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Phytocomponents analysis and antioxidant activity of Malacca fruit extract (*Phyllanthus emblica*) using three different solvents

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Abstract. *Nazaruddin, Rosmaidar, Siregar TN, Wahyuni S, Sutriana A. 2024. Phytocomponents analysis and antioxidant activity of Malacca fruit extract (*Phyllanthus emblica*) using three different solvents. Biodiversitas 25: 1911-1919.* Malacca plant (*Phyllanthus emblica* L.) grows widely in Aceh Province, Indonesia representing a wide group of phytochemical components of medicinal usage. This study aims to assess the antioxidant activity and bioactive compounds in *P. emblica* fruit extract, which was extracted by subsequent extraction using n-hexane, ethyl acetate, and ethanol. The extracts were then subjected to phytochemical and GC-MS to identify and quantify the bioactive compounds and measure the radical scavenging using DPPH assay to estimate the antioxidant activity. The results showed that *P. emblica* fruit extracted with ethanol and ethyl acetate contained flavonoids, phenolics, terpenoids, and saponin compounds, while n-hexane extract only contained steroids. The antioxidant activity of ethanol, ethyl acetate, and n-hexane extracts was very strong, with IC_{50} values of 18.81, 20.75, and 31.01 ppm, respectively. The highest antioxidant activity was found in the ethanol extract of *P. emblica* fruit. The GC-MS analysis revealed that the most abundant chemical component in ethanol and ethyl acetate extract was 1, 2, 3 benzenetriol, while in n-hexane extract was eugenol. Based on bioactive components and antioxidant activity, ethanol is suggested as the optimal solvent to gain a high content of polyphenols and flavonoids as well as high antioxidants from *P. emblica* fruit for utilization in pharmacognosy.

Keywords: Antioxidant activities, GC-MS analysis, Malacca fruit, phytocomponent

INTRODUCTION

Malacca (*Phyllanthus emblica* L.) is a deciduous tree with a size of small to medium up to 5.5 m that has enormous benefits for health. All parts of *P. emblica* plant (twigs, roots, leaves, flowers, seeds, and fruit) can be used as herbal medicine because they have high medicinal properties and are valuable for therapeutic efficacy (Yadav et al. 2017). All components of the *P. emblica* plant have been greatly utilized in various traditional medicines, including Indian medicine, Greek Arabic medicine, Tibetan medicine, and traditional Chinese medicine (Krishnaveni and Mirunalini 2010; Kumar et al. 2012).

Many pharmacological investigations have found that the therapeutic properties of *P. emblica* plants concentrate in the fruit, which is an excellent source of chemical metabolites, including saponins, flavonoids, tannins, steroids, and glycosides (Poltanov et al. 2009; Luo et al. 2011). Several flavonoid compounds found in *P. emblica* fruit are kaempferol-3-O-α-L-(6″-ethyl) rhamnopyranoside, kaempferol-3-O α-L-(6″- methyl)-rhamnopyranoside, β-amyrin, β-amyrin-3-palmitate, betulinic acid, daucosterol, oleanolic acid, triacontanoic acid, triacontanol, ketone, lupeol acetate, gallic acid, ursolic acid, quercetin, and bisabolane (Habib-ur-Rehman et al. 2007; Pientaweeratch et al. 2016). These secondary metabolite compounds, known as antioxidants, protect biological components such as lipids, proteins, vitamins, and DNA by inhibiting damage by free radical oxidation and providing electron/electron donor or reductant compounds (Liu et al. 2008a). The metabolite content of *P. emblica* fruit has been confirmed to have pharmacological effects such as anticancer (Chatterjee et al. 2011), anti-aging (Chaikul et al. 2021), hepato-protective, immuno-modulatory (Khan 2009), to cure Alzheimer's disease (Jang et al. 2017), anti-oxidation, anti-inflammation, anti-viral and lowering blood pressure, blood lipid, and blood glucose (Yan et al. 2022).

The bioactive components of a plant material can be obtained by extraction. The extraction technique greatly determines the obtainment of bioactive components in quantity and quality (Gaire and Subedi 2014; Alkaabi et al. 2023). There are various extraction techniques, both conventional and modern, and each extraction technique has its advantages and disadvantages. The extraction

technique and solvent influence the extraction result and the produced extracts' biological activity (Mahdi-Pour et al. 2012; Kaur et al. 2023). Many individual solvents have been used to extract the bioactive compounds from *P. emblica* fruit, including polar, semi-polar, and non-polar solvents (Albadwawi et al. 2022). However, due to the diversity of bioactive substances in plant materials and their varying solubility traits in different solvents (Ajanal et al. 2012), the optimal solvent for extracting *P. emblica* fruit must be evaluated. The choice of hexane in the extraction process as the first solvent so that the fat components in the material can be separated first, aims not to hinder the release of the active ingredient in the extraction process with other solvents. The hexane solvent also functions to release active steroid/terpenoid compounds. The ethyl acetate solvent can attract components such as phenols, terpenoids, and alkaloids, while the methanol solvent can attract components such as alkaloids, phenolics, and carotenoids (Sulasmi et al. 2020; Abubakar and Haque 2022; Halim et al. 2022).

To examine and quantify the levels of bioactive compounds found in natural and biological systems based on their polarity, molecular size, and chemical nature, researchers have widely used the Gas Chromatography-Mass Spectroscopy (GCMS) method (Aldhanhani et al. 2022). GC-MS is a simple, sensitive, and effective method to separate and detect the biologically active substances contained in the plant extract (Uma and Balasubramaniam 2012; Meechai et al. 2016). No research has identified and compared the phytocomponents of the ethanol, ethyl acetate, and n-hexane solvents using GC-MS. Therefore, this research was carried out to screen the active compounds in *P. emblica* fruit using 3 different solvents and analyze the antioxidant activity of *P. emblica* fruit using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method before being applied as therapy for streptozotocin-induced diabetic rat.

MATERIALS AND METHODS

Plant sample authentication and preparation

The *P. emblica* fruit samples were collected in Krueng Raya, Aceh Besar, Indonesia. The fresh matured fruits were chosen based on similar size, shape, color, and ripening stages which were assessed by visual appearances. An acknowledged taxonomist at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia (No: 713/UN11.1.8.4/TA.00.01/2022) identified and authenticated the samples.

A fresh sample of *P. emblica* fruit was then washed using running water to remove dirt attached to the fruit sample. The washed samples were air-dried without being exposed to direct sunlight. The dried samples were crushed using a blender to obtain fine powder, sieved using a sieve, and then weighed.

