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Physicochemical Determination of Ethyl Acetate Extract and n-Hexane Extract of Azadiractha indica A. Juss Leaves

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ABSTRACT:

Background: Neem (Azadiractha indica A. Juss) as a tropical and subtropical plant has been widely used in traditional medicine since prehistoric times. Several biological activities, such as antibacterial, anti-inflammatory and antioxidant properties, have been demonstrated in Neem extract.

Aim: The study aims to research was carried out on the physicochemical properties of neem leaves (Azadirachta indica A. Juss) using ethyl acetate and n-hexane as solvents.

Methode: In this study, Azadiractha indica A. Juss leaves was extracted using a maceration method with two fluids namely ethyl acetate a n-hexane. The extract based on six parameters from determining physicochemical properties, namely % yield, organoleptic, determination of dissolved compound levels, phytochemical screening, determination of drying Shrinkage and determination of specific weight.

Result : The results of this study showed, the yield obtained for each solvent is 6.218% ethyl acetate extract and 0.968 % n-hexane extract. Organoleptic examination (form, colour, smell and flavor) for ethyl acetate extract was thick, blackish green, distinctive smell and bitter, and than n-hexan extract was thick, brownish yellow, odorless and bitter. In the ethyl acetate extract, the levels of compounds dissolved in water and ethanol solvents were 16.84 % ± 0.061 and 68.29 % ± 0.042 respectively. The levels of compounds in the n-hexane extract dissolved in water and ethanol were 4.26% ± 0.064 and 23.18% ± 0.049, respectively. The results of the phytochemical screening for ethyl acetate extract of neem leaves are alkaloids and flavonoids. Meanwhile, n-Hexane extract from neem leaves contains alkaloids and terpenoids. The drying loss values obtained from ethyl acetate extract and n-Hexan extract of neem leaves were 4.748% ± 0.257 and 5.219% ± 0.089. Specific gravity obtained from The dilution of ethyl acetate extract and n-hexane extract of neem leaves was 1.0389 g/mL ± 0.039 and 1.0289 g/mL ± 0.038.

Conclusion: This study showed that variations in solvents in extraction can affect the physicochemical properties. The extract with ethyl acetate solvent showed better physicochemical properties where the extract obtained in % yield, soluble compound content, phytochemical screening, determination of drying Shrinkage and determination of specific weight was better compared to the extract with n-hexane.

KEYWORDS: Azadiractha indica A. Juss, Neem Leaf, Physicochemical Determination.

I. INTRODUCTION

Plants such as Neem originate from India, while others can be found in the forests of Southeast Asia, including Mauritius and the Caribbean; Fiji; America & South Asia; Sri Lanka; Malaysia/Pakistan; Thailand; Andhra Pradesh. The northern coast of Lombok, Bali, Subang and East Java in Indonesia are places where neem is widely planted [1]. Neem as a tropical and subtropical plant has been widely used in traditional medicine since prehistoric times. Several biological activities, such as antibacterial, anti-inflammatory and antioxidant properties, have been demonstrated in Neem extract [2].

Almost all parts of the neem plant, including stems, leaves and seeds, contain bioactive compounds [3]. Bioactive compounds found in neem include azadirone, promeliacin, limonoid, gedunin, bilasinin, C-secomeliacin, azadirachtin, nimbin, salanin and other non-osprenoids, amino acids/proteins, polysaccharides, flavonoids, β-sitosterol, hyperosid, nimbolide , quercetin, quercitrin [4]. The neem plant, especially the seeds and leaves, contains several secondary metabolite compounds such as flavonoids,

saponins, tannins, azadirachtin, salanin, meriantriol, nimbin, and nimbidin which are useful in agriculture (peptides and fertilizers) and in medicine, cosmetics and medicines are said to be beneficial [5].

Maceration extraction is a simple extraction method that uses a solvent and repeats shaking and stirring at room temperature. We chose the maceration extraction method because the procedures and equipment used are simple, and do not require heating so the natural ingredients will not be degraded. Cold extraction can extract many compounds, but some compounds have limited solubility in the extraction solvent at room temperature [6]. The solvent used for extraction, namely ethyl acetate, can dissolve several compounds such as sterols, terpenoids, saponins, tannins, flavonoids and phenolic compounds. On the other hand, n-hexane was chosen as a solvent because it is stable and volatile, dissolves substances selectively and dissolves non-structural compounds such as waxes, fats and terpenoids [7]. Based on this background, research was carried out on the physicochemical properties of neem leaves (Azadirachta indica A. Juss) using ethyl acetate and n-hexane as solvents.

