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Original Research Article

Phytochemicals and Antioxidant Properties of Ethanol Extract of Terap Fruit Seeds *(Artocarpus odoratissimus)*

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ABSTRACT

Artocarpus odoratissimus commonly called Terap plant is used in ethnomedicine for the treatment of many diseases. The plant contains several secondary metabolites including terpenoids, flavonoids, stilbenoids, arylbenzofurans, and neolignans found in different parts of the plant. However, little research has been conducted on the chemical profiles and antioxidant activity of Terap fruit seeds extract. This study aimed to investigate the chemical profile, and the antioxidant activity of Terap fruit seeds. Terap fruit seeds were extracted by maceration in ethanol (70%). The total phenolic and flavonoid contents of the extract was determined by colorimetric methods. The extract was subjected to liquid chromatography-high resolution mass spectrometry (LC-HRMS/MS) analysis to identify the compounds present. The antioxidant activity was evaluated using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging, and *cupric ion reducing antioxidant capacity* (CUPRAC) assays. The total phenolic and flavonoid contents of the extract were 0.1178 ± 0.0099 mg GAE/g and 0.0598 ± 0.0385 mg RE/g, respectively. LC-HRMS/MS analysis identified 44 compounds, including amino acids, fatty acids, phenolics, nitrogen bases, vitamins, organic acids, carbonyl, and thiazole compounds, with five specific phenolic compounds being noted. The extract demonstrated significant antioxidant activity with IC_{50} values of 25.0984 ± 0.7707 ppm and 17.9385 ± 0.0986 ppm for DPPH and ABTS radical scavenging activity, respectively. The extract also demonstrated high CUPRAC with trolox equivalent antioxidant capacity (TEAC) of 288.9660 ± 3.3264 ppm. These findings suggest that Terap fruit seeds has high antioxidant potential, which can be harnessed for the management of oxidative stress-related diseases.

Keywords: *Terap Plant, Phytochemicals, Total Flavonoid Content, Total Phenolic Content, Antioxidants.*

Introduction

Indonesia is home to one of the highest levels of biodiversity in the world. The country's protected forest areas cover approximately 27.4 million hectares, including 50 national parks, 250 nature reserves, 75 wildlife sanctuaries, 115 natural tourist parks, 23 forest parks, 13 hunting parks, and marine water areas.[1] Although, a rich biodiversity exists with many herbs, most are underutilized, and the public is unaware of their potential benefits.[2]

Artocarpus odoratissimus, commonly known as Terap, is a species of tree in the genus Artocarpus (Moraceae) that occurs in many tropical regions of Southeast Asia, including Borneo. Nearly all parts of this plant are known to possess pharmacological properties, the plant is historically essential to many communities as both an edible plant and as ingredient in traditional medicine.[3] Terap plant is of immense ethnobotanical significance to indigenous populations. [4] In addition to its nutritional and health benefits, the fruits of Terap are also culturally and economically important. Terap seeds can be prepared in many ways: boiled, roasted, salted, dried, or ground to flour form for culinary use.[5] Terap fruit seeds have been shown to possess several pharmacological potentials, such as antioxidant, antibacterial, and anti-cancer activities.[4,6] Literature reports suggest that the conditional metabolites produced by Artocarpus species are terpenoids, flavonoids, stilbenoids, arylbenzofurans, and neolignans,[7] with flavonoids being the most abundant. Essential biological activities combined with significant antioxidant activity characterize Terap plant.[8–12] For example, it was found that the peel, flesh, and seeds of Terap fruit exhibited antioxidant capacity in the ferric reducing antioxidant power (FRAP) assay, with FRAP values of 378.93 ± 20.25 µM/g, 17.92 ± 0.74 µM/g, and 68.06 ± 2.93 µM/g for the peel, flesh and seeds, respectively.[13] Additionally, the antimicrobial and therapeutic potentials of Terap fruit seeds have been demonstrated.[14,15]

