

# Synergistic Effect of Alcohols, Ketone and Water on the Yield of Soluble Compounds and Tannins from the Barks of *Azadirachta indica* A. Juss. and *Moringa oleifera* Lam.

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## Abstract

Investigations into the most efficient solvent or solvent mixtures, which yield substantial quantities of tannins for the production of adhesives for wood composite industries have been ongoing. However, knowledge of the synergistic effect of ketones, alcohols, and water on the yield of soluble compounds and tannins from plants is lacking. Using the Soxhlet extraction method, the synergistic effect of ketone, alcohol and water mixtures in the extraction of soluble compounds and tannins from the barks of *Moringa oleifera* and *Azadirachta indica* was examined. Ketone, water and alcohol did not have a definite synergistic effect on the yields of soluble compounds and tannins from the two plants. Mixtures of some solvents, as opposed to their individual solvents, recorded high yield of soluble compounds. The reverse was also observed. Water extract recorded the most substantial quantity of soluble compounds in *M. oleifera* while ethanol-methanol-water mixture (60:20:20) extracted the highest quantity of soluble compounds in *A. indica*. The mixtures containing either a ketone or the alcohols produced comparatively high tannin yields in *A. indica*. This trend was not so for *M. oleifera*. The efficiency of solvents to extract soluble compounds depended mostly on the species.

**Keywords:** Adhesive, lewis acid, phenol, plant extract, polar solvent, stiasny number, wood composite

## Introduction

Synthetic glues such as urea-formaldehyde, resorcinol-formaldehyde and phenol-formaldehyde play important roles in the production of wood composites (Jetuah *et al.* 2001). National and international politics of oil-producing countries such as Russia, Iran, Saudi Arabia and the United States have led to the increase in the monetary value of crude oil components (resorcinol and phenol) used for glue production. This has necessitated the extraction of polyphenolic compounds such as tannins, from several plant parts (e.g. leaves, bark, fruits and stem) to replace the expensive crude oil phenol and resorcinol components (Readel *et al.* 2001) of adhesives. The demand for tannins for the production of adhesives has increased significantly due to their renewability and availability (Ugovsek *et al.* 2010). Many research has focused on the determination of efficient ways of extracting these tannins from plant parts. Conventionally, the extraction of tannins from plants is done with solvents such as methanol, acetone, water, ethanol, petroleum ether and sodium hydroxide or alkali (Darkwa and Jetuah 1996; Shi *et al.* 2021). The non-conventional method includes the use of ultrasound and microwave systems among others (Azwandia 2015).

Singh *et al.* (2014) asserted that the selection of solvents for extraction is an important phase for obtaining extracts with acceptable yields. Over the past decades, researchers have sought to determine the best solvents that improve the efficiency of extracting tannins. Darkwa and Jetuah (1996) reported 1% sodium hydroxide as a very efficient solvent for extracting tannins from *Rhizophora spp.* Antwi-Boasiako and Animapauh (2012) recorded a higher

amount of tannins for distilled water than 1% sodium hydroxide and thus concluded that water is highly efficient for tannin extraction from the leaves, bark and fruits of *Tetrapleura tetraptera*. Downey and Hanlin (2010) examined the relative effectiveness of aqueous mixtures of acetone and ethanol (in ratios ranging from zero to 100%) in the extraction of tannins. Acetone-water extracted more condensed tannins than ethanol-water. A solvent combination of acetone-water (80:20, v/v) used by Chavan and Amarowicz (2013) also extracted considerably higher amount of condensed tannins from beach pea (*Lathyrus maritimus L.*). According to Fraga-Corral *et al.* (2020), unlike the high polar hydrolysable tannins, condensed tannins have limited solubility in polar organic solvents such as water and would likely be efficiently extracted using low polar solvents including acetone.

Stefanelli *et al.* (2013), Singh *et al.* (2014) and Dailey and Vuong (2015) found that the extracting efficiencies of solvents were enhanced when they were mixed together. When solvents are combined, the range of soluble compounds, which they can extract broadens. Therefore, Arranz *et al.* (2009) explained that total plant polyphenols have often been underestimated due to the choice of extracting solvents and methods which could leave compounds behind. According to Fraga-Corral *et al.* (2020), the combination of water and either ethanol, sodium hydroxide (NaOH), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), sodium bisulphite (NaHSO<sub>3</sub>), or sodium sulphite (Na<sub>2</sub>SO<sub>3</sub>) improves extraction efficiency. Singh *et al.* (2014) found that the yield of extracts was higher for a mixture containing ethanol, ether and water compared to acetone only and ether only. Dailey and Vuong (2015) also indicated that the combination

of methanol, ethanol, acetonitrile and acetone with water resulted in a high yield of extracts compared to absolute ethanol, acetonitrile, water and acetone.