II. RESEARCH METHODE

A. Tools and materials

This research used tools including chemical beakers, watch glasses, measuring flasks, micropipettes, volume pipettes, drop pipettes, horn spoons, UV-Vis spectrophotometers, analytical scales, jars, vials, aluminum foil, porcelain cups, stirring rods, and funnels.
This research used materials in the form of neem leaves (Azadirachta indica A. Juss), aluminum chloride ($AlCl_3$), ammonia (NH_3), acetic anhydride (C_4H_6O3), sulfuric acid (H_2SO_4) 2N, distilled water (H_2O), ethanol (C_2H_5OH), Iron (II) chloride ($FeCl_2$) 1%, Concentrated hydrochloric acid (HCl), Sulfuric acid (H_2SO_4), chloroform ($CHCl_3$), Iron (III) chloride ($FeCl_3$) 1% solution, methanol (CH_3OH), Sodium hydroxide (NaOH) 1M, Sodium nitrite ($NaNO_2$), Mayer's reagent, Wagner's reagent, Dragendrof's reagent, Mg powder, and quercetin.

B. Sample Processing

Samples of neem leaves (Azadirachta indica A. Juss) were obtained from the Ikhtiar Mosque on Jalan Sunu, Unhas Complex, Makassar City, South Sulawesi Province. The neem leaves that have been harvested are then sorted and separated between the dry leaves and the fresh leaves, and washed with water until clean. Then chop to reduce the size of the neem leaves and dry them in indirect sunlight. Drying can be continued in a drying cabinet with the aim of speeding up the drying process without being influenced by weather conditions, then powdered using a blender to obtain sample powder.

C. Extraction of Active Compounds

A total of 250 g of simplicia was put into 2 different containers, adding ethyl acetate solvent to the first container and n-hexane to the second container, 3 L each. The soaking process was for 3 x 24 hours. Every 24 hours the solvent is replaced and stirred every 8 hours. After 72 hours, the results of the maceration are filtered. The dregs from the extraction results were re-macerated with the same solvent, 1 L each, for 2 days. The liquid extract obtained is collected and then concentrated using a rotary evaporator until a thick extract is obtained.

$$\text{Yield } (\%) = \frac{\text{Extract weight}}{\text{Dry Simplicia Weight}} \text{ X } 100\ \%$$

D. Organoleptic Examination

Organoleptic examination of the extract includes shape, odor, taste and color. The statements “odorless”, “practically odorless”, “weak characteristic odor” or others are applied by observation after the material has been exposed to air for 15 minutes, or 15 minutes is calculated after the container containing ≤5 g of the material is opened. For containers containing ≤25 g of material, open. For containers containing >25 g of material, the determination is carried out after ±5 g has been transferred into a 100 mL evaporating cup, the odor mentioned is only descriptive and cannot be considered as a standard for the purity of the material in question [6].

E. Dissolved Compound Content Analysis is the determination of water soluble essence content

One grams of dry extract is weighed carefully, put into a stoppered flask, add 20 mL of chloroform saturated water. Shake frequently for the first 6 hours, leave for 18 hours. The filtrate was filtered and evaporated to dryness in a shallow, flat-bottomed cup that had been heated to 105ºC and leveled, the weight of the container used had been kept constant. The remainder is heated at 1050C until the weight remains constant. Calculate the content in % water soluble essence [6].

F. Analysis of Dissolved Compound Content, namely Determination of ethanol soluble essence content

One grams of dry extract is weighed carefully, put into a stoppered flask, add 20 mL of ethanol P. Shake repeatedly for the first 6 hours, leave for 18 hours. The filtrate was filtered and evaporated to dryness in a shallow, flat-bottomed cup that had been heated to 105^0C and leveled, the weight of the container used had been kept constant. The remainder is heated at 105^0C until the weight remains constant. Calculate the content in % of ethanol soluble essence [6].

G. Phytochemical Screening

1. Alkaloid Testing

A total of 1 ml of extract was mixed with 5 mL of chloroform and 5 mL of ammonia then heated, shaken and filtered. Five drops of 2 N sulfuric acid were added to each filtrate, then shaken and left to stand. The top of each filtrate was taken and tested with Mayer, Wagner, and Dragendorf reagents. The formation of orange, brown and white precipitates indicates alkaloids [8].