Studies on extracts of the peel, seeds, and fruit of *A. odoratissimus* suggest that the plant parts contain phenols and flavonoids with significant alpha-glucosidase inhibitory activity. Compared to the fruit peel, *A. odoratissimus* seed extract contained significantly lower phenol and flavonoid contents.[16] Chemical entities from the plant have been shown to possess significant cytotoxicity activity against human promyelocytic leukemia (HL-60) and breast cancer (MCF-7) cell lines, as well as antioxidant activity against 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical.[17]

Despite the numerous pharmacological properties that has been reported for Terap plant, only a few studies have been done on its antioxidant activity, total phenolic, and flavonoid contents. For this reason, the present study aimed to investigate the phytochemicals and the antioxidant properties of *A. odoratissimus* fruit seeds.

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Materials and Methods

Extraction of Terap fruit seeds

The dried powdered Terap fruit seeds (500 g) were macerated in 70% ethanol at a plant material-to-solvent ratio of 4:5 (g/mL) at room temperature for 24 hours. The extract was filtered and the marc was re-extracted with the same solvent. The process was done three times. The combined extract was concentrated at 55°C using a rotary evaporator (Buchi Rotavapor R-300 from Switzerland) at reduced pressure.

Determination of total phenolic content

The total phenolic content (TPC) was determined following procedures as described in the Indonesian Herbal Pharmacopoeia, Edition II, 2017.[18] The sample solution (100 µL of 500 ppm) was placed in an Eppendorf tube, followed by the addition of 500 µL of 7.5% Folin-Ciocalteu solution. The mixture was incubated for 8 min, and afterward, 400 µL of 1% sodium hydroxide (NaOH) was added and incubated for 1 hr. The absorbance of the resulting mixture was measured at 730 nm. Similarly, blank measurements were performed without the addition of the sample solution. All measurements were performed in triplicate. Gallic acid was used as the reference standard and a calibration curve was prepared using 160, 140, 120, 100, and 80 ppm of gallic acid. The absorbance data obtained were used to calculate the total phenolic content using the formula shown in equation 1 below. The results were expressed in milligram gallic acid equivalent per gram of the sample (mg GAE/g sample).

$$C = \frac{(C1xV)}{w} \quad \text{---------- (1)}$$

Where;
C = Total phenolic content (mg GAE/g sample)
C1 = Gallic acid concentrations obtained from the regression curve (mg/mL)
V = Extract volume (mL)
w = Extract weight (g)

Determination of total flavonoid content

Total Flavonoid Content (TFC) was determined according to the procedure described in Indonesian Herbal Pharmacopoeia Guidelines, Edition II, 2017.[18] To 100 µL of the test sample (500 ppm) in an Eppendorf tube was added 20 µL of 10% aluminum chloride ($AlCl_3$) solution, 20 µL of 1 M ammonium acetate (CH_3COONH_4) solution, 300 µL of ethanol, and 560 µL of distilled water. The mixture was incubated for 30 minutes, and then the absorbance was measured at 413 nm. Rutin at concentrations of 450, 400, 350, 300, 250, and 200 ppm was used as the reference standard for the preparation of calibration curve. The experiment was performed in triplicate. The total flavonoid content was calculated using the formula in equation 2. The result was expressed as milligram rutin equivalent per gram of sample (mg RE/g sample).

$$C = \frac{(C1xV)}{w} \quad \text{---------- (2)}$$

Where;
C = Total flavonoid content (mg RE/g sample)
C1 = Rutin concentrations obtained from the regression curve (mg/mL)
V = Extract volume (mL)
w = Extract weight (g)

LC-HRMS/MS analysis
The liquid chromatography-high resolution mass spectrometry (LC-HRMS/MS analysis of Terap fruit seed extract was conducted using a Vanquish™ UHPLC Binary Pump and a Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer (Thermo Scientific™, Germany) equipped with a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm ID × 2.6 µm). Approximately 5-10 mg of the sample was weighed and dissolved in 1 mL of solvent (water, methanol, or MS-grade acetonitrile). The solution was sonicated for 30 minutes, and filtered using a PTFE syringe filter with 0.22 µm pore size.