Extensive review of past research on tannin extraction revealed that most often, the solvent combinations have been made from either water and the alcohols or ketones and water. It is not clear from literature whether there would be an improvement in the extraction efficiencies of the solvents when two or more ketones only or alcohols only are combined. These combinations could be explored to determine their efficiencies in the extraction of tannins from plants.

Tannins, which are secondary compounds are readily available as they are widely distributed in plants (Antwi-Boasiako and Animapauh 2012). They are often found in the growth areas of trees, such as the secondary phloem and xylem and the bark (Hagerman 2002). *Moringa oleifera* and *Azadirachta indica* possess tannins in their barks which can be exploited for adhesive production (Prakash *et al.* 2002).

The objective of this research was to evaluate the efficiencies of ketones, alcohols and water and their combinations in extracting tannins from the barks of *Moringa oleifera* and *Azadirachta indica*. This was achieved by measuring the yield of soluble compounds and the amount of tannins (i.e. stiasny number) obtained from the barks of the two species by the solvents. Results from this research would provide the composite industry with knowledge on the possible range of solvent combinations, which can improve the amount of tannins obtained from plants for adhesive production.

## Materials and Methods

### Study Area

This study was conducted at the General Chemical Laboratory of the Faculty of Renewable Natural Resources (FRNR), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Bark samples of 33-year-old *A. indica* trees and 27-year-old *M. oleifera* trees were collected from the demonstration farms of the FRNR and the Department of Horticulture, KNUST. The sites are located in the moist semi-deciduous forest zone with an average altitude of 260 m and dominated by sandy loam soil. The zone has an average annual rainfall of 1,270 mm (Nolan and Twumasi 1992) and an average temperature of 25.9°C.

### Sampling and Processing of Materials

The bark samples were collected from five randomly selected trees each of *A. indica* and *M. oleifera*. The samples were obtained from the stem of the trees at a distance of 4 feet from the ground along the boles of the trees, air-dried for 1 week, milled in the Wiley mill and sieved through a 0.5µm mesh. The milled samples were kept in airtight containers.

### Preparation of Solvent Mixtures

Two alcohols [ethanol (Model Number: MFCD00003568) and methanol (Model Number: MFCD00004595)], one ketone [acetone (Model Number: MFCD00008765)] (all from VWR chemicals) and water were the solvents used for the extraction.

Table 1. Ratio of the individual solvents mixed together for the extraction of tannins

Solvent/Solvent mixtures ID	Ratio of the individual solvents in the mixture (%)				Total (%)
	Acetone	Ethanol	Methanol	Water	
A	25	25	25	25	100
B	60	20	20	0	100
C	0	60	20	20	100
D	20	0	60	20	100
E	20	20	0	60	100
F	0	0	0	100	100
G	100	0	0	0	100
H	0	100	0	0	100
I	0	0	100	0	100
J	50	50	0	0	100
K	50	0	50	0	100
L	50	0	0	50	100
M	0	50	50	0	100
N	0	50	0	50	100
O	0	0	50	50	100

These solvents were all miscible according to the solvent miscibility table. The ratios of these solvents in Table 1 were adopted from the ratios used by previous authors (e.g. Downey and Hanlin 2010; Antwi-Boasiako and Animapauh 2012) in similar works. About 250ml each of

fifteen (15) solvent mixtures at 90% concentration were prepared in a conical flask for the extraction process.

### Tannin Extraction Process

Five grams of the milled samples from each species was weighed and loaded into the thimble of Glas-col Soxhlet

Extraction apparatus (Model: Glas-Col 100D RJ30424). The extraction solvent was placed in a distillation flask and heated to reflux until the solvent in the thimble chamber turned colourless. The extraction process was replicated three times for each species and solvent(s).