Extraction of *Phyllanthus emblica* fruit

The extraction process was performed by successive macerations using increasing polarity solvents (n-hexane, ethyl acetate, and 96% ethanol). The ratio of *P. emblica* fruit powder and solvent was 1:10. A total of 250 g fine powdered *P. emblica* fruit was weighed, put into a tube container, and then macerated with 2.5 L of n-hexane solvent for 72 hours. The n-hexane solution of *P. emblica* fruit was filtered by filter paper to separate the simplicia and filtrate of *P. emblica* fruit. After filtration, the *P. emblica* fruit was re-macerated with 2.5 L of ethyl acetate solvent for 72 hours, after which it was filtered again. Finally, this simplicia was macerated using ethanol as performed in the maceration process with n-hexane and ethyl acetate solvents. The filtrates from all solvents were then evaporated at 40-60°C temperature using a vacuum rotary evaporator to remove the remaining solvents. The obtained extracts were then packaged in a well-closed dry container and stored at 4°C for further analysis.

The yield of the concentrated extract was calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of concentrated extract}}{\text{Weight of simplicial}} \times 100\%$$

Phytochemical screening

Phytochemical screening of active compounds from simplicia powder of *P. emblica* fruit extract includes examination of alkaloid, flavonoid, steroid, terpenoid, tannin, saponin, and phenolic compounds. This screening was performed on 3 types of extracts dissolved in 3 different solvents. This phytochemical screening was performed to follow the method of Harborne (1984).

Antioxidant activity analysis

Antioxidant activity was performed as described previously by Fidrianny et al. (2018) with some modifications. The extract stock solution was made by dissolving 50 mg of extract in 500 mL of methanol to obtain a 1000 ppm solution. The solution was then diluted into five different concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm). At each concentration, methanol was added to a 10 mL volumetric flask up to 10 mL. Next, 0.5 mL of the extract solution prepared for the antioxidant activity test was placed into a 10 mL volumetric flask. Add 1.5 mL of 40 ppm DPPH solution to each concentration and vortex for 2 seconds. After that, the samples were incubated for 30 minutes at 37°C in the dark room. This was conducted to optimize DPPH activity so that the reaction occurs between DPPH and the tested sample (Sharma and Bhat 2009). Each extract was performed in triplicates. Next, a UV-Vis spectrophotometer was used to read the absorbance with a wavelength of 517 nm. The ascorbic acid preparation as a standard solution was performed by diluting 5 mg of ascorbic acid in 100 mL methanol (100 ppm). The solution was then diluted to obtain concentrations of 0 ppm, 2 ppm, 6 ppm, 8 ppm, and 10 ppm. Each standard concentration was processed similarly to the extracted sample. The percentage inhibition was measured using the formula as follows:

$$\text{\% Inhibition} = \frac{(\text{A DPPH} - \text{A sampel}) \times 100\ \%}{\text{A DPPH}}$$

Where: A is absorbance.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was conducted at the Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Banda Aceh. Samples were analyzed using an ISQ™ 7000 Single Quadrupole GC-MS System (Thermo Scientific) Triple off-axis Thermo Scientific™ DynaMax™ XR detection system. The GC system has the Thermo Scientific™ AI/AS 1310 Series Autosampler and System Qualification Column (SQC) column with a 30 m ×0.25 mm ID × 0.25 µm column length. Helium was used as the gas carrier at a constant 1 mL/min flow rate. The time was 60 minutes with an injector temperature of 260°C, detector 250°C, and column 325°C. Commercial mass spectral library (latest edition) options, including NIST Mass Spectral Library with RI and MS/MS, Wiley Mass Spectral Library, and Maurer/Pfleger/Weber Mass Spectral Library for drugs, poisons, pesticides, pollutants, and their metabolites.

Data analysis

Data were analyzed descriptively.

RESULTS AND DISCUSSION

Phytochemical screening

One parameter that influences the extract's quality is the extract yield percentage. The extract yield was determined by dividing the extract weight by the weight of the simplicia used. Therefore, achieving high extraction yields and different biological activities depends on the extraction medium with solvents with appropriate soluble affinity (Lefebvre et al. 2021). In this study, the *P. emblica* fruit was extracted using 3 different solvents with different polarities: n-hexane, ethyl acetate, and ethanol. The yield of the resultant *P. emblica* fruit extract, which was extracted using different solvents, ranged from 1.55 to 3.25 % (Table 1). Among the solvents used, the ethanol extract yielded the highest, followed by n-hexane and ethyl acetate.

In a previous report, El Husna et al. (2022) reviewed that the extract yields of *P. emblica* fruit ranged from 0.7% to 96%. The extraction yield obtained in this study was considered low for all solvents. Under the same raw material conditions (fruit powder), the yield is affected by the extraction time, which is 21.5% for extraction using methanol for 24 hours (Liu et al. 2008b) and reaches 56.25% when extracted with alcohol for 7 days (Alagar et al. 2014). The longer soaking time softens the cell wall of the material, thus releasing more bioactive components (El Husna et al. 2022). However, Ghosh et al. (2021) observed that *P. emblica* fruit extracted with ethanol for 7 days produced a 23.1 % yield. The difference in yield might be due to the different conditions of the raw materials used and differences in extraction time and solvents used. Kumari and Khatkar (2016) stated that the compatibility of solvents and extracted compounds regarding their degree of polarity is very important in assisting better and maximum extraction, which is also evident in this study.

The phytochemical screening tests showed that *P. emblica* fruit contains secondary metabolite compounds such as flavonoids, terpenoids, steroids, phenolics, tannins, alkaloids, and saponins (Table 2). Different solvents used in the successive extraction process will produce different active compounds. The results of extraction with hexane solvent only obtained steroid compounds; ethyl acetate solvent obtained flavonoid, steroid, phenolic, tannin, and saponin compounds; while extraction with ethanol solvent obtained bioactive components of flavonoids, terpenoids, phenolics, tannins, and saponins.

The present study showed that solvents had a great effect in extracting bioactive components from *P. emblica* fruit. Due to the different degrees of polarity of solvents and compatibility of the compound with the solvents, extraction of flavonoid, phenolic and tannin compounds was varied. Ethanol and ethyl acetate were efficient solvents for extracting flavonoid, phenolic, and tannin compounds (Table 2). Similarly, Do et al. (2014) reported that ethanol was an effective solvent for extracting polyphenols linked to the polar fibrous matrix and was safe for human consumption. Polyphenols illustrate at least 10,000 compounds containing aromatic rings with over one hydroxyl group attached (Rudrapal et al. 2022). The most common dietary polyphenols are flavonoids, phenolic acids, tannins, lignans, anthocyanidins, catechins, and stilbenes (Rudrapal et al. 2022). As a secondary plant metabolite, they are abundant in most vegetables, herbs, and fruits (Roy et al. 2022).