MS grade Mobile Phase A was 0.1% formic acid in water, and Mobile Phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.3 mL/min. In the conditioning phase, a mobile phase of 0.1% formic acid in acetonitrile was injected at 5% and increased to 90% over 16 min. This concentration was maintained at 90% for 4 minutes before returning to 5% for 25 minutes. The column temperature was 40°C, and the injection volume was 3 µL.
The XCalibur 4.4 software facilitated data acquisition, and the HRMS mode was configured to enable MS/dd-MS2 capture in positive ionization mode. Nitrogen gas was used as sheath (32 AU), auxiliary (8 AU), and sweep gas (4 AU) during the analysis. The capillary temperature was 320°C, the auxiliary gas heater was 30°C, and the spray voltage was 3.30 kV. It was maintained over the m/z range of 66.7−1000 in Full MS data at 70,000 (dd-MS2, 17,500).
Finally, the sample and solvent blank raw data were analyzed using Compound Discoverer™ 3.2. Compounds identification was achieved by automated database searches and spectral library comparisons (mzCloud™ and ChemSpider™). Identified compounds were organized based on established criteria. The results were tabulated and displayed identified compounds by name and structure in the mzCloud™ and ChemSpider™ databases. Mass annotations were accepted within a range of -5 ppm to 5 ppm, and only named compounds and favored ions from MS2 were reported.

Determination of antioxidant activity
DPPH radical scavenging assay

A stock solution of DPPH (20 mM) was prepared by dissolving 99 mg of DPPH in 12.5 mL of methanol. The stock solution was diluted with methanol (1:66 v/v) to obtain a working solution (0.3 mM) with an absorbance of 0.74 ± 0.02 at 515 nm. A stock solution of the sample was prepared at a concentration of 1000 ppm. Then, 10 µL of the sample solution was added to 190 µL of the DPPH working solution. The reaction mixture was incubated by shaking in a microplate reader for 30 minutes at room temperature. The absorbance was measured at 515 nm.[19] The percentage scavenging activity of DPPH radical (% inhibition) was calculated for different concentrations of the extract and standards using the formulae in equation (3):

% DPPH radical scavenging activity = (A0–A1/A0) × 100 ---------- (3)

Where;
A0 is the absorbance of the control (DPPH without the sample), and A1 is the absorbance of the sample + DPPH.
A linear regression equation was constructed from the concentration and percentage inhibition values on the x and y axes, respectively. The IC_{50} value (50% inhibitory concentration) was determined using the linear regression equation expressed as y = a + bx.

ABTS radical scavenging assay

Potassium persulfate ($K_2S_2O_8$) solution (2.45 mM) was prepared by dissolving 13.25 mg of $K_2S_2O_8$ in 20 mL of distilled water and stored in a dark place at 0-4°C for 6 hours. A stock solution of ABTS (7 mM) was prepared by dissolving 96.02 mg of ABTS in 25 mL of distilled water and stored in a dark place at 0–4°C for 6 hours. The $K_2S_2O_8$ solution was mixed with the ABTS solution in a 1:1 volume ratio and allowed to stand at room temperature for 12–16 hours to produce $ABTS^{\bullet+}$ cation radicals. This solution was diluted with distilled water in a 7:93 (v/v) ratio, resulting in an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 190 µL of the $ABTS^{\bullet+}$ working solution was added to 10 µL of the sample (1000 ppm). The mixture was agitated in a microplate reader for 10 minutes at ambient temperature. Thereafter, the absorbance was measured at 734 nm. Ascorbic acid and Trolox were used as standards. The percentage scavenging of ABTS radical (% inhibition) was calculated for different concentrations of the extract and standards using the formulae in equation (2).[20]

% ABTS scavenging activity = (A0–A1/A0) × 100 ---------- (2)

Where;
A0 is the absorbance of the control and A1 is the absorbance of the sample.
A linear regression equation was constructed from the concentration and percentage inhibition values on the x and y axes, respectively. The *IC_{50} value (50% inhibitory concentration)* was determined using the linear regression equation expressed as y = a + bx.

