Ultrasound assisted extraction and biological profile evaluation of Java plum extract

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Abstract: The present study was designed to evaluate the antioxidant activity of acetone and methanol extracts from different parts of Syzygium jambolanum in aspirin induced ulcerogenic effect. Red leaves, seed and bark of the plant were extracted by several methods (Soxhlet, ultrasound-assisted agitated bed with or without combination) for extracting bioactive compounds. The extracts also differ in TPC and TFC values based on chemical analyses. A great symbolism of its antioxidant power were the highest TPC and TFC values landed by mango peel aqueous ethanol extracts. DPPH radical scavenging assay revealed a different level of inhibition seen within each extract and intra-extracts, with the aqueous ethanol extracts demonstrated the strongest antioxidant properties compared to their other solvent counterparts based on calculated IC50 values. Significance of the results was confirmed using statistical analysis ($p<0.05$). These findings demonstrate important pharmacological applications of java plum which could act as a potent bio-antioxidative agent and also useful in food industry to protect against oxidative stress-related diseases.

Keywords: Syzygium jambolanum, java plum, antioxidant activity, total phenolics, total flavonoids, DPPH assay.

INTRODUCTION

Demand for natural antioxidants has been increasing because of its well established role in reducing the oxidative stress and frailties related degenerative diseases (Leyane et al., 2022; Liguori et al., 2018). Due to the above mentioned remarkable medical outcomes of polysaccharides, they are most certainly appealing for their possible utility concerning functional foods and nutraceuticals (Salanță et al., 2020). Java plum *(Syzygium cumini*), an indigenous source of phytochemicals, especially phenolic and flavonoid derivatives (Alvi et al.; Hafeez, Mansha, et al., 2021) are predominant among various sources (Singh et al., 2018).

Conventional extraction of bioactive compounds from plant materials are done typically using organic solvents and the process is also time consuming (Usman et al., 2022). Such methods can lead to the degradation of sensitive compounds and are not friendly towards the environment (Rasheed et al., 2019). Ultrasound-assisted extraction (UAE) allows higher yields to be obtained in shorter extraction times and with less solvent at atmospheric pressure (Rutkowska et al., 2017). Ultrasonic waves disintegrate plant cell walls & enables bioactive compounds to be released in the solvent (Sanjaya et al., 2022). The application of UAE on several plant materials has been more successful but for Java plum it remains to be exhaustively exploited (Sharma & Dash, 2022).

Although Java plum has been reported to have antioxidant potential, no comprehensive study is available on the optimization of UAE methodology for Java plum along with its biological evaluation (Mahindrakar & Rathod, 2020; Prajapati et al., 2022). Unfortunately, limited studies have been conducted on the use of UAE instead for extracting bioactive compounds from various part such as leaves (Donn et al., 2023), peel or bark of Java plum compared to conventional extraction methods (Arif et al., 2018). In addition, the antioxidant activities of these extracts should be compared based on plant organs and extraction methodology (Yang et al., 2012). This study was designed to gap the information cascaded on literature by applying UAE for extraction of phenolic and flavonoid compounds from leaves (Rrucaj et al., 2024), peel, stem bark in Java plum (Gul et al., 2024). We studied response surface methodology employing UAE in the UAE, and we also compared these with extraction efficiencies using ethanol and methanol extracts (Dahmoune et al., 2014). We also investigated the radical scavenging activity of these extracts using DPPH method to support their antioxidative potential (Aliyu et al., 2013). showed a considerable increase in the extraction of phenolic and flavonoids (Pulungan et al., 2020) with EPA as confirmed by our results when compared to being conventionally treated according to most practice procedures mentioned elsewhere (Ulrich et al., 2019).