Antioxidant activity test

DPPH test is generally conducted to evaluate the antioxidant activity of crude extracts. DPPH assay is considered a widely used, rapid, and economical method to evaluate the antioxidant activity of different foods using different solvents. Determination of the absorbance of each sample concentration was determined by a spectrophotometer at a wavelength of 517 nm. At this wavelength, the extract has the antioxidant ability to inhibit DPPH free radicals, which are stable free radicals in solution and the oxidized form with strong absorption capabilities (Kaur and Kapoor 2002). The antioxidants' ability to inhibit free radicals is defined as % inhibition. Figure 1 depicts the correlation between sample concentration and % inhibition of free radicals from *P. emblica* fruit extract for each solvent.

The parameter used to demonstrate antioxidant activity is the Inhibition Concentration (IC_{50}) value, which indicates DPPH radical scavenging activity (Senevirathne et al. 2006). The lower the IC_{50} value is the higher the antioxidant activity (Molyneux 2004). Table 3 shows the antioxidant activity of *P. emblica* fruit extract using different solvents.

Table 1. The yield of *Phyllanthus emblica* fruit extract after extraction using ethanol, ethyl acetate, and n-hexane

Solvents	Simplicia (g)	Extract (g)	Extract yield (%)
Ethanol	200	6.5	3.25
Ethyl acetate	200	3.1	1.55
n-Hexane	200	4	2.00

Table 2. Phytochemical screening of *Phyllanthus emblica* fruit extracted with ethanol, ethyl acetate, and n-hexane solvents

Compound	Ethanol extract	Ethyl acetate extract	n-Hexane extract
Flavonoids	+	+	-
Terpenoids	+	-	-
Steroids	-	+	+
Phenolic	+	+	-
Tannin	+	+	-
Alkaloids			
DD	-	-	-
Meyer	-	-	-
Wagner	-	-	-
Saponin	+	+	-

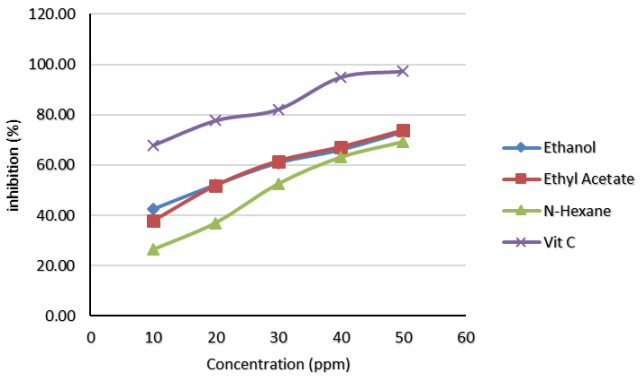

Figure 1. Antioxidant activities (% inhibition) of various concentrations of *Phyllanthus emblica* fruit extracted with different solvents

Table 3. The IC_{50} values (ppm) of DPPH scavenging activity of *Phyllanthus emblica* fruits extracted with different solvents

Extract	IC_{50} (ppm)
Ethanol	18.81±0.34
Ethyl acetate	20.75±5.16
N-Hexane	31.01±1.06

Based on the IC_{50} calculation results, it showed that the *P. emblica* fruit extract with all the solvents used has an IC_{50} value below 50 (n-hexane extract=31.01; ethyl acetate=20.75; ethanol=18.81), which indicated that the extract with all these solvents was classified as an antioxidant with very strong abilities. According to Molyneux (2004), the classification of antioxidant activity was split into 5 categories: >200 ppm (very weak), 150-200 ppm (weak), 100-150 ppm (moderate), 50-100 ppm (strong), and <50 ppm (very strong). This study revealed that the lowest IC_{50} values were displayed in ethanol extract, tailed by ethyl acetate extract, and the lowest was found in n-hexane extract. Addina and Harahap (2023) have confirmed that the use of different solvents influenced the IC_{50} of the *P. emblica* bark and fruit extract, whereby *P. emblica* fruit extracted using ethanol possessed IC_{50} of 729.66±117.03 ppm while using n-hexane was 3,345.85±278.38 ppm.

This study also revealed that the antioxidant activity (% inhibition) of *P. emblica* fruit extracted using ethanol and ethyl acetate was stronger than that of *P. emblica* fruit extracted using n-hexane. Generally, phenols and flavonoids are polar compounds, so the compound will easily dissolve in polar or semi-polar solvents. However, those compounds could not be extracted perfectly in an n-hexane solvent (a non-polar compound), consequently cannot optimally reduce the DPPH radical activity (Mustarichie et al. 2017) which resulting in lower antioxidant activity, as observed in this study. Earlier, Kumari and Katkhar (2016) had been observed that the ethanolic extract of *P. emblica* fruit possessed higher antioxidant activity than the ethyl acetate extract.

Ngo et al. (2017) stated that solvents used significantly affect the extraction yield and bioactive compound content and will consequently affect the biological activity of the extract, which is also evident in this study. The ethanolic extract was the most potent in the present study regarding IC_{50} values of DPPH scavenging activity among the extracts tested. This might be because the ethanolic extract of *P. emblica* fruit contained the highest level of flavonoid, phenolic, alkaloid, and terpenoid compounds (Kuppusamy et al. 2015). These compounds have strong antioxidant activity and hence remove various reactive oxygen species to protect the body from oxidative stress, including peroxynitrite, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and superoxide anions (Chao et al. 2014; Chaudhary et al. 2023).

Previously, the antioxidant activity of *P. emblica* fruit was reported by Halim et al. (2022), who demonstrated *P. emblica* fruit ethanolic extract ability as an antioxidant with an IC_{50} value of 7.63 µg/dL. Several researchers have also pointed out that *P. emblica* fruits are a potential source of strong antioxidants (Liu et al. 2008b; Saha and Verma 2015; Cahyaningrum et al. 2020; Prananda et al. 2023). In addition, Scartezzini et al. (2006) found that processed *P. emblica* fruit poses higher antioxidant activity than Vitamin C.

