Bioactive Compounds and Anticancer Activities of *Moringa Oleifera* of East Nusa Tenggara Origin

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Abstract

This research aimsto know the bioactive compound content of Moringa leaves and seeds so that later it can be developed as a candidate for anticancer drugs. The extraction process results showed n-hexane, ethyl acetate, and ethanol extract levels in the Moringa leaves, respectively; 11.38%; 25.56%, and 40.1%. The LC_{50} values of the ethyl acetate extract of Moringa leaves and seeds were 127.95 ppm and 117.52 ppm. Furthermore, the LC_{50} values of the ethanol extract of Moringa leaves and seeds were 34.58 and 60.69 ppm. The levels of bioactive compounds of the ethyl acetate extract of Moringa leaves and seeds were 137.5 ppm and 6.5 ppm. Based on the anticancer activity test results, all extracts of both Moringa leaves and seeds have potent anticancer activity (<1000 ppm). The n-hexane extract and the ethanol extract of Moringa leave from Kupangcan be developed as ananticancer.

Keywords: *Moringa leaves, anticancer activities, bioactive and Phytopharmacy*

Introduction

The rapid development in the field of isolation and identification of phytochemical compounds has increased the research trend over the last two decades towards treatments that use herbal plants as a potential source of anticancer agents[1,2].Pharmacological studies show that herbal extracts consist of essential nutrients, several anti-tumor compounds, antioxidant and anti-mutant activity, and various targets that work synergistically[3–9].

Moringa is a plant in the form of shrubs or trees that are widely available in Indonesia. The community uses this plant to overcome various complaints of diseases. Timor Island's indigenous people use the Moringa plant to treat skin diseases, respiratory problems, ear and mouth infections, hypertension, diabetes, and anemia. Several studies identified bioactive compounds from the leaves of Moringa olefera, including vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins and oxalates, and phytates[4].Moringa can grow in tropical and industry areas on all soil types and is resistant to dry seasons with drought-tolerance of up to six months[10].

The characteristics of this Moringa plant are very suitable for the climate in the Kupangarea.Moringa plants from Kupang have several advantages: the harvest is fast, easy to grow in with minimal water, can be planted in large land areas, and bear fruit at ease. Phytochemical studies show that moringa plants contain polyphenol compounds, routine, kaempferol, chlorogenic acid, ellagic acid, and vanillin[11].Content of moringa plant bioactive compounds is strongly influenced by soil moisture and pH, so it is possible that Moringa from Kupang, NTT contains different bioactive compounds

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from other places. Medicinal plants will be encouraged to produce bioactive compounds on dry land compared to fertile and water-abundant land.

Moringa plant has not impacted the economy and quality of public health in East Nusa Tenggara. The Natives use Moringa leaves by cooking them. This way of processing Moringa leaves can reduce the efficacy of Moringa leaves' efficacy due to reduced active content due to heating [12,13].Several studies have shown that the water and alcohol extract of Moringa oleifera leaves exhibits anticancer activity in several cell lines in terms of cancer. Aqueous and ethanol extracts of Moringa oleifera leaves inhibited the viability of acute myeloid leukemia, acute lymphoblastic leukemia, and hepatocellular carcinoma cells[14]. Aqueous extract of Moringa oleifera leaves could inhibit proliferation and induce apoptosis of human KB tumor cells[15]. A study also reported apoptosis induction and activity to inhibit tumor cell growth in lung cancer [8].

Methods

Extraction of the leaves and seeds of Moringa plants using solvents with differentpolarities (n-hexane, ethyl acetate, and ethanol).A total of 500 grams of dry powder of Moringa leaves macerated with 2.5 L of n-hexane solvent for four days. After four days, the mixture of extract and pulp and the solvent are filtered. The extract that still contained the solvent then evaporated using a vacuum rotary evaporator to obtain a solvent-free leaf extract. Then the pulp was macerated again with 2.5 L of ethyl acetate for four days. The mixture was filtered, and the filtrate evaporated to obtain solvent-free ethyl acetate extract. The waste was extracted again with 2.5 L of 96% ethanol, filtered and evaporated to obtain a solvent-free ethanolextract.We processed the Moringa plant seeds with the same procedure, then weighing each dry extract to obtain each part of the Moringa plant's extracted content, stored at 4ºC.

Anticancer activity testing using the *Brine Shrimp Lethality Test* (BSLT)*method*

This test intended to obtain an initial description of the presence of bioactive (anticancer) compounds in each extract. As a bioindicator, we use *Artemia salina Leach* shrimp larvae to obtaineach extract's LC 50 value. The extract, which has a small LC_{50} value, has good biological activity. The test procedure consists of three steps: the hatching of *Artemia salina L* eggs*,* toxicity testing, and calculating the percent mortality oflarvae.