2. Flavonoid Testing

A total of 1 mL of extract was mixed with 3 mL of 70% ethanol then shaken, heated and shaken again then filtered. The filtrate obtained was added with 0.1 g mg powder and 2 drops of concentrated HCl. The formation of a red color in the ethanol layer indicates the presence of flavonoids [9].

3. Steroid/Triterpenoid Testing.

A total of 1 ml of extract is mixed with 3 mL of chloroform or 3 mL of 70% ethanol and added with 2 mL of concentrated sulfuric acid and 2 mL of anhydrous acetic acid. A color change from purple to blue or green indicates the presence of steroid compounds [9].

4. Saponin Testing

A total of 1 ml of extract was put into a test tube, 10 mL of hot water was added, then cooled and shaken vigorously for 10 seconds. It is positive for containing saponin if foam is formed as high as 1–10 cm for no less than 10 minutes and when 1 drop of 2 N HCl is added, the foam does not disappear [10].

5. Tannin Testing

A total of ± 1 mL of the sample extract is boiled with 20 l of water on a water bath, then filtered. The filtrate obtained was added with a few drops (2-3 drops) of 1% FeCl and a greenish brown or blackish blue color was formed indicating the presence of tannins [9].

6. Terpenoid Testing

A total of 1 ml of extract was mixed with 3 mL of chloroform or 3 mL of 70% ethanol and added to 2 mL of concentrated sulfuric acid and 2 mL of anhydrous acetic acid. The brownish color change between the surfaces indicates the presence of terpenoid compounds [11].

A. Drying sequence determination

A total of 2 g of extract was put into a porcelain dish covered and previously heated for 30 minutes at a temperature of 105˚C and has been tarred. The extract is spread evenly in the rate by shaking the rate until a layer forms 5-10 mm thick, then weighed. Put in the oven then the lid is opened, dried at a temperature of 105˚C until a constant weight is obtained. Cooled in a desiccator and calculate the percentage value [12] [13].

H. Determination of specific weight

The specific gravity of the extract was determined by diluting the 5% extract in ethanol solvent using a pycnometer. The pycnometer used must be clean, dry and calibrated. by determining the weight of the pycnometer and the weight of water that has just been boiled at a temperature of 25˚C. The temperature is adjusted until the liquid extract is approximately 20˚C, then put into the pycnometer. Adjust the temperature of the extract in the pycnometer until it reaches 25˚C, then weigh it [13].

G. Data analysis

The independent variable in this study was a variety of solvents, while the dependent variables were % yield, organoleptics, F dissolved compound content (determination of water soluble essence content and determination of ethanol soluble essence content), and phytochemical screening of neem leaf extract. The data collected in this research is primary data, namely data obtained from each parameter determination. The data analysis technique for this research is based on the results obtained for each physicochemical test parameter.

III. RESULT AND DISSCUSION

The sample used in this research was neem leaf extract (Azadiractha indica A. Juss). The extract is obtained from the extraction results using a variety of filter fluids, namely ethyl acetate and n-hexane. This research aims to determine the good physicochemical properties of various filter fluids.

B. Extraction Results

The maceration process is predicated on the application of various polarising fluids, such as n-hexane and ethyl acetate, according to polarisation levels. The choice of polarised fluids is determined by the polarity of the chemicals found in neem leaves. Then there are ethyl-acetate polarising fluids, where ethyl acid has a low level of polarity so that it is predicted to attract molecules with a low degree of polarisation, and n-hexane polarising fluids, which are non-polar because n-hexane has special qualities that will attract only nonpolar chemicals. It is anticipated that the active ingredients in neem leaves will be drawn in accordance with polarised

levels by employing various polarising fluids based on polarisation levels [6].

The extraction results showed that there were differences in the yield of the extract with the variations in the filter fluid used (table 1). The research results obtained showed that ethyl acetate extract produced a higher % yield compared to ethyl acetate extract. The difference in yield values produced can be caused by the different polarity properties of the filter used (Priyanto, 2010).

Table 1. Data on the yield of n-Hexane and Ethyl Acetate of Neem Leaves

Sample	Simplicia Weight (g)	Extract Weigh (g)	% Yield
Ethyl Acetate Extract	250	15.545	6.218 %
n-hexan Extract	250	2.421	0.968 %

The yield obtained for each solvent is 0.968 % n-hexane extract and 6.218% ethyl acetate extract. The small size of the yield indicates the efficiency of the extraction process. The extraction efficiency can be influenced by the type of solvent, sample particle size, extraction time and extraction method used. Most yields are obtained from etil asetat Extract [14].