GC-MS analysis from ethanol extract of *P. emblica* fruit

The ethanolic extract chromatogram of *P. emblica* fruit using GC-MS is displayed in Figure 2. The chemical compounds from the ethanol extract of *P. emblica* fruit that can be identified using GS- are listed in Table 4. There are 6 chemical compounds identified, the most being 1,2,3-Benzenetriol (68.85%) and lupeol (21.44%). 1,2,3-Benzenetriol (synonym pyrogallol) is an organic compound with the molecular formula of $C_6H_6O_3$ and a molecular weight of 126.11 (g/mol) belonging to the polyphenol group (Alonso et al. 2022). Pyrogallol has shown a beneficial

effect on health issues since the compound can inhibit some factors that cause cardiovascular diseases, provide protection against several types of cancer, and assist in inhibiting inflammatory processes in chronic degenerative diseases such as hypercholesterolemia and diabetes (Asuzu et al. 2019; Leri et al. 2020). In a previous study, Balasubramanian et al. (2014) reported a similar result as found in this study, in which the major bioactive compound found in the methanolic extract of *P. emblica* leaves was 1, 2, 3-benzene-triol.

Lupeol is a Triterpenoid naturally found in many edible vegetables, fruits, and medicinal plants. Its molecular formula is C30H50O, and its molecular weight is 426.72 (g/mol) (Nguyen et al. 2021). Lupeol is important in various pharmacological activities, such as antioxidant, anti-inflammatory, anticancer, and antimicrobe (Liu et al. 2021) and accelerating wound healing (Beserra et al. 2018).

GC-MS analysis of Ethyl Acetate extract of *P. emblica* fruit

The chromatogram of the ethyl acetate extract of *P. emblica* fruit using GC-MS is presented in Figure 3. The chemical compounds of the ethyl acetate extract of *P. emblica* fruit are shown in Table 5. There were 37 chemical compounds identified, with the largest compound being 1,2,3-Benzenetriol (29.93 %) and 3-Allyl-6-methoxyphenol (19.20%). 3-Allyl-6-methoxyphenol (synonym eugenol) is a phenolic constituent (C10H12O2 or CH3C6H3) with a molecular weight of 164.20 g/mol (Nejad et al. 2017) which can be found in a diversified plant source. Like other phenolic compounds, eugenol exhibits free radical scavenging activity (Nejad et al. 2017). It plays an important role in various life-threatening conditions, including cancer, inflammation, hyperglycemia, and elevated cholesterol levels (Khalil et al. 2017).

GC-MS analysis of n-Hexane extract of *P. emblica* fruit

The results of the chromatogram of the n-hexane extract of *P. emblica* fruit are shown in Figure 4; the chemical compounds that were successfully identified using GC-MS are shown in Table 6. The dominant chemical compounds in the n-hexane extract were eugenol (18.89%) and ß-Amyrone (15.38%). *β*-Amyrone is a triterpene compound with a formula of C30H50O, and the molecular weight is 426 (Ali et al. 2022). Previously, it has been reported that *β*-Amyrone contained in some plant materials possessed antioxidant activity (Ali et al. 2022) and showed systemic anti-inflammatory action in rats (de Almeida et al. 2015).

Overall, the GC-MS results showed that the most dominant compound found among the 3 solvents used was 1,2,3 benzenetriol, identified in ethanol extracts of *P. emblica* fruit (68.5%). The higher polyphenol compound found in the extracted ethanol of *P. emblica* fruit using GC-MS analysis agrees with DPPH analysis results, in which the higher antioxidant activity was observed in ethanol extract. Phenolic groups have been reported to have the ability as antioxidants and prevent free radical damage through donating hydrogen ions, acting as direct free radical scavengers, and chelating metal cations. Plant-derived polyphenols act as reducing agents and antioxidants due to their characteristic of donating hydrogen from their hydroxyl groups to form stable radicals (Olszowy 2019). This stable radical formation occurs because the free electrons in the radical are stabilized by electron delocalization in the presence of resonances in the aromatic ring (Mustarichie et al. 2017). Therefore, we assumed that the polyphenol compounds are responsible for the observed antioxidant activity in this study.

Table 4. Bioactive compounds from ethanol extract of *Phyllanthus emblica* fruit

Name of the compound	Retention time (Min)	Peak area (%)
1,2,3-Benzenetriol	19.993	68.85
3-Furanacetic acid, 4-hexyl-2, 5-dihydro-2,5dioxo	22.194	0.67
Z-2-Tridecen-1-ol	30.026	0.64
Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)	47.627	4.44
Thunbergol	52.651	3.96
Lupeol	53.314	21.44