### Qualitative Test for Tannins Obtained from the Extraction Process

The extracts obtained by the different solvents were subjected to 3 drops of iron (III) chloride (FeCl<sub>3</sub>) solution and the colour changes were recorded and compared to the Axis Gear Standard Colour Chart ([www.axisgear.ca](http://www.axisgear.ca)), which served as a reference point. A blue to green or blue-black colouration indicated the presence of tannins (Darkwa *et al.* 1996). The test was repeated three times for each extract.

### Quantitative Test for Soluble Compounds

**Percentage Yield of Soluble Compounds Obtained from *A. indica* and *M. oleifera* by the Solvents.** The weights of five empty beakers were recorded and the beakers were filled with 5g of the milled samples and oven-dried at 105 ± 2°C for 24 h. The oven-dried milled samples were cooled in a desiccator for 1h and re-weighed. Oven-dried weight of the milled samples were determined from the difference between final weight of filled beakers and empty beakers. About 200ml of the extracts from the different solvents were oven-dried at 105 ± 2°C for 4 h, cooled in a desiccator for 1 h and weighed. The percentage yield of soluble compounds was determined by the formula (Antwi-Boasiako and Animapauh 2012);

$$\text{Yield (\%)} = \frac{\text{Weight of extracts after oven-drying}}{\text{Oven-dried weight of milled samples}} \times 100$$

**The Amount of Tannins (Stiasny Number or Formaldehyde Precipitate number) Contained in the Soluble Compounds.** Stiasny number or formaldehyde precipitate number, which relates directly to the amount of tannins in the soluble compounds was determined. Stiasny number determination described by Wissing (1955) was adopted in quantifying the amount of tannins. A mixture of concentrated hydrochloric acid (250 ml) and Formaldehyde (40%) (500 ml) (1:2 v/v) was prepared in a volumetric flask. 50 ml of the extracts obtained from the extraction process was oven dried at 105 ± 2°C for 4 h, cooled in a desiccator for 1 h and the oven-dried weight recorded. Another 50 ml of the extracts were reacted individually with 10 ml of the Hydrochloric acid and Formaldehyde mixture to form a suspension. The suspension was then heated under reflux for 30 min. The suspension was allowed to cool to enable the formed precipitates to settle. The suspension was then filtered and the precipitate washed with hot water and oven-dried at 105 ± 2°C until constant weight. This process was replicated three times for each solvent extract. The stiasny number was determined by the formula (Antwi-Boasiako and Animapauh 2012);

$$\text{Stiasny number (\%)} = \frac{\text{Weight of precipitate after oven-drying}}{\text{Oven-dry weight of extract}} \times 100$$

### Data Analysis

The data were subjected to ANOVA and LSD test to determine the significant differences ( $p < 0.05$ ) between the amount of tannins extracted by the solvents and their mixtures.

## Results

### Qualitative Test for Tannins Obtained from the Extraction Process

The colour of the extracts from *A. indica* and *M. oleifera* before their reaction with iron (III) chloride varied from cinnamon to gold. After the reaction, there was a change in colouration, which ranged from black to army (Table 2). This indicated the presence of tannins in the extracts.

### Quantitative Test for Soluble Compounds in *A. indica* and *M. oleifera*

**Percentage Yield of Soluble Compounds Obtained from *A. indica* and *M. oleifera* by the Solvents.** More soluble compounds were obtained from *A. indica* than *M. oleifera* for all the solvents (Figure 1). In *A. Indica*, the mixture containing the alcohols (ethanol and methanol) and water (i.e., E-M-W) extracted the highest soluble compounds (97.96 ± 1.71%) followed by the ketone and water mixture (i.e., Acetone-Water) (69.01 ± 8.36%). The least yield of soluble compounds was obtained by the ketone and the alcohols mixture (i.e., A-E-M) (23.78 ± 0.34%). ANOVA showed significant differences between the solvents. For *M. oleifera*, water gave the highest yield of soluble compounds (39.39 ± 1.5%) followed by the mixture of ketone and water (i.e., Acetone-Water) (30.74 ± 3.79%) (Table 3). The difference was not significant ( $p > 0.05$ ). The least yield of soluble compounds was obtained from the ketone/acetone only (3.29 ± 0.21%). ANOVA showed significant differences between the solvents.