Cupric ion reducing antioxidant capacity

CUPRAC solution was prepared by mixing copper (II) chloride solution (10 mM), neocuproine solution (7.5 mM), and 1 M ammonium acetate (pH 7) in equal volume. Sample solution (50 µL of 1000 ppm) was mixed with 150 µL of the CUPRAC solution. Subsequently, the reaction mixture was incubated by agitating in a microplate reader for 30 minutes at room temperature. The absorbance was measured at a wavelength of 450 nm. The same procedure was carried out to measure the blank by replacing the sample solution with methanol solvent. Trolox was used as the standard. Subsequently, a Trolox standard curve was constructed with the x-axis representing concentration (ppm) and the y-axis representing absorbance. The antioxidant activity was estimated from the linear regression equation of the calibration curve, and measured in terms of Trolox equivalent antioxidant capacity (TEAC).[9]

Statistical analysis

Values were presented as mean ± standard deviation (SD) of triplicate determination. Data were analyzed using one-way analysis of variance (ANOVA).

Results and Discussion

Total phenolic content (TPC)

Artocarpus odoratissimus (Family Moraceae) commonly known as the Terap plant has been extensively studied for its phenolic and flavonoid contents. Three notable flavonols have been isolated from the chloroform fraction of the Terap leaf extract.[21] The present study determined the total phenolic content of a 70% ethanol extract of Terap fruit seeds using the Folin-Ciocalteu colorimetric method. The Folin-Ciocalteu method is one of the most common approaches to determining total phenolic content in a sample. In this assay, the Folin–Ciocalteu reagent reacts with phenolic compounds to form a blue phosphotungstate–phosphomolybdenum complex (Figure 1). It is a reduction process where the oxidation state of tungsten is reduced from +6 to +5, along with a wavelength shift from yellow to blue. The phenolic content is then quantified by measurement of the absorbance of the complex at 730 nm. It is important to mention that this reaction occurs in an alkaline environment, hence the addition of sodium hydroxide. The basic conditions favours the dissociation of protons in phenolic compounds to form phenolate ions.[22]

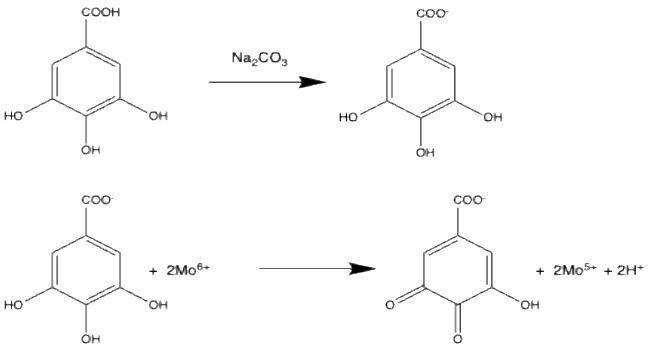

Figure 1: Reaction of Folin-Ciocalteu with a phenolic compound

The total phenolic content of the sample was estimated from the gallic acid calibration curve. The linear regression equation of the curve was obtained as y = 0.0953x-0.0629 with a correlation coefficient (r) of 0.998 (Figure 2). As shown in Table 1, the total phenolic content of Terap fruit seeds ethanol extract was 0.1178 ± 0.0099 mg GAE/g. This value is significantly lower compared to value 13.72 ± 0.87 mg GAE/g previously reported for the fruit seed extract of Terap extracted using 80% ethanol.[13] This suggests that 70% ethanol may be less effective in extracting the phenolic compounds from Terap fruit seeds.

