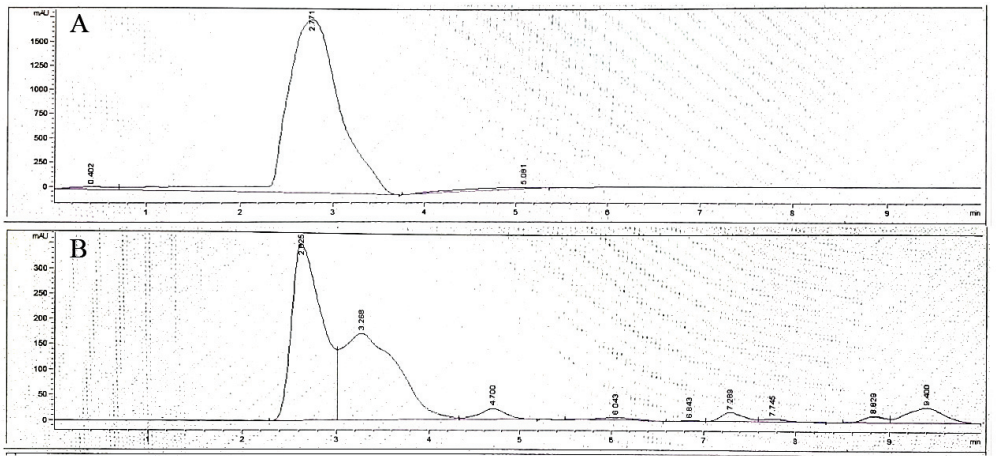


Figure 6: HPLC-DAD phenolic profile of jujube leaves ethanolic extract at 50% (A) and 100% (B)

Table 3: The retention time and peak area of caffeic acid and samples analyses using HPLC

Standard (mg/mL caffeic acid)		Retention Time (mins)	Peak Area (cm^2)
0.01		2.822	785.1
0.02		3.091	1296.2
0.03		2.809	1390.2
0.04		2.790	1981.1
0.06		2.817	2713.3
0.08		2.819	3975.1
Average ± SD		2.858 ± 0.115	
Jackfruit leaves	50%	2.749 2.860	5332.1 9260.8
	100%	–	–
Jujube leaves	50%	2.771	73047.9
	100%	–	–

Table 4: The concentration of caffeic acid in samples extract

Samples	Ethanol Concentration (%)	Concentration of Caffeic Acid (mg/mL)
Jackfruit leaves	50%	0.15 ± 0.06
	100%	–
Jujube leaves	50%	1.49 ± 0.00
	100%	–

extract and not the 100% ethanolic extract. This is due to the additional water component. The presence of water in a 50% ethanol solution compared with 100% ethanol. When combined with ethanol, water—a highly polar solvent—enhances the overall polarity of the solution. This increased polarity improves the solvent's ability to interact with polar substances through hydrogen bonding and other polar interactions.

Caffeic acid (3,4-dihydroxy-cinnamic acid) is a notable member of the hydroxycinnamic acid subgroup, which is a subset of the broader class of polyphenols, known for their potent antioxidant qualities. Found naturally in a variety of foods and drinks, including nuts, herbs, vegetables, fruits and oils, caffeic acid, which is present in plants, is also released when other molecules, such as caftaric acid in grapes or the chlorogenic acids in coffee, are metabolised. Caffeic acid offers several health benefits, including antiviral, anti-inflammatory and anticancer effects. It acts as an antioxidant by mitigating oxidative stress caused by free radicals and has been shown to protect against cancer development. Because of its naturally occurring bioactive compounds, caffeic acid, like other antioxidants, may have a positive anti-wrinkle effect and improve skin elasticity. Caffeic acid's antimicrobial activity further highlights its potential in the treatment of skin diseases, alongside its role in enhancing

collagen production and preventing premature ageing (Birková *et al*., 2020).