Whereas the combination of the solvents resulted in a higher quantity of soluble compounds than the yield from the individual solvents in some instances, the reverse was also recorded for some mixtures. For instance, in *A. indica*, acetone, methanol and water independently produced a soluble compound yield of 31.44%, 34.06% and 34.54% respectively while their combination extracted 43.03% of soluble compounds. Similarly, in *M. oleifera*, while methanol and ethanol independently produced 11.58% and 7.97% of soluble compounds respectively, their mixture extracted up to 12.62% of these compounds. Nevertheless, while ethanol and water separately extracted 7.97% and 39.39% of soluble compounds from *M. oleifera*, their combination extracted only 13.53% of soluble

compounds. The mixture of three or more solvents did not improve the yield of soluble compounds in *M. oleifera*. Likewise, the yields of soluble compounds from acetone (31.44%), ethanol (43.51%) and methanol (34.06%) were higher than that from their mixture (23.78%) in *A. indica*. For example, while acetone, methanol and water independently extracted 3.29%, 11.58% and 39.39% of soluble

compounds respectively, their mixture extracted only 7.94% of soluble compounds.

With the exception of Ethanol-Water, Methanol-Water and A-E-M-W mixtures, all combinations containing water generally produced high yield of soluble compounds in *A. indica*. This was not so in *M. oleifera*.

Table 2. Colour change of extracts before and after reaction with Iron (III) chloride

Species	Solvent(s) used for the extraction	Extract colour before reaction with $FeCl_3$	Extract colour after reaction with $FeCl_3$	Inference
<i>A. indica</i>	Acetone (A)	Cinnamon	Black	Tannin present
<i>A. indica</i>	Methanol (M)	Brick	Forest	Tannin present
<i>A. indica</i>	Ethanol (E)	Brick	Forest	Tannin present
<i>A. indica</i>	Water (W)	Brick	Forest	Tannin present
<i>A. indica</i>	Acetone-Ethanol	Brick	Forest	Tannin present
<i>A. indica</i>	Acetone-Methanol	Brick	Forest	Tannin present
<i>A. indica</i>	Acetone-Water	Cocoa	Black	Tannin present
<i>A. indica</i>	Ethanol-Methanol	Brick	Black	Tannin present
<i>A. indica</i>	Ethanol-Water	Cinnamon	Black	Tannin present
<i>A. indica</i>	Methanol-Water	Brick	Black	Tannin present
<i>A. indica</i>	A-E-M-W	Cinnamon	Forest	Tannin present
<i>A. indica</i>	A-E-M	Brick	Black	Tannin present
<i>A. indica</i>	E-M-W	Brick	Black	Tannin present
<i>A. indica</i>	A-M-W	Brick	Forest	Tannin present
<i>A. indica</i>	A-E-W	Brick	Black	Tannin present
<i>M. oleifera</i>	Acetone	Gold	Forest	Tannin present
<i>M. oleifera</i>	Methanol	Army	Army	Tannin present
<i>M. oleifera</i>	Ethanol	Army	Army	Tannin present
<i>M. oleifera</i>	Water	Gold	Gold	Tannin present
<i>M. oleifera</i>	Acetone-Ethanol	Citrus	Forest	Tannin present
<i>M. oleifera</i>	Acetone-Methanol	Citrus	Forest	Tannin present
<i>M. oleifera</i>	Acetone-Water	Coffee	Forest	Tannin present
<i>M. oleifera</i>	Ethanol-Methanol	Citrus	Forest	Tannin present
<i>M. oleifera</i>	Ethanol-Water	Gold	Army	Tannin present
<i>M. oleifera</i>	Methanol-Water	Gold	Army	Tannin present
<i>M. oleifera</i>	A-E-M-W	Citrus	Forest	Tannin present
<i>M. oleifera</i>	A-E-M	Lemon	Army	Tannin present
<i>M. oleifera</i>	E-M-W	Tangerine	Army	Tannin present
<i>M. oleifera</i>	A-M-W	Lemon	Army	Tannin present
<i>M. oleifera</i>	A-E-W	Tangerine	Army	Tannin present

Table 3. Mean percentage yield of soluble compounds from *A. indica* and *M. oleifera*