C. Organoleptic Examination

Organoleptic observations of extracts aim to provide an initial introduction to simplicia and extracts using the five senses by describing shape, color, smell and taste . Based on observations made, it shows differences in color and odor produced by the filter fluid used [15]

Table 2. Results of organoleptic observations of neem leaf extract

Observation	Result	
	Maceration	
	Ethyl Acetate	n-hexan
Form	Thick	Thick
Colour	Blackish Green	Brownish Yellow
Smell	Distinctive Smell	Odorless
Flavor	Bitter	Bitter

D. Dissolved Compound Content Analysis

The levels of dissolved compounds from the extract were determined using two solvents, namely water and ethanol. The assay results shown in table 3 and table 4 show that ethyl acetate extract has the highest compounds compared to n-hexane extract. Thus, the polar compounds contained in the extract are thought to be much more abundant than non-polar compounds. This is proven by the large levels of dissolved compounds in water and the levels of dissolved compounds in ethanol from the ethyl acetate extract.

Tabel 3. Determination of water soluble essence content

Parameter	Dissolved Compound Content Analysis (%)				
	Maserasi				
	Replication	Ethyl Acetate Extract	Average ± SDV	n-hexan Extract	Average ± SDV
Determination of water soluble essence content	1	16.11	16.84 ± 0.061	4,21	4.26 ± 0.064
	2	16.21		4.23	
	3	16.22		4.33	

Tabel 4. Determination of ethanol soluble essence content

Parameter	Dissolved Compound Content Analysis (%)				
	Maceration				
	Replication	Ethyl Acetate Extract	Average ± SDV	n-hexan Extract	Average ± SDV
Determination of ethanol soluble essence content	1	68.30	68.29 ± 0.042	23.11	23.18 ± 0.049
	2	68.23		23.21	
	3	68.33		23.22	

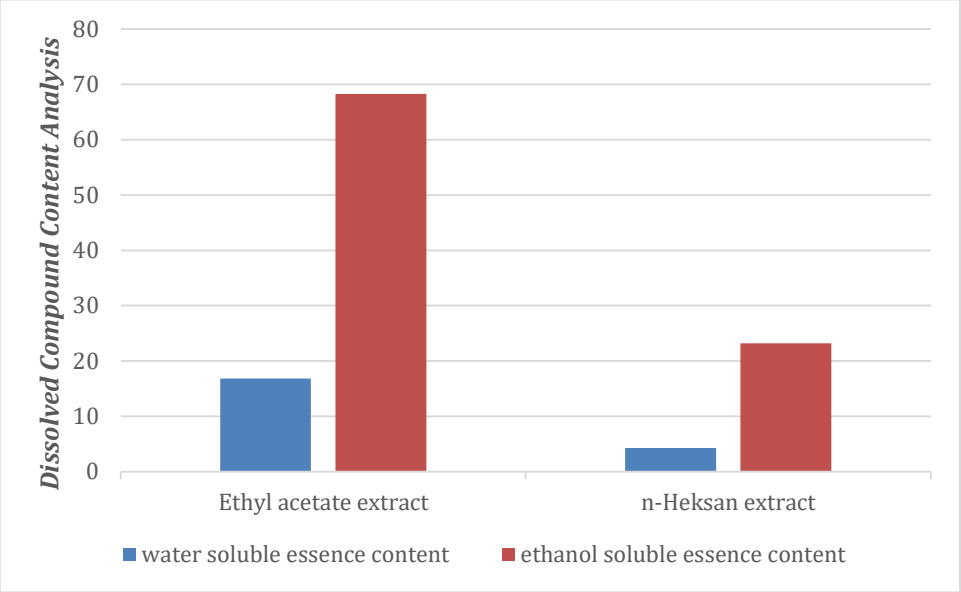

Figure 1. Dissolved Compound Content Analysis was Water soluble essence content (%) and Determination of ethanol soluble essence content (%) ethyl acetate Extract and n-Hexan Extract of Neem Leave