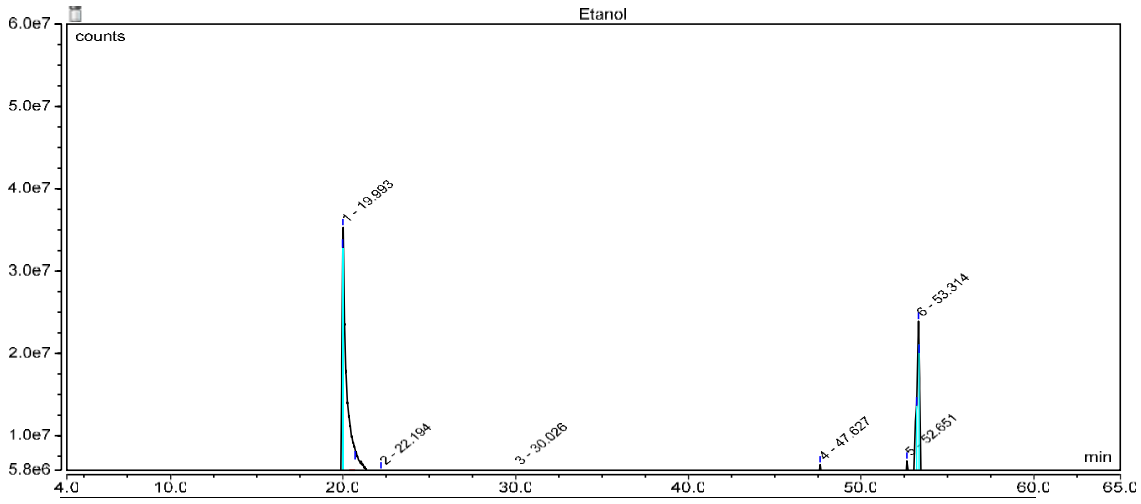

Figure 2. GC-MS chromatogram of ethanol extract of *Phyllanthus emblica* fruit

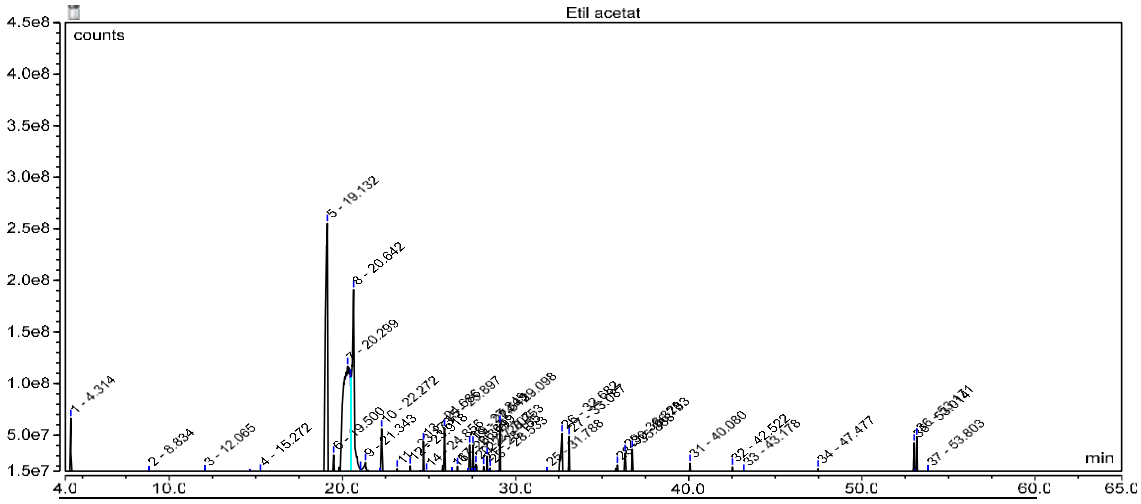

Figure 3. GC-MS chromatogram of ethyl acetate extract of *Phyllanthus emblica* fruit

Table 5. Bioactive compounds from ethyl acetate extract of *Phyllanthus emblica* fruit

Name of the compound	Retention time (Min)	Peak area (%)
Acetic acid, butyl ester	4.314	1.28
2H-Pyran-2,6(3H)-dione	8.834	0.71
1,2,3-Propanetriol, 1-acetate	12.065	0.75
Glycerin	15.272	2.36
3-Allyl-6-methoxyphenol	19.132	19.20
Copaene	19.500	0.63
1,2,3-Benzenetriol	20.299	29.93
1,2,4-Benzenetriol	20.642	18.26
2-Propenoic acid, 3-phenyl-	21.343	1.27
3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5dioxo	22.272	1.50
Naphthalene, 1,2,3,4-tetrahydro-1,6dimethyl-4-(1-methylethyl)-, (1S-cis)	23.173	0.37
1H-Indene, 1-ethylideneoctahydro-7amethyl-, cis-	23.918	0.42
1-Hexadecanol	24.686	0.99
Diethyl Phthalate	24.856	0.28
11,11-Dimethyl-4,8 dimethylenebicyclo[7.2.0]undecan-3-ol	25.897	2.27
Isoaromadendrene epoxide	26.349	0.27
Caryophyllene oxide	26.649	0.46
Benzenesulfonamide, N-adamantan-1ylmethyl-2-chloro-5-nitro-	27.349	1.15
Phenol, 4-(1,1-dimethyl propyl)-	27.543	1.22
Phenol, 2-(1,1,3,3-tetramethyl butyl)-	27.707	0.74
3,5,7-Nonatrien-2-one, 8-methyl-7-(1methylethyl)-, (E,E)	28.155	0.29
Phenol, 2-(1,1,3,3-tetramethyl butyl)-	28.353	0.66
4-(2-Methyl-cyclohexane-1-enyl)-but-3-en-2-one	28.533	0.33
E-15-Heptadecenal	29.098	1.48
Hexadecanoic acid, methyl ester	31.788	0.32
n-Hexadecanoic acid	32.682	3.05
5-Eicosene, (E)-	33.087	1.10
trans-13-Octadecenoic acid	35.883	1.80
Octadecanoic acid	36.328	1.32
1-Heneicosanol	36.733	0.80
Heptacos-1-ene	40.080	0.49
Phthalic acid, di(2-propyl pentyl) ester	42.522	0.39
9-Hexacosene	43.178	0.28
Pentacosane	47.477	0.41
?-Sitosterol	53.014	1.34
ß-Amyrone	53.171	1.53
Lup-20(29)-en-3-one	53.803	0.32

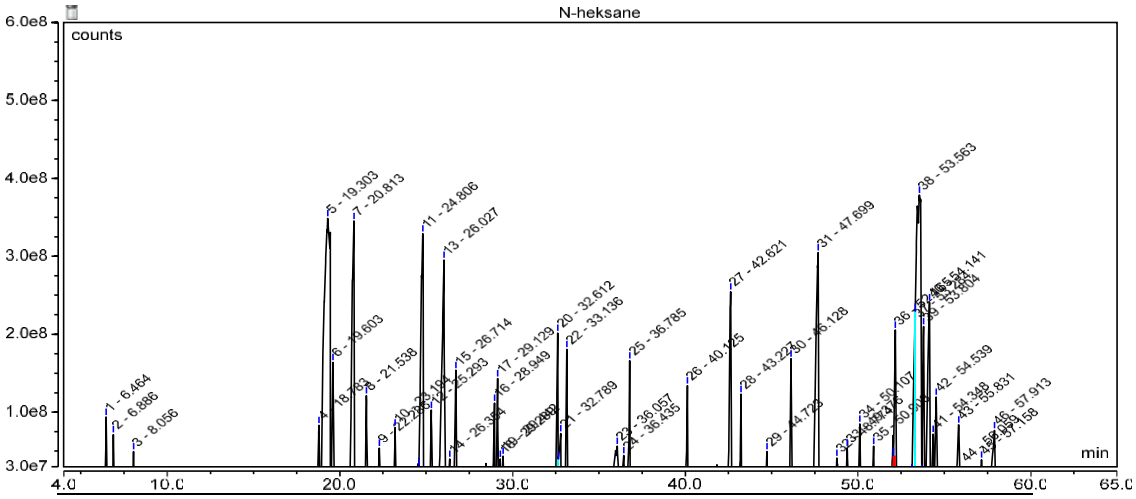

Figure 4. GC-MS chromatogram of n-hexane extract of *Phyllanthus emblica* fruit

Table 6. Bioactive compounds from ethyl acetate extract of *Phyllanthus emblica* fruit