Hatching Eggs

250 mL of seawater put into the hatchery, 2 grams of *Artemia salina L.* eggs aerated by giving an aerator into the hatchery vessel. We cover the eggs with *aluminum foil,*and the lights are turned on for 48 hours to hatch the eggs in the darkroom. The hatched larvae will go to a lighter area through the bulkhead. Healthy larvae are phototropic and ready to be used as test animals after 48 hours of age.

Toxicity Test

We carried out toxicity test treatment three times for each sample extract. All extracts obtained at the extraction stage weighed as much as 1000 mg, then diluted with distilled water in a 1000 mL volumetric flask to the limit (concentration of 1000 ppm or mother liquor). Tests carried out at extract concentrations of 10, 50, and 100 ppm, for testing the concentration of 10 ppm, first 0.1 mL of the mother liquor solution taken. Furthermore, ten shrimp larvae added, one drop of yeast solution with 1% concentration, and seawater added to the volume of 10 mL. As a control (0 ppm), 10 mL of seawater put in the test tube, ten shrimp larvae, and one drop of yeast. All test tubes filled with shrimp larvae are covered with aluminum foil and given air holes after 24 hours observed and counted the number of shrimp larvae that died.

Quercetin standard curve creation

Standard curves are made by connecting thequercetin standard solution's concentration with the absorption results obtained from measurements using a UV-Vis spectrophotometer at a wavelength of 437nm.Testing the levels of the sample bioactive compoundsThe sample of Moringa plant extract weighed as much as 1 mg dissolved in 9.8 mL 96% ethanol, then added with 0.1 mL of 10% aluminum (III) chloride, 0.1 mL of sodium acetate 1 M $AlCl_3$. The sample solution incubated for 30 minutes at room temperature, and then the absorbance was measured at a wavelength of 437 nm.

Isolation of Bioactive Compounds in Active Extracts

Bioactive compounds were isolated from extracts that had the best anticancer activity (test results with BSLT).The first *step* is to prepare a small chromatography *chamber*, enter into it a solvent (eluent) ethyl acetate: ethanol in a ratio (3: 1), the *chamber is* closed and allowed to saturate with eluent. In thin-layer chromatography (TLC) separation, a *silica gel* GF $_{254}$ plate was used, which had been activated in an oven at 105ºC for 30 minutes. We made each plate in size 8x2 cm, marked with a line along with the plate at the lower limit (2 cm from the lower edge of the plate) and the upper border (1.5 cm from the plate's upper edge).

We observed the plates under a UV lamp with wavelengths of 254 nm and 366 nm. We record the color of the *spots* formed, then measure the distance traveled by each *spot*. All TLC results calculated with the Rf value, seen in the chromatogram column and the spot's color. The eluent which produced the best separation used for preparative TLC. The separation results with preparative TLC tested with a UV-Visspectrophotometer

Results and Discussion

Extraction

The extraction process results are in the form of qualitative data, namely the color of the extract and quantitative data in the form of extract content. The extraction process results are shown in Table 1, indicating that the leaf extract's color using n-hexane, ethanol, and ethyl acetate solvents is green. The n-hexane extract of Moringa seeds is yellow due to the extraction of Moringa seed oil, as is known from previous studies that the oil content in dry moringa seeds is 38.08%.

The ethanol extract content is higher than the ethyl acetate extract and n-hexane extract. Meanwhile, in the seed extract, the content of n-hexane extract is relatively high, above 40%, this is due to the high oil content of the moringa seeds. The number of oils attracted to the n-hexane solvent is due to the extractor's compatibility and the extracted substance. Oil is nonpolar, so it will quickly draw n-hexane, which is nonpolar aswell.

Anticancer Activity

In this study, anticancer activity testing used the *Brine Shrimp Lethality Test* method (using Artemia salina L). As in Table 2, the BSL test results show that the n-hexane extract of moringa leaves has an LC value of $_{50,}$ which is smaller than the n-hexane extract of moringa seeds, almost a third. The anticancer activity of n-hexane extract is three times stronger than that of moringa seed's n-hexane extract. The oil content (triglycerides) in Moringa seeds is almost 40%. The anticancer potential of the ethyl acetate extract of Moringa leaves and seeds were the weakest compared to n-hexane and ethanol extracts. Bioactive compounds tend to be attracted by nonpolar and polar solvents. The anticancer potential of the ethanol extract of Moringa leaves is almost twice that of the ethanol extract of Moringa seeds. In general, all extracts have the potential as anticancer because a chemical compound is said to have bioactive potential if it has an LC_{50} value of less than 1,000 µg / ml.