The extracts of peel also possessed greater total phenolics and flavonoids, which further confirmed the superior antioxidant activity (AyeshaTariq et al.; Imran et al.). Concentration-dependent improving radical scavenging activity of DPPH was observed, with the peel extract being more effective than leaf and bark extracts which showed highest antioxidant capacity (Noor et al.; Noureen et al.). This observed increase in bioactive compound contents and antioxidant activities provide evidence for the significantly enhanced phenolic content following optimization of UAE conditions.

We conclude from the present study that elevated extraction efficiency and better quality of bioactive compounds like phenolics can be obtained with enhanced recoverability via ultrasound-assisted-extraction in Java plum which itself makes UAE renewable, rapid as well as

efficient method for extractions. The effective free radical scavenging activity of the ethanolic extract of Java plum peel (EJPE) demonstrates its promising application for and as a rich natural source of antioxidant agents in functional food systems or nutraceutical formulations. Further confirmation of the health benefits and therapeutic potential should be provided using in vivo evaluation by future research.

MATERIALS AND METHOD

Sample Collection

Java plum fruits (*Syzygium cumini*) were collected from a local market and sun-dried for two days. Materials and methods The reagents were of high-performance liquid chromatography (HPLC) or at least analytical grade and were used without further purification (Hafeez, Zahoor, et al., 2021; Khushnood et al.). All solvents, Sigma Aldrich and Merck solutions U.S.A., USA The SPE adsorbent used was Amberlite XAD-7 HP resin (Sigma Aldrich, USA) (Wathon et al., 2023a).

Extraction from plant materials

Extraction process used different techniques (Soxhlet, ultrasound-assisted extraction-UAE, agitated bed extraction-ABE and combination of UAE with ABE) (F Hafeez et al., 2022; Iftikhar et al., 2023). Soxhlet extraction: 5 g sample with ethanol or acidified ethanol were extracted in Soxhlet apparatus for 8 h. The UAE was performed as follows: 2.5 g of the sample mixed with 25 mL of ethanol during room temperature for a period that lasts for 2 h, ABE used 2.5 g of sample with 25 mL ethanol and sonicated for two hours at 30 °C in the combined UAE + ABE method to improve yields (in a glass cylinder reactor, Figure S1) Full size image Filtered extracts were then evaporated under vacuum and placed at -18 °C for chemical analysis (Rohadi et al., 2020).

Determination of total phenolic content (TPC)

Total phenolic content (TPC) analysis using Folin-Ciocalteau method One ml extract was mixed with 1 mL of Folin-Ciocalteu reagent. After three minutes, 1 mL of a saturated (50 % w/w) sodium carbonate solution was introduced into the reaction mixture and adjusted to a final volume up to 10 mL with purified water. Thereafter, the mixture was incubated in dark for 90 min at room temperature and scanned an absorbance peak of 725 nm with a UV-visible spectrophotometer (Raza et al., 2015).

Total monomeric anthocyanin (TMA)

Total monomeric anthocyanin (TMA) content was determined by pH differential method. The extract was diluted at 20 mg/10 mL in distilled water. Accordingly, two dilutions (sodium-acetate/acetic acid buffer pH = 4.5 and hydrochloride-potassium chloride-salt solution-buffer pH = 1) were prepared. UV-Vis spectrophotometer was used to measure the absorbance at certain wavelengths, for pH changes (Wathon et al., 2023b).

Total Flavonoids content (TFC)

Total Flavonoid Content (TF) The content of total flavones was measured by mixing 1 mL extract and adding a solution containing 5 %$NaNO_2$ and distilled water to the flask up to final volume of 10mL (Bizuayehu et al., 2016). Then 0.6 mL of aluminum chloride (10%) was added, and after a further five minute incubation 2 ml NaOH (1 M) pup up solution Finally, the volume was made to 100ml with purified water and Absorbance of this solution measured at 510nm (Mahindrakar & Rathod, 2020).