Solvents/Solvent Mixtures	Yield of soluble compounds (%)	
	<i>A. indica</i>	<i>M. oleifera</i>
Acetone	31.44 ± 3.67 <sup>abcdef</sup>	3.29 ± 0.21 <sup>abc</sup>
Methanol	34.06 ± 1.88 <sup>ghijklmn</sup>	11.58 ± 0.81 <sup>defghij</sup>
Ethanol	43.51 ± 0.59 <sup>opqrstu</sup>	7.97 ± 1.33 <sup>klmnopq</sup>
Water	34.54 ± 1.59 <sup>bgovwxyzab</sup>	39.39 ± 1.50 <sup>*</sup>
Acetone-Ethanol	42.77 ± 0.52 <sup>hpyvpθmδ</sup>	6.61 ± 0.69 <sup>alrstuvw</sup>
Acetone-Methanol	42.55 ± 0.63 <sup>iqwyΔμ&amp;η</sup>	8.76 ± 2.22 <sup>ekrxyzab</sup>
Acetone-Water	69.01 ± 8.36 <sup>*</sup>	30.74 ± 3.79 <sup>*</sup>
Ethanol-Methanol	45.42 ± 6.9 <sup>trpΔ12</sup>	12.62 ± 2.08 <sup>ixypθπ</sup>
Ethanol-Water	33.29 ± 1.49 <sup>cjx345</sup>	13.53 ± 0.18 <sup>γδ</sup>
Methanol-Water	33.11 ± 0.33 <sup>dky367</sup>	15.94 ± 0.22 <sup>ρδ</sup>
A-E-M	23.78 ± 0.34 <sup>f</sup>	4.41 ± 0.02 <sup>εηΔ1</sup>
E-M-W	97.96 ± 1.71 <sup>*</sup>	9.38 ± 0.10 <sup>houzθμz23</sup>
A-M-W	43.03 ± 0.77 <sup>mtam&amp;18ε</sup>	7.94 ± 0.05 <sup>pvα&amp;124</sup>
A-E-W	40.60 ± 0.72 <sup>nuβδγ2579ε</sup>	9.50 ± 0.43 <sup>iqwβηπ34</sup>
A-E-M-W	35.58 ± 1.17 <sup>elszθμ4689</sup>	6.71 ± 0.05 <sup>bmsyΔμ&amp;η</sup>

Means with the same superscripts in the same column are not significantly different at 5% confidence level

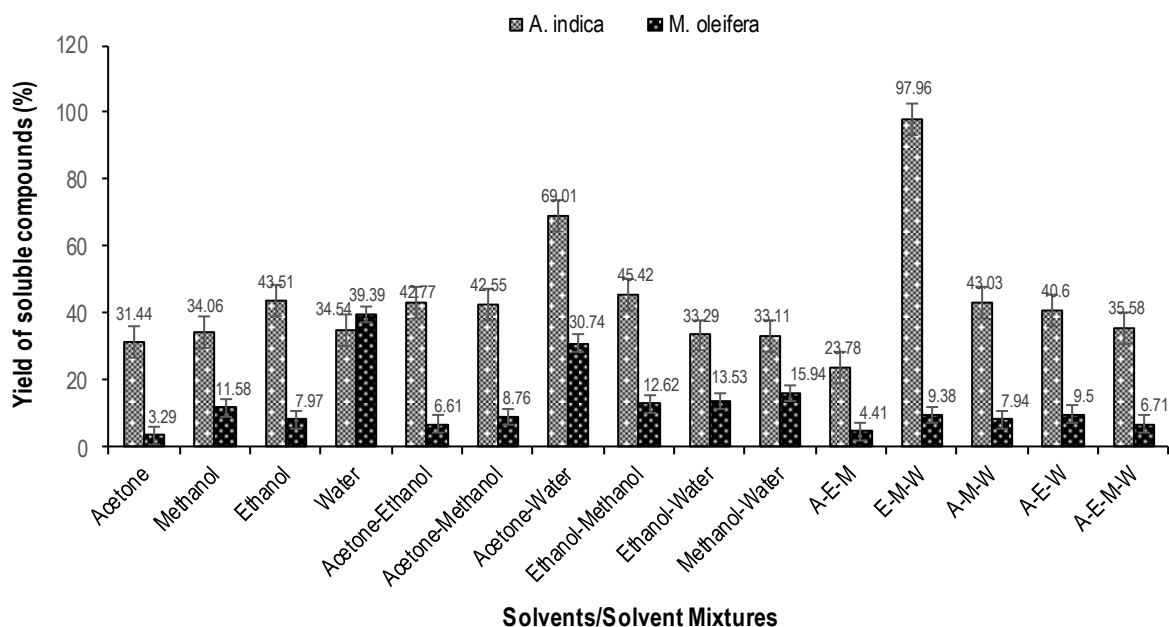


Figure 1. Mean percentage yield of soluble compounds obtained from *A. indica* and *M. oleifera*