The BSLT is a basis for a toxicity test for cell lines, anti-tumor, and anticancer activity[16]. This test's advantage is that it is fast and comfortable; the results can be repeated and do not require expensive costs[17].Moringa leaf extract has good potential, both ethanol extract, and n-hexane extract, because it has the smallest LC $_{50}$ value, meaning that it has the best biological activity. Both extracts had much better activity than Binahong leaf extract (*Anrederacordifiola*(Ten) Steenis), which had toxicity levels of ethyl acetate extract and n-hexane extract 106.99 ppm and 175.80 ppm.

Bioactive Levels

Moringa bioactive compounds areflavonoids, polyphenolic compounds that are known to have good activity in the field of medicine. Flavonoid compounds that function as anticancer are quercetin and kaempferoldue to their ability to prevent cell damage caused by free radicals. Its ability as an antioxidant is due to its ability to release oxygen radicals,neutralizing harmful radicals. Flavonoids are included in polyphenol compounds, secondary metabolites of plants, and have acted as an anticancer. Flavonoids contain quercetin, which comes from the flavonol subclass: Quercetin, genistein, or flavopiridol, used asan ingredient in cancer

drugs [18].

The results of determining the total flavonoid content of the moringa plant extract shown in Figure 1 show that the n-hexane leaf extract and the ethanol extract of the moringa leaf fruit had the highest total flavonoid levels, namely 1792.5 ppm and 1766.5 ppm. If the value of total flavonoids converted to units of percent w / w, the total flavonoids in the extract n-hexane leaves and fruit ethanol extract are 7.79% and 8.44%. Every 20 g of leaves a total of 1.559 grams of flavonoids, and every 20 g of the fruit contains 1.689 grams of flavonoids—the lowest levels of flavonoids found in the ethanol extract of bark, which was 4.5 ppm.Based on Figure 1, the content of bioactive compounds in all seed extracts is shallow compared to the leavesbecause more bioactive compounds on the leaves than the seeds. Compared with the results of previous studies, the total flavonoid content in the n-hexane leaf extract and the ethanol extract of Moringa leaves is higher than the total flavonoid content in the n-hexane extract of the Kupa fruit *Shyzygiumpolycephalum*Miq, which is 6.06% [19] and the ethanol extract of Bidara leaves was 1.531% [20].The bioactivity of the n-hexane and ethanol extracts of Moringa leaves had the smallest LC_{50} value (the best bioactivity) compared to the seed extract. Of these compounds, several *prenylated flavonoids* from plants show cytotoxicity activity against several cancer cells, such as artelastin, artelastochromene, artelasticin.

Bioactive Compound Isolation

The bioactive compounds' isolation results in the n-hexane extract showed that the n-hexane extract with ethyl acetate: ethanol as eluent separated from one compound n-butanol: acetic acid: water (4: 1: 5) separated two compounds. The separation results using the preparative thin-layer chromatography (TLC) method presented in Table 3. The isolates obtained were Isolate-1 (Eluene ethyl acetate - ethanol) and Isolated-2(n-butanol - acetic acid - water). Both are measured at the same wavelength range from 200 to 80 nm. For Isolate-1, it is measured at a wavelength of 200-600 nm so that the compounds that are attracted to the wavelength range are nonpolar and semipolar compounds, including alkaloids, terpenoids, and steroids, with each wavelength in the range 210-340 nm, 200-400 nm. Isolate-2 was measured in the 200-800 nm wavelength range so that the compounds that attracted to the wavelength range are polar, that is, flavonoids with a wavelength of 250-550nm.The Spectro results show the maximum absorption at a wavelength of 224 nm. The absorbance value is 2.410 and has an electron transition of $\pi\pi^*$ in the presence of a conjugated bond C=C─C=C.