Determination of DPPH

DPPH (1, 1- diphenyl -2- picrylhydrazyl) assay assesses antioxidant potentials with ascorbic acid serving reference. Dissolve 10 mg extract in 10 mL of methanol and vortexing till fully dissolved. The extract was prepared at different concentrations. All test samples were combined with 1 mL of DPPH and 5 ml methanol, vortexed for even mixing than incubated in darkness at room temperature for thirty minutes. The absorbance was recorded using UV-Vis spectrophotometer at 515nm. The positive control was ascorbic acid which the analyses were performed in triplicate (Nurcholis et al., 2022).

(DPPH) Radical Scavenging Analysis

This research was primarily designed to profile IC50 values for DPPH radical scavenging of the CCES/PRFs from each mango component (leaves, peel or bark) (Freeha Hafeez et al., 2022; Nazeer et al., 2023). Samples (0.10-5 mg/mL) were mixed with 5 mL methanol-DPPH solution (0.004%), and the reaction mixture was allowed to equilibrate at room temperature for 30 min. Each solution was measured at 517 nm optical density (OD) (Hidayati et al., 2020).

Reducing Power of CCEs/PRFs

The colorimetric assaying for the reducing power of CCES/PRFS according to their ability so reduce potassium ferricyanide (1.0%) was determined by incubation in sodium phosphate buffer (pH 6.6, 0.2 M) at 50°C for another additional final period of time of twenty minutes The solution was then centrifuged for 10 min, supernatant (980 g) and treated with trichloroacetic acid 10% (5 mL). To 2.5 mL of the supernatant were added distilled water with FeCl3 at 0.1% (0,5mL). The optical density (OD) of the final reaction solution was measured at 700 nm by spectrophotometer (Umamahesh et al., 2016).

Inhibition of Peroxidation by CCEs/PRFs

5 mg of various CCCS/PRFS was ground and homogenized, respectively with 10 mL pure ethyl alcohol; further also added was 0.1 ml linoleic acid under to pH7 by use of sodium phosphate buffer (0.2 m). 5 mL of distilled water was added to the solution, and oxidation was allowed by keeping it at 40°C. Preparation of sample solution 10 mL of 75 % ethanol, 0.2 mL of30 % ammonium thiocyanate and 0.2 ml

magnesium chloride (20 mM in3.5% HCl) were mixed to make up the reagent blank (Ahmad et al., 2023), and the inhibition (Didunyemi et al., 2020), oxidation was calculated as follows:

$$\text{inhibition } \% = 100 - [\frac{As}{Ac}] - 100$$

Statistical Analysis

Data of chemical analysis and antioxidant activity tests in the study on ultrasound-assisted extraction (Siddique et al.) and biological profile were statistically analyzed with AMOVA (Analysis of Variance) using SPSS software. Mean ± SD were calculated and significance of the results for different extraction methods or antioxidant assays was determined by comparison (Lameirão et al., 2020).

RESULTS

Yield Extract

Table 1 shows the yield of various extracts of S. jambolanum leaves, seeds and bark varied from 6.13 – 14.57 g /100g of leaves, 22.36 -34.19 g/100g of seeds and 9.65 –g / 100g of bark. Peel of Malabar plum embody the highest r extraction potency of 36%with aqueous ethanol and 23% with distilled water being the lowest extraction usage. Similarly, java plum or jamun yields were 7.46-13.83 g /100g of leaves, 23.43-34.67 g/100g of seed, and 7.48-15.46 g/100g of bark. Aqueous ethanol extraction embodies the yields of 35 % from jamun seeds. Distilled water features the lowest extraction usage of 23.43%.

The abilities of solvent extraction in decreasing TPC were as follows: Decoction > absolute ethanol > absolute methanol > aqueous ethanol and distilled water. Three types of 80% ethanol extracts - leaves, seeds and bark that was the highest yields extract for each part aiming a potential to extract antioxidants. Skin and bark were superior to leaves in terms of seed extraction. Solvent system and plant part had a significant effect ($p<0.05$) on the yield, with aqueous ethanol having yielded highest (36%), while distilled water was lowest for Malabar plum seeds. Table 2 The seeds of jamun had higher phenolic and flavonoid content as compared to leaves or bark. The concentration of total phenolics (47.54 mg/100g) and flavonoids (55.8 mg/100g) was highest in seeds as aqueous ethanol extract (Noreen et al., 2022; Shoaib et al.).