**The Amount of Tannins (Stiasny Number or Formaldehyde Precipitate Number) Contained in the Soluble Compounds.** The amount of tannins obtained from *A. indica* by all the solvents (i.e.,  $27.24 \pm 1.61 - 98.74 \pm 0.27\%$ ) were generally higher than those from *M. oleifera* ( $1.61 \pm 0.2 - 34.56 \pm 3.4\%$ ) (Figure 2). The alcohols and water mixture (E-M-W) produced the highest yield of tannins in *A. indica* ( $98.74 \pm 0.27\%$ ) while Ethanol only gave the highest yield ( $34.56 \pm 3.4\%$ ) in *M. oleifera*. The least amount of tannins was obtained by water in *A. indica* ( $27.24 \pm 1.61\%$ ) and Methanol-Water in *M. oleifera* ( $1.61 \pm 0.2\%$ ) (Table 4).

The mixtures containing either a ketone (i.e., acetone) or the alcohols (i.e., methanol and ethanol) produced comparatively high tannin yield in *A. indica* (Figure 2). This

trend was not so for *M. oleifera*. With the exception of water only and, acetone, ethanol and methanol mixture (A-E-M), the individual solvents performed better than their various mixtures in *M. oleifera*. For *A. indica*, however, the mixtures largely produced high yield of phenols than the individual solvents except for some few mixtures involving either Methanol or Ethanol. The differences between the tannin yields of the solvents were significant for both species except between E-M-W and Acetone-Water in *A. indica* and, Acetone and Methanol, and Acetone and Ethanol in *M. oleifera*.

Table 4. Total amount of tannins (Stiasny number) contained in the soluble compounds from *A. indica* and *M. oleifera*

Solvents	Total amount of tannins (%)	
	<i>A. indica</i>	<i>M. oleifera</i>
Acetone	33.31 ± 2.51 <sup>a</sup>	30.63 ± 1.05 <sup>abcde</sup>
Methanol	77.53 ± 4.27 <sup>bcdde</sup>	33.44 ± 3.56 <sup>afgh</sup>
Ethanol	92.83 ± 0.88 <sup>ghijkl</sup>	34.56 ± 3.40 <sup>bfi</sup>
Water	27.24 ± 1.61 <sup>a</sup>	3.94 ± 0.83 <sup>iklm</sup>
Acetone-Ethanol	97.87 ± 0.77 <sup>mnopq</sup>	24.03 ± 1.54 <sup>no</sup>
Acetone-Methanol	93.16 ± 1.15 <sup>qrstuv</sup>	12.48 ± 0.53 <sup>pqr</sup>
Acetone-Water	98.03 ± 0.27 <sup>hnnwxy</sup>	27.20 ± 2.85 <sup>gnst</sup>
Ethanol-Methanol	70.48 ± 5.88 <sup>bz</sup>	8.94 ± 1.44 <sup>juvw</sup>
Ethanol-Water	82.38 ± 4.60 <sup>coβ</sup>	3.16 ± 0.64 <sup>kuxy</sup>
Methanol-Water	71.27 ± 1.42 <sup>dz</sup>	1.61 ± 0.20 <sup>lz</sup>
A-E-M	88.44 ± 0.24 <sup>isβyp</sup>	43.90 ± 0.31 <sup>*</sup>
E-M-W	98.74 ± 0.27 <sup>otwθπ</sup>	7.43 ± 3.58 <sup>mnyz</sup>
A-M-W	97.72 ± 0.32 <sup>kpuvθδ</sup>	24.49 ± 4.32 <sup>dos</sup>
A-E-W	93.28 ± 0.43 <sup>qvypπδ</sup>	31.50 ± 1.14 <sup>ehit</sup>
A-E-M-W	82.62 ± 1.17 <sup>eoγ</sup>	14.97 ± 2.29 <sup>qv</sup>

Means with the same superscripts in the same column are not significantly different at 5% confidence level.