Name of the compound	Retention time (Min)	Peak area (%)
Thanone, 1-(3-ethyloxiranyl)-	6.464	0.71
Pentanal, 2,2-dimethyl-	6.886	0.36
2-Pentene, 2,4-dimethyl-	8.056	0.24
a-Cubebene	18.783	0.49
Eugenol	19.303	18.59
Copaene	19.603	1.27
Caryophyllene	20.813	6.56
1,4,7,-Cyclounderatrieme, 1,5,9,9-tetramethyl-, z,z,z,-	21.538	0.74
3- Furanacetic acid, 4-hexyl-2,5-dihydro-2,5- dioxo-	22.286	0.46
Cis-Calamenene	23.194	0.44
Caryophyllene oxide	24.806	6.51
(1R, 3E, TE, 11R)-1,5,5,8-Tetramethyl-12- oxabicyclo[9.1.0]dodeca-3,7-diene	25.293	0.66
11,11-Dimethyl-4,8- dimethylenebicyclo[7.2.0] undecan-3-ol	26.027	5.96
Tricyclo[5.2.2.0(1,6)] undecan-3-ol, 2- methylene-6,8,8-trimethyl-	26.354	0.31
Caryophyllene oxide	26.714	1.25
Humulenol-1	28.949	0.89
E-15-Heptadecenal	29.129	0.99
cis-Z-a-Bisabolene epoxide	29.289	0.29
Tetradecane, 2,6,10-trimethyl-	29.442	0.31
Dibutyl phthalate	32.612	1.87
n-Hexadecanoic acid	32.789	0.93
1-Heneicosanol	33.136	1.34
cis-Vaccenic acid	36.057	1.57
Octadecanoic acid	36.435	0.34
Heptacos-1-ene	36.785	1.22
n-Tetracosanol-1	40.125	0.93
Phthalic acid, di(2-propyl pentyl) ester	42.621	2.71
1-Heptacosanol	43.227	0.99
Pentacosane	44.723	0.38
1-Heptacosanol	46.128	1.74
Nonacosane	47.699	6.89
Octacosyl trifluoroacetate	48.774	0.38
Octacosanal	49.376	0.33
Tetratriacontane	50.107	0.55
13,27-Cycloursan-3-one	50.903	00:34
Nonacosanal	52.165	1.83
Olean-12-en-3-ol, acetate, (3ß)-	53.284	2.66
B-Amyrone	53.563	15.38
B-Amyrin	53.804	1.89
Lup-20(29)-en-3-one	54.141	3.94
Lupeol	54.348	0.60
Glutinol	54.539	1.19
Glutinol	55.831	1.19
1-Heptatriacotanol	56.059	0.11
24-Norursa-3,12-dien-11-one	57.158	0.36
2,6-Di-tert-butyl-4-methoxyphenol, trifluoroacetate	57.913	1.32

In conclusion, based on bioactive components and antioxidant activity, ethanol is suggested as the optimal solvent for obtaining a higher content of polyphenols and flavonoid compounds as well as antioxidant activity from *P. emblica* fruit for utilization in pharmacognosy.

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REFERENCES

Abubakar AR, Haque M. 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci 12 (1): 1-10. DOI: 10.4103/jpbs.JPBS_175_19.

Addina S, Harahap ES. 2023. Analysis of antioxidant activity of balakka barks and fruits (*Phyllanthus emblica*) from South Tapanuli. IOP Conf Ser: Earth Environ Sci 1241: 012098. DOI: 10.1088/1755-1315/1241/1/012098.

Ajanal M, Gundkalle MB, Nayak SU. 2012. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Anc Sci Life 31 (4): 198-201. DOI: 10.4103/0257-7941.107361.

Alagar RM, Shailaja V, Banji D, Rao KNV, Selvakumar D. 2014. Evaluation of standardization parameters, pharmacognostic study, preliminary phytochemical screening and in vitro antidiabetic activity of *Emblica officinalis* fruits as per WHO guidelines. J Pharmacogn Phytochem 3 (4): 21-28.

Albadwawi MAOK, Ahmed ZFR, Kurup SS, Alyafei MA, Jaleel A. 2022. Comparative evaluation of aquaponic and soil systems on yield and antioxidant levels in basil, an important food plant in lamiaceae. Agronomy 12 (12): 3007. DOI: 10.3390/agronomy12123007.

Aldhanhani ARH, Ahmed ZFR, Tzortzakis N, Singh Z. 2022. Maturity stage at harvest influences antioxidant phytochemicals and antibacterial activities of jujube fruits (*Ziziphus mauritiana* Lamk. and *Ziziphus spina-christi* L.). Ann Agric Sci 67 (2): 196-203. DOI: 10.1016/j.aoas.2022.12.003.

Ali N, Khan F-A, Salawu KM, Irshad R, Jabeen A, Zhang C-L, Choudhary MI, Liu X-M, Wang Y. 2022. Phytochemical characterizations of *Maranthes polyandra* (Benth.) Prance. Molecules 27 (4): 1316. DOI: 10.3390/molecules27041316.

Alkaabi RAS, Bani Matar HH, Karthishwaran K, Ahmed ZFR, Kurup S, Salem Alyafei M, Jaleel A. 2023. Extraction of nutrients from *Rumex vesicarius*, a wild indigenous edible plant from United Arab Emirates. Not Sci Biol 15 (3): 11658. DOI: 10.55779/nsb15311658.

Alonso A-M, Reyes-Maldonado OK, Puebla-Pérez AM, Arreola MPG, Velasco-Ramírez SF, Zúñiga-Mayo V, Sánchez-Fernández RE, Delgado-Saucedo J-I, Velázquez-Juárez G. 2022. GC/MS analysis, antioxidant activity, and antimicrobial effect of *Pelargonium peltatum* (Geraniaceae). Molecules 27 (11): 3436. DOI: 10.3390/molecules27113436.

Asuzu PC, Besong SA, Aryee ANA. 2019. Polyphenols and other phytochemicals in cancer prevention and management. FASEB J 33 (S1): 802.43-802.43. DOI: 10.1096/fasebj.2019.33.1_supplement.802.43.

Balasubramanian S, Ganesh D, Panchal P, Teimouri M, Surya Narayana VV. 2014. GC-MS analysis of phytocomponents in the methanolic extract of *Emblica officinalis Gaertn* (Indian Gooseberry). J Chem Pharm Res 6 (6): 843-845.