Table 1: Extract Yields (g/100g Dry Weight) from Malabar Plum Parts

Extracts	Bark	Leaves	Peel
Ethanol 100%	14.71±0.17	10.63±0.29	31.33±0.13
Ethanol 80%	16.12±0.2	14.57±0.18	34.19±0.1
Methanol 100%	11.97±0.14	8.92±0.32	29.79±0.13
Methanol 80%	15.53±0.11	12.61±0.21	32.13±0.11
Distilled Water	9.65±0.24	6.13±0.3	22.36±0.15
Decoction	10.74±0.13	7.07±0.27	27.21±0.12

Table 2: Extract Yields (g/100g Dry Weight) from Jamun Parts

Solvents	Bark	Leaves	Peel
Ethanol 100%	12.4±0.13	10.73±0.31	30.46±0.17
Ethanol 80%	15.46±0.17	13.83±0.2	34.67±0.14
Methanol 100%	11.81±0.24	9.75±0.24	31.73±0.18
Methanol 80%	13.94±0.21	11.86±0.26	29.31±0.15
Distilled Water	7.48±0.22	7.46±0.26	23.43±0.24
Decoction	9.36±0.18	8.39±0.24	27.51±0.16

Total flavonoid and phenolic content

Phenolic and flavonoid content of Dosheri and Anwar

Ratol mango leaves, peel, and bark was analyzed in Table 3 & 4. The results for Dosheri were 8.43-17.96 CE and 7.97-14.79 GAE mg/100g in leaves, 32.62-52.72 GAE and 18.14-30.55 CE mg/100g in peel, and 23.39-36.47 GAE and 8.11-14.73 GAE mg/100g in bark. Anwar Ratol values were 6.98-14.32 GAE and 7.39-14.86 CE mg/100g in leaves, 31.18-38.80 GAE and 35.74-55.80 CE mg/100g in peel, and 9.63-22.19 GAE and 7.83-16.62 CE mg/100g in bark. Aqueous ethanol extract gave higher phenolic and flavonoid content, with s Dosheri peel at 52.72 GAE and 30.55 CE mg/100g and Anwar Ratol at 47.54 GAE and 55.80 CE mg/100g. Phenolic content had a significant difference on the type of solvent was used at $p<0.05$, and the peel had higher phenolic content. Solvent effectiveness decreased in the order distilled water > decoction > absolute ethanol > absolute methanol > aqueous ethanol expressed as. Ethanol is the solvent of choice since it is nontoxic and most effective in extracting phenolics.

Table 3: Total phenolic and flavonoid content in Java plum extracts

Extracts	Total Flavonoid (TFC)			Total Phenolic (TPC)		
	Bark	Peel	Leaves	Bark	Peel	Leaves
Ethanol 100%	12.16± 0.01	48.66± 0.02	10.23± 0.01	16.83± 0.01	42.59 ±0.01	9.58± 0.02
Ethanol 80%	16.62± 0.01	55.8± 0.02	14.86 ±0.02	22.19± 0.02	47.54± 0.01	14.32 ±0.01
Methanol 100%	10.16 ±0.02	46.29± 0.02	9.83 ±0.01	13.04± 0.01	38.8± 0.02	8.3± 0.01
Methanol 80%	15.27± 0.01	52.37 ±0.02	12.47± 0.02	20.53± 0.02	43.63 ±0.01	12.13 ±0.02
Distilled Water	7.83 ±0.02	35.74± 0.01	7.39± 0.01	9.63 ±0.02	31.18± 0.02	6.98± 0.01
Decoction	8.34 ±0.02	41.27 ±0.01	8.82 ±0.01	10.21 ±0.01	34.31± 0.02	7.49 ±0.01

Flavonoids and phenolic content, in peel of Java plum was significantly higher than that observed in leaf or bark. In ethanol peel extract levels of 52.72 mg/100 g in phenolics and 30.55 mg/100 g for flavonoids were the highest detected (Table 3).