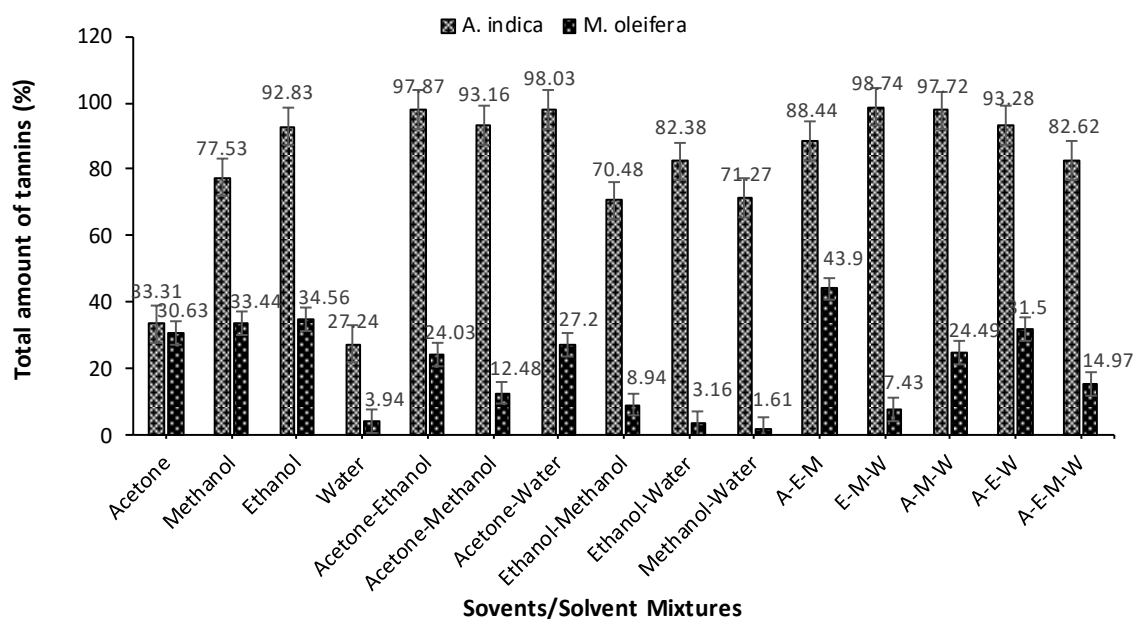


Figure 2. Total amount of tannins (Stiasny number) contained in the soluble compounds from *A. indica* and *M. oleifera*

## Discussions

### Qualitative Test for Tannins Obtained from the Extraction Process

Tannins are phenols, which are tested qualitatively using ferric salts. They form compounds with ferric salts such as iron (III) chloride, which possess a metal centre bound to ligands (atoms, ions or molecules). The ligands donate electrons to the metal ( $Fe^{3+}$ ) and this transfer of charges, gives a very strong spectral signal in the visible range of the electromagnetic spectrum. Phenols contain hydroxyl groups (hydrogen and oxygen) and are relatively strong acids as iron (III) chloride which is a Lewis acid. According to Sarhan and Bolm (2009), all oxygen containing

compounds act as bases in the presence of Lewis acids. This results in an acid-base reaction, with the formation of a precipitate and ligand exchanges, which causes a colour change.

In determining the presence of tannins in plant extracts Darkwa and Jetuah (1996) and Lysyuk (2011) explained that when iron (III) chloride is added to the extracts and the colour of the extract changes to blue-black or green, it indicates that tannins are present. When 3 drops of iron (III) chloride were added to the extracts from the barks of *Moringa oleifera* and *Azadirachta indica*, their colours changed to shades of green and black (Table 2). The colour change could be attributed to the ligand exchanges between the phenols and iron (III) chloride. Dhawan and Gupta (2017) similarly observed these colour

changes with *Datura metel* leaves extract after reaction with iron (III) chloride, and concluded that the extract contained tannins.

The difference in the colours of the extracts after their reaction with the Lewis acid represents the variations in the amount of tannins found in the plants. Deep green to black colouration shows a high tannin content while light blue to green or no shade of green indicates a low or no tannin content (Antwi-Boasiako and Animapauh 2012). *A. indica* bark gave deeper colour shades of forest (green with black undertone) and black than *M. oleifera* where the extract colours were gold, and mostly army (light shade of green). Thus, *A. indica* bark extracts were expected to have a higher tannin content than *M. oleifera*.