Beserra FP, Xue M, de Azevedo Maia GL, Rozza AL, Pellizzon CH, Jackson CJ. 2018. Lupeol, a pentacyclic triterpene, promotes migration, wound closure, and contractile effect *in vitro*: Possible involvement of PI3K/Akt and p38/ERK/MAPK pathways. Molecules 23 (11): 2819. DOI: 10.3390/molecules23112819.

Cahyaningrum PL, Yuliari SAM, Putra C, Suta IBP. 2020. Antioxidant activity of loloh Malaka fruit *(Phyllanthus emblica L.)* in Ayurveda medication: How it supports environmental conservation. J Phys: Conf Ser 1469: 012115. DOI: 10.1088/1742-6596/1469/1/012115.

Chaikul P, Kanlayavattanakul M, Somkumnerd J, Lourith N. 2021. *Phyllanthus emblica* L. (amLa) branch: A safe and effective ingredient against skin aging. J Tradit Complement Med 11 (5): 390-399. DOI: 10.1016/j.jtcme.2021.02.004.

Chao P-Y, Lin S-Y, Lin K-H, Liu Y-F, Hsu J-I, Yang C-M, Lai J-Y. 2014. antioxidant activity in extracts of 27 indigenous Taiwanese vegetables. Nutrients 6 (5): 2115-2130. DOI: 10.3390/nu6052115.

Chatterjee A, Chattopadhyay S, Bandyopadhyay SK. 2011. Bhiphasic effect of *Phyllanthus emblica* L. extract on NSAID-induced ulcer: An antioxidative trail weaved with immunomodulatory effect. Evid Based Complement Alternat Med 2011: 146808. DOI: 10.1155/2011/146808.

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi-Rad J. 2023. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front Chem 11: 1158198. DOI: 10.3389/fchem.2023.1158198.

de Almeida PDO, de A Boleti AP, Rüdiger AL, Lourenço GA, da Veiga Junior VF, Lima ES. 2015. Anti-Inflammatory activity of triterpenes isolated from *Protium paniculatum* oil-resins, Evid Based Complement Alternat Med 2015: 293768. DOI: 10.1155/2015/293768.

Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y-H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 22 (3): 296-302. DOI: 10.1016/j.jfda.2013.11.00.

El Husna N, Noor E, Fahma F, Sunarti TC. 2022. Teknik ekstraksi dan nanoenkapsulasi komponen bioaktif buah malaka: Tinjauan literatur. Agrointek 16 (2): 164-178. DOI: 10.21107/agrointek.v16i2.12433. [Indonesian]

Fidrianny I, Anggraeni NAS, Insanu M. 2018. Antioxidant properties of peels extract from three varieties of banana (*Musa* sp.) grown in West Java-Indonesia. Intl Food Res J 25 (1): 57-64

Gaire BP, Subedi L. 2014. Phytochemistry, pharmacology and medicinal properties of *Phyllanthus emblica* Linn. Chin J Integr Med 2014: 1-8. DOI: 10.1007/s11655-014-1984-2.

Ghosh R, Barua P, Sikder O, Saha S, Mojumder S, Sikdar D. 2021. Comparison of phenolic content and antioxidant activity of two common fruits of Bangladesh in solvents of varying polarities. Food Res 5 (6): 187-196. DOI: 10.26656/fr.2017.5(6).253.

Habib-ur-Rehman, Yasin KA, Choudhary MA, Khaliq N, Atta-ur-Rahman, Choudhary MI, Malik S. 2007. Studies on the chemical constituents of *Phyllanthus emblica*. Nat Prod Res 21 (9): 775-781. DOI: 10.1080/14786410601124664.

Halim B, Syahputra RA, Adenin I, Lubis HP, Mendrofa F, Lie S, Nugraha SE. 2022. Determination of phytochemical constituent, antioxidant activity, total phenol and total flavonoid of *Phyllanthus emblica* fruit ethanol extract. Pharmacogn J 14: 63-67. DOI: 10.5530/pj.2022.14.9.

Harborne JB 1984. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. Springer, Dordrecht. DOI: 10.1007/978-94-009-5570-7.

Jang H, Srichayet P, Park WJ, Heo HJ, Kim D-O, Tongchitpakdee S, Kim T-J, Jung SH, Lee CY. 2017. *Phyllanthus emblica* L. (Indian gooseberry) extracts protect against retinal degeneration in a mouse model of amyloid beta-induced Alzheimer's disease. J Funct Foods 37: 330-338. DOI: 10.1016/j.jff.2017.07.056.

Kaur C, Kapoor HC. 2002. Antioxidant activity and total phenolic content of some Asian vegetables. Intl J Food Sci Technol 37 (2): 153-161. DOI: 10.1046/j.1365-2621.2002.00552.x.

Kaur N, Shahwar D, Hassan FE, Ahmed ZFR. 2023. Antioxidant and antibacterial activities of date palm fruit (*Phoenix dactylifera* L.) in response to postharvest application with natural elicitors. Acta Hortic 1364: 187-194. DOI: 10.17660/ActaHortic.2023.1364.25.

Khalil AA, ur Rahman U, Khan MR, Sahar A, Mehmood T, Khan M. 2017. Essential oil eugenol: Sources, extraction techniques and nutraceutical perspectives. RSC Adv 7 (52): 32669-32681. DOI: 10.1039/C7RA04803C.

Khan KH. 2009. Roles of *Emblica officinalis* in Medicine - A review. Bot Res Intl 2 (4): 218-228.

Krishnaveni M, Mirunalini S. 2010. Therapeutic potential of *Phyllanthus emblica* (amLa): The ayurvedic wonder. J Basic Clin Physiol Pharmacol 21 (1): 93-105. DOI: 10.1515/jbcpp.2010.21.1.93.

Kumar KPS, Bhowmik D, Dutta A, Yadav APd, Paswan S, Srivastava S, Deb L. 2012. Recent trends in potential traditional Indian herbs *Emblica officinalis* and its medicinal importance. J Pharmacogn Phytochem 1 (1): 24-32.

Kumari P, Khatkar BS. 2016. Assessment of total polyphenols, antioxidants and antimicrobial properties of aonla varieties. J Food Sci Technol 53: 3093–3103. DOI:10.1007/s13197-016-2282- 0.

Kuppusamy P, Yusoff MM, Parine NR, Govindan N. 2015. Evaluation of in-vitro antioxidant and antibacterial properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. Saudi J Biol Sci 22 (3): 293-301. DOI: 10.1016/j.sjbs.2014.09.016.