Table 4: Total phenolic and flavonoid content in Anwar Ratol mango extracts

Extracts	Total Flavonoid Content (TFC)			Total Phenolic Content (TPC)		
	Bark	Peel	Leaves	Bark	Peel	Leaves
Ethanol 100%	12.53±0.01	24.16 ±0.01	11.93± 0.02	32.49 ±0.01	47.48 ±0.02	11.37 ±0.02
Ethanol 80%	17.96± 0.02	30.55± 0.01	14.79± 0.01	36.47 ±0.01	52.72 ±0.01	14.73 ±0.01
Methanol 100%	11.71 ±0.01	21.61 ±0.02	10.62 ±0.02	30.39± 0.02	45.71 ±0.01	10.68 ±0.03
Methanol 80%	15.53± 0.02	28.71± 0.01	13.43 ±0.01	33.61± 0.01	50.16 ±0.01	12.35 ±0.01
Distilled Water	8.43± 0.01	18.14± 0.02	7.97± 0.02	23.39 ±0.02	32.62 ±0.02	8.11± 0.02
Decoction	10.52± 0.02	20.88 ±0.01	9.54± 0.02	29.73± 0.02	38.36 ±0.02	9.41 ±0.02

DPPH Radical Analysis Scavenging Activity

Malabar plum leaf, seed and bark extracts exhibited IC50 values in DPPH radical scavenging assay of 0.071-0.318 mg/ml, 0.251-0411mg/ml and 0177-0407mg/mg (Table), respectively Ethanolic leaf showed the highest activity with IC50 value of 0.071 mg/ml followed by seed extract in distilled water which exhibited lowest activity (IC50-0.411 mg/ml). Jamun extracts of leaves, peel and bark exhibited IC50 values ranging from 0.047-0.181 mg/ml for leaves, 0.331-0.419mg/ml for Peel and 0.186-372mg/mg data not shown concentration range. The highest scavenging activity was observed in the ethanolic leaf extract (IC50 of 0.047 mg/ml) and lowest of which can be seen with distilled water peel extracts (IC50 to 0.419 mg/ml). The hierarchy set in the scavenging ability of extracts was as follows: Decoction > Absolute ethanol > absolute methanol> aqueous ethanol distilled water. The antioxidant activity of all extracts were smaller compared to the synthetic BHT. Table 5 showed that the aqueous-ethanol extract with Malabar plum leaf produced an IC50

of 0.071 mg/mL, showing greater radical scavenger potential. On the other hand, peel extract dissolved in distilled water recorded a higher IC50 value (0.411 mg/mL), showing slower radical scavenging power.
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Table 5: DPPH IC50 Values of Malabar Plum Parts

Extracts	IC50 Value (mg\mL)		
	Bark	Peel	Leaves
Ethanol 100%	0.219 ±0.63	0.0387 ±0.63	0.073± 0.24
Ethanol 80%	0.186± 0.33	0.331 ±0.57	0.047 ±0.53
Methanol 100%	0.237 ±0.31	0.392± 0.46	0.089± 0.72
Methanol 80%	0.201 ±0.28	0.357± 0.47	0.068± 0.37
Distilled Water	0.372± 0.13	0.419± 0.31	0.181± 0.21
Decoction	0.319 ±0.47	0.407± 0.38	0.146± 0.28