Table 1: Total phenolic content of *Artocarpus odoratissimus* (Terap) fruit seeds extract

Concentration (ppm)	Replicate	Absorbance	TPC (mg GAE/g)	Average TPC (mg GAE/g)
200	1	0.267	0.115	0.1178 ± 0.0099
	2	0.258	0.111	
	3	0.242	0.103	
300	1	0.291	0.127	
	2	0.294	0.128	
	3	0.283	0.123	

TPC = Total phenolic content

The choice of the gallic acid compound as a control compound was because this compound is a simple phenolic compound which is often found in food and plants, where this simple compound has quite good stability.[50] The reactions that occur in this measurement are as follows.[51]

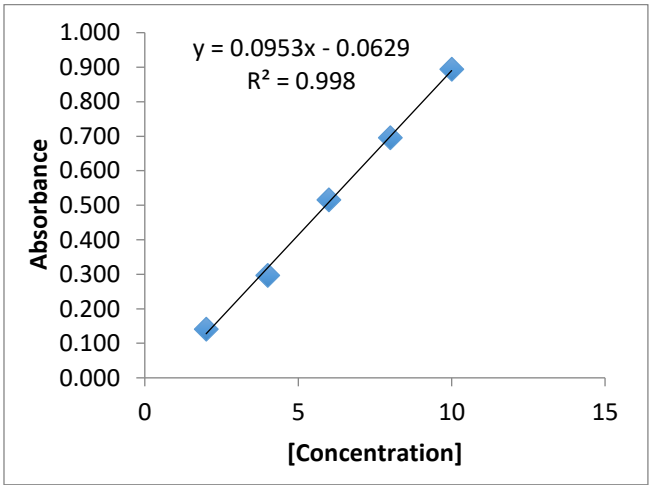

Figure 2: Gallic acid calibration curve

Phenolic compounds are secondary metabolites of plants consisting of at least one aromatic ring and one or more hydroxyl groups.[23,24] They are extracted from various parts of the plants such as the leaves, roots, seeds, flowers, stems, and fruits, they are critical for plants to protect themselves from biotic and abiotic stressors. Various biological activities of plants including antioxidant, antibacterial, anti-inflammatory, and anti-cancer activities has been attributed to their phenolic compounds.[24,25] The biological activity of these compounds depends on factors such as the number and configuration of the hydroxyl group, and the position of double bonds.[25]

Total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method. This method is based on the principle of complex formation between aluminium chloride ($AlCl_3$) and the hydroxyl and ketone groups of flavonoids. Aluminium chloride specifically reacts with ketone group at C-4 and OH group at C-3

(flavonols) or C-5 (flavones or flavonols), yielding a stable yellow complex. Flavonoid contents are usually estimated using quercetin and rutin as reference standards, which are flavonoid compounds containing a ketone group at C-4 and hydroxyl group in neighboring C-3 and C-5 atoms.[26–28] The reaction of $AlCl_3$ with a flavonoid compound is shown in Figure 3.[29]

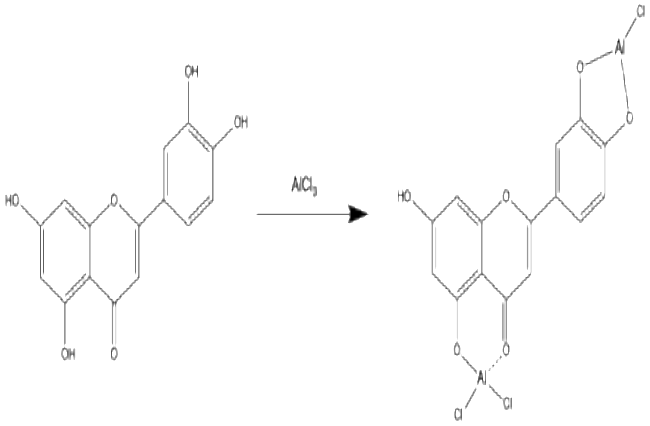

Figure 3: Reaction of $AlCl_3$ with flavonoids

A linear regression equation (y = 0.0014x - 0.0172) with a correlation coefficient (r) of 0.9988 was derived from the rutin calibration curve (Figure 4). The flavonoid contents were determined at concentrations of 50 and 100 ppm, with three replicates for each concentration. The results, as presented in Table 2, showed an average total flavonoid content (TFC) of 0.0598 ± 0.0385 mg RE/g for Terap fruit seeds. This value was again lower compared to that obtained from a similar study where a total flavonoid content of 10.18 ± 0.81 mg RE/g was found in Terap seeds.[13]