The purpose of determining the levels of dissolved compounds in water and ethanol solvents is to estimate the amount of active compounds that are polar (soluble in water) and polar – non-polar (soluble in ethanol) [16]. In the ethyl acetate extract, the levels of compounds dissolved in water and ethanol solvents were 16.84 % ± 0.061 and 68.29 % ± 0.042 respectively. The levels of compounds in the n-hexane extract dissolved in water and ethanol were 4.26% ± 0.064 and 23.18% ± 0.049, respectively. The results obtained showed that compounds from ethyl acetate extract and n-hexane extract of neem leaves were more soluble in ethanol solvent than water. Meanwhile, the levels of dissolved compounds in the water solvent and ethanol solvent in the ethyl acetate extract were greater than those in the n-hexane extract of neem leaves. This shows that there are more polar compounds contained in neem leaves than non-polar compounds.

D. Phytochemical Screening Results

Table 5. Phytochemical Screening of ethyll acetate and n-Hexane Extracts of Neem Leaves

Phytochemical screening		**Maceration**	
		Ethyl Acetat	**n-hexan**
Alkaloids	Mayer	White sediment (+)	White sediment (+)
	wagner	brown sediment (+)	brown sediment (+)
	Dragendorf	Orange sediment (+)	Orange sediment (+)
Flavonoids		Brick red (+)	Yellow (-)
Saponins		No foam (-)	No foam (-)
Terpenoids		Black (-)	Brownish (+)
Steroids		Black (-)	Brown (-)
Tannin		Black (-)	Yellow (-)

The results of phytochemical screening based on variations in the filter fluid used showed that there were differences in the compound components detected qualitatively (Table 5) because the two filters used in the extraction process had different polarity levels. It can be seen that the results of the phytochemical screening for ethyl acetate extract of neem leaves are alkaloids and

flavonoids. Meanwhile, n-Hexane extract from neem leaves contains alkaloids and terpenoids.

E. Drying sequence determination

Table 6. Drying sequence determination of ethyll acetate and n-Hexane Extracts of Neem Leaves

Parameter	Determination of Drying Shrinkage (%)				
	Maceration				
	Replication	Ethyl Acetate Extract	Average ± SDV	n-Hexan Extract	Average ± SDV
Determination of Drying Shrinkage	1	4.395	4.748 ± 0.257	5.113	5.219 ± 0.089
	2	4. 999		5.212	
	3	4.849		5.332	

Drying loss is one of the parameters which aims to provide a maximum limit (range) regarding the amount of compounds lost in the drying process. The drying shrinkage parameter is basically a measurement of the remaining substance after drying at a temperature of 105ºC to a constant weight, which is expressed as a percent value. The drying loss values obtained from ethyl acetate extract and n-Hexan extract of neem leaves were 4.748% ± 0.257 and 5.219% ± 0.089. The mass that could be lost due to heating included water molecules, essential oils, ethyl acetate solvent and n-hexane solvent.

F. Determination of specific weight

Table 7. Determination of specific weight of ethyll acetate and n-Hexane Extracts of Neem Leaves

Parameter	Determination of specific weight (g/mL)				
	Maceration				
	Replication	Ethyl Acetate Extract	Average ± SDV	n-Hexan Extract	Average ± SDV
Determination of specific weight	1	1.0442	1,0389 ± 0.039	1.0341	1.0289 ± 0.038
	2	1.0351		1.0253	
	3	1.0373		1.0272	

Specific gravity is defined as a density ratio a substance to the density of water with a mass value per volume unit. The purpose of determining this type of weight is to: provides an overview of the chemical content dissolved in an extract [17]. Measurement of the specific gravity of ethyl acetate extract and n-hexane extract of neem leaves was determined using a pycnometer. The extract used is an extract that has been diluted 5% with water. Specific gravity obtained from The dilution of ethyl acetate extract and n-hexane extract of neem leaves was 1.0389 g/mL ± 0.039 and 1.0289 g/mL ± 0.038.

IV. CONCLUSIONS

G. Based on six parameters from determining physicochemical properties, namely % yield, organoleptic, determination of dissolved compound levels, phytochemical screening, determination of drying Shrinkage and determination of specific weight carried out on neem leaf extract with various solvents, it can be concluded that:

1. Variations in solvents in extraction can affect the physicochemical properties
2. The extract with ethyl acetate solvent showed better physicochemical properties where the extract obtained in % yield, soluble compound content, phytochemical screening, determination of drying Shrinkage and determination of specific weight was better compared to the extract with n-hexane.

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