Table 6: DPPH IC50 Values of Jamun Plum Parts

Extracts	IC50 Value (mg\mL)		
	Bark	Peel	Leaves
Ethanol 100%	0.259± 0.23	0.0387 ±0.63	0.086 ±0.72
Ethanol 80%	0.177± 0.17	0.331 ±0.57	0.071 ±0.67
Methanol 100%	0.266 ±0.65	0.392± 0.46	0.11±0.71
Methanol 80%	0.275± 0.71	0.357± 0.47	0.083± 0.39
Distilled Water	0.417 ±0.57	0.419± 0.31	0.318± 0.49
Decoction	0.375± 0.38	0.407± 0.38	0.207 ±0.13

Table 6 strongest radical scavenging activity was recorded in amun leaf extract in aqueous ethanol mixture with IC50 value 0.047 mg/mL. So the seed extract with purified water exhibited highest IC50 value (0.419 mg/mL), indicating its lowest free radical-scavenging efficiency among all extracts tested (Hidayati et al., 2020)..

Antioxidant activity in linoleic acid system

Linoleic acid is a polyunsaturated fatty acid that forms peroxides with oxygen and consequent conversion of Fe^{2+} to Fe^{3+}. Formation of SCN – permits quantification via spectrophotometry at 500 nm. Greater absorbance implies elevated peroxide concentrations and reduced antioxidant activity. The extract from Malabar plum leaf, seed, and bark suppressed lipid peroxidation. The inhibition was 56.81% to 83.45% for leaves, seed inhibition was from 66.21% to 93.17%, and bark inhibition was 67.39% to 92.19%. The aqueous ethanol extract performed excellently with lipid peroxidation inhibition recorded at 93.17%. Seed extracts also had a higher antioxidant activity compared to leaves. For Jamun, linoleic acid oxidation inhibition was 76.43% to 96.12% for leaves, 77.05% to 96.13% for peel, and 68.23% to 88.27% for bark. The aqueous ethanol extract reduced oxidation to 96.13%. Seeds showed the highest antioxidant activity from the order: leaves showed the least activity as absolute ethanol, absolute methanol, extracted in a distillation manner, decoction, and aqueous ethanol. There is a value of 93.17%. The Phenol components rich leaves, seeds and bark significantly ($p<0.05$) inhibited the formation of peroxidation place 96.13% inhibition by their aqueous ethanol extract. Seeds also showed in Table 7 higher antioxidant potential than leaves and bark related to linoleic acid oxidation (Nurcholis et al., 2022).

Table 7: Inhibition of Linoleic Acid Oxidation (%) by Malabar Plum Extracts

Extracts	% Inhibition of linoleic acid oxidation		
	Bark	Peel	Leaves
Ethanol 100%	82.84± 0.59	0.0387 ±0.63	88.43± 0.3
Ethanol 80%	88.27 ±0.71	0.331 ±0.57	96.12 ±0.57
Methanol 100%	79.43± 0.27	0.392± 0.46	84.73 ±0.41
Methanol 80%	85.57 ±0.38	0.357± 0.47	93.51± 0.52
Distilled Water	68.23± 0.47	0.419± 0.31	76.43 ±0.42
Decoction	73.16± 0.52	0.407± 0.38	80.14± 0.47

CONCLUSION

Finally, in the present study we have investigated and characterized thoroughly antioxidant activity of different parts extracts from Syzygium jambolanum (Java Plum) using various assays. Leaves, seeds and bark were the most representative plant parts selected for investigation to understand their antioxidant potential by applying diverse extraction methods and solvents. However, the results showed that different parts and extraction solvents have significantly distinct antioxidant activity. The ethanol extracts, especially the 80%, always presented more total phenolics and flavonoids suggesting that this is a better solvent for extracting these substances. The DPPH radical scavenging assay showed strong antioxidant activity of all extracts; IC50 values below 100 mg means that the extract has potent antioxidative potential. These findings highlight the medicinal values of Syzygium cumini extracts which exhibit strong antioxidant activity and may therefore be beneficial for diseases where oxidation stress plays an important role. Further investigations about those bioactive compounds, which are elicit such effects in the system can be carried out to use them functional foods or Pharmaceutics.

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