Table 2*:* Total flavonoid content of Artocarpus odoratissimus (Terap) fruit seeds extract

Concen-tration (ppm)	Replic ate	Absor bance	TFC (mg RE/g)	Average TFC (mg RE/g)
	1	0.094	0.010	
50	2	0.120	0.040	
	3	0.084	0.030	0.0598 ±
	1	0.144	0.105	0.0385
100	2	0.134	0.078	
	3	0.132	0.096	

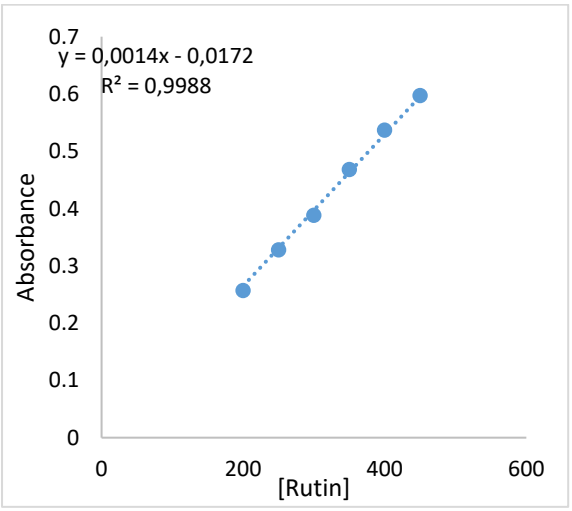

Figure 4: Rutin calibration curve

Compounds identified from LC-HRMS/MS analysis

A total of forty-four (44) compounds ranging from amino acids, fatty acids, phenolics, nitrogen bases, vitamins, organic acids, carbonyl, and thiazole compounds were identified from the LC-HRMS/MS analysis of Terap fruit seeds extract (Table 3). Among the phenolic compounds identified are chlorogenic acid, 4-aminosalicylic acid, kojic acid, scopoletin, and 5-hydroxymethylfurfural were also identified.

Chlorogenic acid (CGA), a phenolic compound from the hydroxycinnamic acid family, is present in tea and coffee. CGA, an ester of quinic and caffeic acids is an important intermediate in lignin biosynthesis.[30] According to Santana-Gálvez et al. (2017), CGA has potential health benefits, mainly acting as antibacterial, antidiabetic, anti-inflammatory, and antioxidant agent.[31] The antidiabetic effects of CGA include inhibition of the enzymes α-amylase and α-glucosidase.[32] It has been suggested that CGA can modulate glucose and lipid metabolism and acts positively in metabolic disorders.[30] The flavour of roasted coffee is attributed to the thermal processes that CGA undergoes during coffee roasting. CGA, together with caffeic and ferulic acids, prevent the formation of various Maillard reaction products (thiazoles, thiophenes, alkylpyrazines) during coffee roasting process by substitution of free amino groups present in the substrates or by binding to an amino group after thermal cleavage of the ester groups.[33]

The compound 4-Aminosalicylic acid (4-ASA), is a complex molecule with interesting pharmacological properties, and it is used in medicine as antituberculosis and antimalarial agent. Recently, studies have been geared towards synthesizing novel 4-ASA derivatives with enhanced anti-inflammatory and antitubercular potentials. Some of these derivatives have been shown to strongly influence cyclooxygenase-2 and 5-lipoxygenase activity, and one derivative was found to be two and a half times more effective against *Mycobacterium tuberculosis* than 4-ASA. 5-aminosalicylic acid (5-ASA) on the other hand is particularly useful in the treatment of inflammatory bowel disease and has a distinct profile among salicylates as a pharmacological agent.[34–36]