Antioxidant Activities

The antioxidant radical scavenging activities of jackfruit and jujube leaves were assessed using DPPH scavenging activity assays with ethanolic crude extracts in 50% and 100% ethanol at five different concentrations (0.2 to 1.0 mg/mL). The results of the % DPPH radical scavenging activities are summarised in Table 5.

In general, the extracts of jackfruit and jujube leaves in both 50% and 100% ethanol did not demonstrate a concentration-dependent increase in radical scavenging activity. However, at a concentration of 0.6 mg/mL, jackfruit leaves extracted in 50% ethanol showed the highest potential to scavenge free radicals (89.17%). At 0.4 mg/mL, the 100% ethanol extract exhibited the maximum radical scavenging activity of 68.72%. For jujube leaves, the highest radical scavenging activities of 84.94% and 87.29% were observed at 0.4 mg/mL for extracts in 50% and 100% ethanol, respectively. As indicated in Table 5, the 50% ethanolic jackfruit leaf extract showed greater radical scavenging activity compared with the 100% ethanolic jackfruit leaf extract. Meanwhile, there was no significant difference in radical scavenging activity between 50% or 100% ethanol extracts for jujube leaves.

A study by Thakur *et al*. (2020) showed that jackfruit leaf extract in methanol exhibited dose-dependent increase in DPPH radical scavenging activity, with an IC_{50} antioxidant activity value of 20.99 µg/ml. Additionally, Sreeja Devi *et al*. (2021) found in Egypt that jackfruit leaf extracts scavenged DPPH by 70% ethanol, showing radical scavenging activities of 21%, 32%, and 51% at concentrations of 0.2, 0.4, and 0.6 mg/mL, respectively. However, Sanna *et al*. (2012) reported that UV-Vis spectroscopy detected minimal changes in peak absorption with higher water content without evidence of aggregation, which may affect dose-dependent activity.

Furthermore, Riaz *et al*. (2021) reported that jujube leaves showed a 62.5% DPPH inhibition, As the concentration of the methanol extract increased, there was a noticeable increase in the DPPH free radical scavenging activity. At a concentration of 100 mg/ml, the methanol extract demonstrated the highest protection of 94.47%. of the results suggest that the extracts' constituents can effectively scavenge free radicals by means of hydrogen-electron donation mechanisms. This ability to prevent the initiation of harmful chain reactions caused by free radicals in susceptible matrices, such as biological membranes, highlights their potential as therapeutic agents for addressing radical-induced pathological damage and promoting consumer health (Egbe *et al*., 2022).

Table 5: The comparison of % DPPH radical scavenging activities between ascorbic acid and samples

Samples	DPPH Radical Scavenged (%)				
	0.2	**0.4**	**0.6**	**0.8**	**1.0**
Ascorbic acid	95.41 ± 0.24^{a}	95.41 ± 0.01^{a}	94.52 ± 0.28^{a}	95.41 ± 0.01^{a}	95.89 ± 0.42^{a}
Jackfruit leaves (50%)	84.31 ± 0.32^{c}	86.82 ± 0.53^{b}	89.17 ± 0.26^{b}	85.70 ± 0.35^{b}	86.48 ± 0.14^{b}
Jackfruit leaves (100%)	53.29 ± 0.49^{e}	68.72 ± 0.20^{d}	66.29 ± 0.37^{e}	68.46 ± 0.20^{c}	65.68 ± 0.11^{e}
Jujube leaves (50%)	68.08 ± 0.36^{d}	84.94 ± 0.14^{c}	84.58 ± 0.91^{d}	84.76 ± 2.43^{b}	84.31 ± 0.05^{d}
Jujube leaves (100%)	87.11 ± 0.26^{b}	87.29 ± 0.26^{b}	86.25 ± 0.30^{c}	86.06 ± 0.20^{b}	85.75 ± 0.39^{c}

* Mean values in the same concentration with different superscript letters (a, b, c) are significantly different ($p > 0.05$)

DPPH, or free 1,1-diphenyl-2-picrylhydrazil radical, is the most widely used method for estimating antioxidant activity. This assay is based on spectrophotometric measurements of how effectively antioxidants can scavenge DPPH radicals. The process involves the reduction of the nitrogen atom's single electron in DPPH to the corresponding hydrazine through the removal of a hydrogen atom from the antioxidants. The DPPH radical is known for its intense and stable colour, characteristics that have made its solution extensively utilised. This radical has been widely applied in polymer chemistry, especially in EPR spectroscopy and in assessing the antioxidant potential of various compounds (Gulcin & Alwasel, 2023).