Table 1 Extract Content

No.	Extract Name	Colour Extract	Extract Content (% w / w)
1.	Leaf n-hexane extract Moringa	Green	11.55
2.	Moringa leaf ethyl acetate extract	Light green	5,56
3.	Moringa leaf ethanol extract	Dark green	42.70
4.	Moringa seed n-hexane extract	Light yellow	40.35
5.	Ethyl acetate extract of Moringa seeds	Light yellow	7.87
6.	Moringa seed ethanol extract	Light brown	23.79

Table 2.The results of testing for anticancer activity using the BSLT method

No.	Extract name	Concentration extract (ppm)	Shrimp larvaemortalitymean (tail)		IC value 50(ppm)
			Sample	Control	
1.	n-hexane leaf moringa	10	4	0	37.06
		50	7	0	
		100	10	0	
2.	n-hexane seed moringa	10	2	0	97.62
		50	4	0	
		100	6	0	
3.	Ethyl acetate skin of Moringa leaves	10	1	0	127.95
		50	2	0	
		100	4	0	
4.	Ethyl acetate seeds Moringa	10	1	0	117.52
		50	3	0	
		100	4	0	
5.	Ethanol leaf moringa	10	4	0	34.58
		50	8	0	
		100	10	0	
6.	Ethanol seed moringa	10	2	0	60.69
		50	3	0	
		100	5	0	

Table 3 Observations on the separation with KLTP

Eluent	Observationresult			
	Amount Spot	Spot shape	Spot Color	RF
Ethyl Acetate: ethanol	1	Round	Yellowish Green	0.615
BAA	2	Round	Yellowish Green	0.804
		Tail	Yellowish Green	0.713

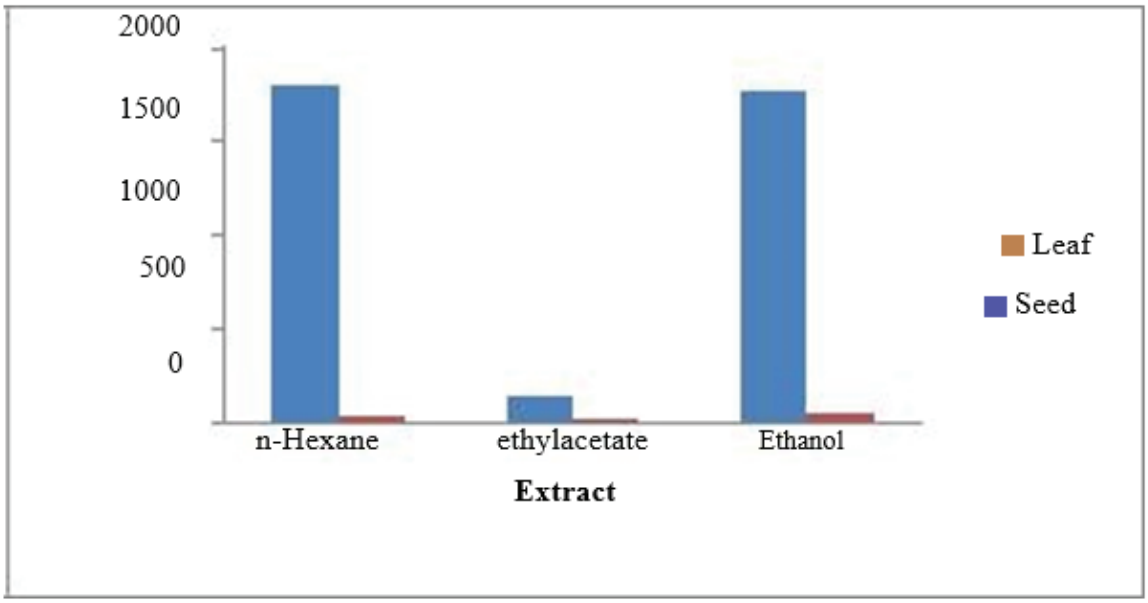

Figure 1 Total flavonoid levels

Conclusion

The extraction process results show n-hexane, ethyl acetate, and ethanol extract in Moringa leaves, respectively; 11.38; 25.56, and 403.1%. The extract levels of n-hexane, ethyl acetate, and ethanol in Moringa seeds were respectively; 9.88; 23.50, and 53.0. The anticancer activity test on the n-hexane extract showed the LC_{50} value of 37.06 ppm (leaves) and 97.62 ppm (seed). The ethyl acetate's LC 50 values were 127.95 (leaves) and 117.52 ppm (seed), while theethanolextractwas34.58 (leaves) and60.69ppm (seed). We found that bioactive compounds in the n-hexane extract are 1792.5 ppm (leaves) and 26.5 ppm (seed). The ethyl acetate level was 137.5 (leaves) and 6.5 ppm (seed). The ethanol extract was 1766.5 (leaves) and 36.5 ppm (seed). More in-depth studies of The Moringa plant are needed to develop it as a potential anticancer drug.

Conflict of Interest: There are no conflicts of interest.

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Ethical Clearance: This study obtained ethical approval from the Institutional Ethics Committee on the Nusa Cendana University in September 2020.

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