Kojic acid has diversified heterocyclic structures and is widely used in cosmetics, medicine, foods, and agriculture. Several fungi of the genus Aspergillus have produced kojic acid, and this compound has been shown to have skin protective properties like UV protection and skin whitening effect; hence, it is used as an ingredient in skincare products.[37]

Various medicinal plants contain scopoletin, a member of the coumarin family of compounds.[38,39] Scopoletin has been shown to possess anti-inflammatory, antioxidant, and anti-tumor effects, and has shown potential for use in the treatment of hypertension, inflammation, thyroid disorders, diabetes, and hyperuricemia.[39]

5-Hydroxymethylfurfural (5-HMF) is an organic compound formed from reducing sugars in honey and other processed foods in the course of the Maillard reaction.[40,41] HMF serves as a chemical marker for assessing the quality of honey and can interfere with various value-added products. HMF has both health benefits and detriments; it plays an important role in the industrial synthesis of furan-2,5-dimethylcarboxylate (FDMC).[42] Studies have demonstrated HMF's antibiofilm activity against Gram-positive pathogens, likely through quorum sensing inhibition. Understanding the structure-function relationships and biosynthesis pathways of HMF is crucial for enzyme management and the industrial production of biobased polymers.[40]

Antioxidant activity

In this study, antioxidant activity was measured using different methods, including 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay, and Cupric ion reducing antioxidant capacity (CUPRAC) assay. The DPPH radical scavenging assay is a sensitive, quick, and simple method for measuring the antioxidant activity of plant extracts. It is based on the scavenging of DPPH radical by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution. The assay measures the reducing ability of antioxidants toward the DPPH radical.[43] The ABTS assay on the other hand is based on scavenging of $ABTS^{\bullet+}$ cation by antioxidants. It is commonly used to evaluate the antioxidant

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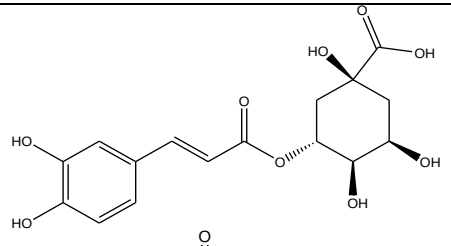
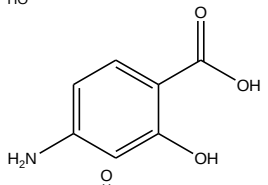
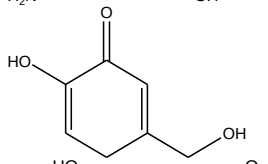
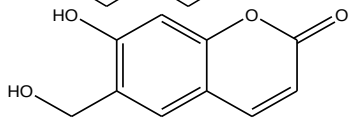
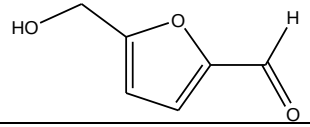
Table 3: Phenolic compounds identified by LC-HRMS/MS analysis of Terap fruit seeds extract

S/N	Compound Name	RT (min)	Molecular Formula	Molecular Weight	mzCloud Best Match	Structure
1	Chlorogenic acid	3.754	$C_{16}H_{18}O_9$	354.09459	99.9%	
2	4-Aminosalicylic acid	1.456	$C_7H_7NO_3$	153.04247	87.8%	
3	Kojic acid	2.172	$C_6H_6O_4$	142.02661	98.7%	
4	Scopoletin	5.773	$C_{10}H_8O_4$	192.04215	96.1%	
5	5-Hydroxymethylfurfural	0.904	$C_6H_6O_3$	126.03166	81.5%	