According to Sinaga *et al*. (2023), free radicals, which are highly reactive atomic species or substances, can cause significant damage to DNA, lipids, proteins, and organs, leading to oxidative reactions. The harm caused by free radicals is associated with a wide range of illnesses, including atherosclerosis, Alzheimer's disease, cancer, liver disorders, neurodegenerative diseases, kidney disease, and premature ageing. Antioxidants play a crucial role in neutralising the effects of free radicals, thereby preventing the onset of these diseases. While free radicals pose a threat to the body, antioxidants offer protection against their damaging effects.

An antioxidant is defined as "any substance that delays, prevents, or removes oxidative damage to a target molecule". These compounds protect against oxidative damage to various molecular targets, including membranes, proteins, lipids, and nucleic acids (Averill-Bates, 2023). Even at relatively low concentrations, antioxidants are effective in inhibiting the oxidation process (Martemucci *et al*., 2022). According to Gulcin and Alwasel (2023), antioxidants neutralise free radicals by reducing oxidative damage in biological processes and donating electrons to the radicals, rendering them harmless. By disrupting the free radical-mediated oxidative process at any of its three main stages—initiation, propagation and termination—antioxidants inhibit the generation of free radicals.

Both external (exogenous) and internal (endogenous) sources of antioxidants are accessible to the body. Exogenous antioxidants, such as vitamins C, A, and E, carotenoids, xanthophylls, flavonoids, phenolics, and polyphenols, are acquired from external sources, while endogenous antioxidants are produced by the body's enzymatic metabolism (Sinaga *et al*., 2023). Diet has increasingly been recognised as a vital source of exogenous antioxidants. as noted by Martemucci *et al*. (2022). Because synthetic antioxidants are toxic, carcinogenic or hepatotoxic to humans, the market for natural antioxidants has supplanted the use of synthetic antioxidants. Naturally occurring dietary antioxidants, found in foods, have garnered a great deal of interest due to their safety. and potential therapeutic and nutritional benefits. Antioxidants are present in various plant parts, including vegetables, fruit, nuts, seeds, leaves (Thakur *et al*., 2020), roots and bark.

Conclusion

Natural food ingredients are safer and more environmentally friendly for human health and the ecosystem. The findings of this study suggest that the ethanolic extracts of *A. heterophyllus* (jackfruit) leaves and *Z. mauritiana* (Indian jujube) leaves, obtained at two distinct solvent concentrations (50% and 100%), could serve as potential natural antioxidant sources that can effectively combat free radicals, when compared with the conventional antioxidant ascorbic acid. The data indicate that both leaves contain significant phytochemical compounds and exhibit active antioxidant properties when extracted in 50% ethanol. It was observed that *Z. mauritiana* leaves had a higher amount of caffeic acid (1.49 ± 0.00 mg/mL) compared with *A. heterophyllus* leaves (0.15 ± 0.06 mg/mL) when extracted in 50% ethanol. Additionally, chlorophyll b and total chlorophyll content were significantly higher in

the 100% ethanolic extracts of *A. heterophyllus* leaves, which also correlated to higher a* values compared with *Z. mauritiana*. Further research on the separation of active ingredients and spectrum analyses could provide deeper insights into the mechanisms of action. This may prove beneficial to the food and pharmaceutical industries in developing a range of products, such as herbal supplements and functional foods, as well as for drug development targeting oxidative stress-related disorders. However, to ensure the long-term effectiveness and safety of these products, careful consideration should be given to assessing their shelf life and stability during the formulation process.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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