Lefebvre T, Destandau E, Lesellier E. 2021. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. J Chromatogr A 1635: 461770. DOI: 10.1016/j.chroma.2020.461770.

Leri M, Scuto M, Ontario ML, Calabrese V, Calabrese EJ, Bucciantini M, Stefani M. 2020. Healthy effects of plant polyphenols: Molecular mechanisms. Intl J Mol Sci 21: 1250. DOI: 10.3390/ijms21041250.

Liu K, Zhang X, Xie L, Deng M, Chen H, Song J, Long J, Li X, Luo J. 2021. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. Pharmacol Res 164: 105373. DOI: 10.1016/j.phrs.2020.105373.

Liu X, Cui C, Zhao M, Wang J, Luo W, Yang B, Jiang Y. 2008a. Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. Food Chem 109 (4): 909-915. DOI: 10.1016/j.foodchem.2008.01.071.

Liu X, Zhao M, Wang J, Yang B, Jiang Y. 2008b. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. J Food Compos Anal 21 (3): 219-228. DOI: 10.1016/j.jfca.2007.10.001.

Luo W, Zhao M, Yang B, Ren J, Shen G, Rao G. 2011. Antioxidant and antiproliferative capacities of phenolics purified from *Phyllanthus emblica* L. fruit. Food Chem 126 (1): 277-282. DOI: 10.1016/j.foodchem.2010.11.018.

Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. 2012. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pac J Trop Biomed 2 (12): 960-965. DOI: 10.1016/S2221-1691(13)60007-6.

Meechai I, Phupong W, Chunglok W, Meepowpan P. 2017. Antioxidant properties and phytochemical contents of *Garcinia schomburgkiana* Pierre. J Appl Pharm Sci 6 (6): 102-107. DOI: 10.7324/JAPS.2016.60618.

Molyneux P. 2004. The use of the stable free radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Tech 26: 211-219.

Mustarichie R, Runadi D, Ramdhani D. 2017. The antioxidant activity and phytochemical screening of ethanol extract, fractions of water, ethyl acetate, and n-hexane from mistletoe tea (*Scurrula atropurpurea* bl. Dans). Asian J Pharm Clin Res 10 (2): 343-347. DOI: 10.22159/ajpcr.2016.v10i2.15724.

Nejad SM, Özgüneş H, Başaran N. 2017. Pharmacological and toxicological properties of eugenol. Turk J Pharm Sci 14 (2): 201-206. DOI: 10.4274/tjps.62207.

Ngo TV, Scarlett CJ, Bowyer MC, Ngo PD, Vuong QV. 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. J Food Qual 2017: 9305047. DOI: 10.1155/2017/9305047.

Nguyen MTH, Ngo QV, Nguyen HTT, Pham QM, Dinh TH, Nguyen HTT, Tinh NV, Nguyen PTM. 2021. Osteogenic activity of lupeol isolated from *Clinacanthus nutans* Lindau: Activity and mode of action. J Chem 2021: 6704999. DOI: 10.1155/2021/6704999.

Olszowy M. 2019. What is responsible for antioxidant properties of polyphenolic compounds from plants? Plant Physiol Biochem 144: 135-143. DOI: 10.1016/j.plaphy.2019.09.039.

Pientaweeratch S, Panapisal V, Tansirikongkol A. 2016. Antioxidant, anti-collagenase and anti-elastase activities of *Phyllanthus emblica*, *Manilkara zapota* and *silymarin*: An in vitro comparative study for anti-aging applications. Pharm Biol 54 (9): 1865-1872. DOI: 10.3109/13880209.2015.1133658.

Poltanov EA, Shikov AN, Dorman HJD, Pozharitskaya ON, Makarov VG, Tikhonov VP, Hiltunen R. 2009. Chemical and antioxidant evaluation of Indian gooseberry (*Emblica officinalis* Gaertn, syn. *Phyllanthus emblica* L.) supplements. Phytother Res 23 (9): 1309-1315. DOI: 10.1002/ptr.2775.

Prananda AT, Dalimunthe A, Harahap U, Simanjuntak Y, Peronika E, Karosekali NE, Hasibuan PAZ, Syahputra RA, Situmorang PC, Nurkolis F. 2023. *Phyllanthus emblica*: a comprehensive review of its phytochemical composition and pharmacological properties. Front Pharmacol 14: 1288618. DOI: 10.3389/fphar.2023.1288618.

Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, Alshahrani MY, Islam S, Islam MR. 2022. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. Biomed Res Intl 2022: 5445291. DOI: 10.1155/2022/5445291.

Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK, Devi R. 2022. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. Front Pharmacol 13: 806470. DOI: 10.3389/fphar.2022.806470.

Saha S, Verma RJ. 2015. Antioxidant activity of polyphenolic extract of *Phyllanthus emblica* against lead acetate induced oxidative stress. Toxicol Environ Health Sci 7: 82-90. DOI: 10.1007/s13530-015-0224-2.

Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. 2006. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. J Ethnopharmacol 104 (1-2): 113-118. DOI: 10.1016/j.jep.2005.08.065.

Senevirathne M, Kim S-H, Siriwardhana N, Ha J-H, Lee K-W, Jeon Y-J. 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food Sci Technol Intl 12 (1): 27-38. DOI: 10.1177/1082013206062422.

Sharma OP, Bhat TK. 2009. DPPH antioxidant assay revisited. Food Chem 113 (4): 1202-1205. DOI: 10.1016/j.foodchem.2008.08.008.

Sulasmi, Astirin OP, Widiyanti T. 2020. Short Communication: The most active fraction of red turi flowers (*Sesbania grandiflora*) on the cytotoxic activity of HepG2 cells. Nusantara Biosci 12: 68-72. DOI: 10.13057/nusbiosci/n120112.

Uma G, Balasubramaniam V. 2012. GC-MS analysis of *Nothapodytes nimmoniana*, Mabberly leaves. J Chem Pharm 4 (9): 4417-4419.

Yadav SS, Singh MK, Singh PK, Kumar V. 2017. Traditional knowledge to clinical trials: A review on therapeutic actions of *Emblica officinalis*. Biomed Pharmacother 93: 1292-1302. DOI: 10.1016/j.biopha.2017.07.065.

Yan X, Li Q, Jing L, Wu S, Duan W, Chen Y, Chen D, Pan X. 2022. Current advances on the phytochemical composition, pharmacologic effects, toxicology, and product development of *Phyllanthi fructus*. Front Pharmacol 13: 1017268. DOI: 10.3389/fphar.2022.1017268.