capacity of a substance by direct reaction of the substance with ABTS$^{\bullet+}$ cation radicals. The shift in hue from blue-green to clear pink serves as a measure of antioxidant activity.[44] ABTS$^{\bullet+}$ is a form of cationic radical produced through a chemical reaction between ABTS and either ammonium persulfate [$(NH_4)_2S_2O_8$] or potassium persulfate ($K_2S_2O_8$).
The ABTS method takes a longer reaction time (12 to 16 hours) compared to the DPPH method which is normally done within 30 minutes.
The quantification of antioxidant activity uses the IC_{50} value, the sample concentration required to inhibit or reduce free radicals by 50%. The strength of antioxidant activity is categorized based on the IC_{50} value: Potent (IC_{50} < 10 µg/mL), strong (IC_{50} between 10 and 50 µg/mL), medium (IC_{50} between 50 and 100 µg/mL), weak (IC_{50} between 100 and 250 µg/mL), and inactive (IC_{50} > 250 µg/mL).[45]
Based on the above, Terap fruit seeds extract is said to have strong antioxidant activity, with IC_{50} values of 17.9385 ± 0.0986 ppm for the ABTS scavenging activity and 25.0984 ± 0.7707 ppm for the DPPH scavenging activity (Table 4).

Table 4: Antioxidant activity of Terap fruit seeds extract

Sample	IC_{50} Value (ppm)		CUPRAC (TEAC in ppm)
	ABTS	DPPH	
Sample	17.9385 ± 0.0986	25.0984 ± 0.7707	288.9660 ± 3.3264
Ascorbic Acid	3.453 ± 0.046	5.109 ± 0.029	
Trolox	8.267 ± 0.104	9.001 ± 0.069	

The observed strong antioxidant activity of Terap seeds extract may be attributed to its phenolic compounds, such as chlorogenic acid, 4-Aminosalicylic acid, kojic acid, scopoletin, and 5-hydroxymethylfurfural as these compounds have been shown to possess significant antioxidant activity.[46-51]
The cupric ion reducing antioxidant capacity (CUPRAC) assay is based on the reduction of Cu(II) to Cu(I) by antioxidant compounds. The chromogen in this colorimetric method is the copper(II) bisneocuproine cation ($Cu(Nc)_2^{2+}$); it changes colour upon complexation with a coordinating species. In this assay, a light blue copper(II) bis(neocuproine) cation is reduced by antioxidant substances to form the orange-yellow chelate of the copper(I) bis(neocuproine) cation, which absorbs UV light at 450 nm.[52]
The rapid oxidation of thiols-type antioxidant by the CUPRAC reagent makes this method superior to other antioxidant measurement techniques.
The result of the CUPRAC assay was expressed in terms of Trolox Equivalent Antioxidant Capacity (TEAC). In this study, a TEAC value of 288.9660 ± 3.3264 ppm was obtained for the antioxidant activity of Terap fruit seeds extract using the CUPRAC assay. This implies that the antioxidant activity of 1 gram of Terap seed extract is equivalent to 288.9660 ± 3.3264 mg of trolox.

Conclusion

Artocarpus odoratissimus commonly known as Terap, has been extensively used ethnobotanically as a medicinal plant. It contains many secondary metabolites such as terpenoids, flavonoids, stilbenoids, arylbenzofurans, and neolignans. LC-HRMS/MS analysis of Terap fruit seeds identified forty-four compounds, five of which are phenolic compounds which include chlorogenic acid, 4-aminosalicylic acid, kojic acid, scopoletin, and 5-hydroxymethylfurfural. Terap fruit seeds ethanol extract has relatively low total phenolic and total flavonoid contents of 0.1178 ± 0.0099 mg GAE/g, and 0.0598 ± 0.0385 mg RE/g. Terap fruit seed extract possesses strong antioxidant activity with IC50 of 25.0984 ± 0.7707 ppm and 17.9385 ± 0.0986 ppm in the DPPH and ABTS radical scavenging activity, respectively. The extract also demonstrated high cupric ion reducing antioxidant capacity (CUPRAC) with Trolox Equivalent Antioxidant Capacity (TEAC) value of 288.9660 ± 3.3264 ppm. These findings suggest that Terap seeds extract has high antioxidant activity which may be attributed to its phenolic content.

Conflict of interest

The author reports no conflicts of interest in this work.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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