#### Quantitative Test for *A. indica* and *M. oleifera*

**Percentage Yield of Soluble Compounds Obtained from *A. indica* and *M. oleifera* by the Solvents.** The yield of soluble compounds from plant parts is a measure of the efficiency of the extracting solvent(s) (Antwi-Boasiako and Animapauh 2012; Boadu *et al.* 2018). Plant extracts contain carbohydrates, alkaloids, terpenoids and phenols (Hagerman 2002); high yield of soluble compounds means there is a high percentage of these components in the extract of the plant. In the present study, more soluble compounds were obtained from *A. indica* than *M. oleifera* for all the solvents. These compounds have varying polarities (Akowuah *et al.* 2005) and their dissolution in specific solvents depends, among others, on the polarity of the solvent (Cuong *et al.* 2020). According to Zuo *et al.* (2002), polar substances easily dissolve in polar solvents and vice versa. The solvents used in the extraction of soluble compounds from *A. indica* and *M. oleifera* had an increasing order of polarity as acetone < ethanol < methanol < water (Tan and Kassim 2011). The yield of soluble compounds in *M. oleifera* also increased as acetone (3.29%) < ethanol (7.97%) < methanol (11.58%) < water (39.39%). Thus, more of the soluble compounds in *M. oleifera* were possibly polar and therefore dissolved in the most polar of all the solvents (i.e., water). Similarly, methanol extraction resulted in a higher yield of soluble compounds from *Datura metel* leaves than acetone (Dhawan and Gupta 2017). In *A. indica*, however, ethanol, which is less polar than water had a high yield of soluble compounds (43.51%) than methanol (34.05%) and distilled water (34.53%). Thus, unlike *M. oleifera*, more of the soluble compounds in *A. indica* could have been less polar and were, therefore, likely attracted to ethanol than the highly polar solvents such as water.

Chavan and Amarowicz (2013) explained that the yield of soluble compounds is often improved by the use of multiple solvents for extraction. This is due to the synergistic strength of the solvents produced by the mixture. This assertion was found to be partially true in the current study since not all the solvent mixtures improved the soluble compound yield from *A. indica* and *M. oleifera*. This work

has found that the type of plant species contributes to the extracting efficiencies of the solvents and their mixtures.

Mixtures containing water extracted the highest quantities of soluble compounds in both species. Darkwa and Jetuah (1996) explained that water is able to dissolve a large range of compounds including the low molecular weight carbohydrates compared to the other solvents. Its combination with the alcohols and ketone broadened the range of compounds that could be dissolved by the mixture. Hence, the high yield of soluble compounds recorded for mixtures containing water.

**The Amount of Tannins (Stiasny Number or Formaldehyde Precipitate Number) Contained in the Soluble Compounds.** The Stiasny number of extracts is an estimate of the amount of reactive tannins that get attached to formaldehyde in the process of quantifying the phenols (Simon *et al.* 1992; Paridah 2002; Pandey and Rizvi 2009). Thus, a high stiasny number of a plant extract means that most of the soluble compounds in the extract that got attached to formaldehyde are tannins (Hagerman, 2002; Antwi-Boasiako and Animapauh 2012). For all solvents, more tannins were obtained from *A. indica* (i.e.,  $27.24 \pm 1.61 - 98.74 \pm 0.27\%$ ) than *M. oleifera* ( $1.61 \pm 0.2 - 34.56 \pm 3.4\%$ ). For the single solvents, Darkwa and Jetuah (1996) recorded higher tannin content (74%) in the soluble compounds extracted from *Rhizophora spp.* with water than those extracted with ethanol (61%) and petroleum ether (3.2%). On the contrary, water produced lower amount of tannins (27.24% in *A. indica* and 3.94% in *M. oleifera*) in the present study than ethanol (92.83% and 34.56% respectively). The mixtures containing either a ketone (i.e., acetone) or the alcohols (i.e., methanol and ethanol) produced comparatively high tannin yield in *A. indica*. Thus, although water extracted more soluble compounds in both species, smaller percentage of these compounds were tannins. Tannins have low polarity (Tian *et al.*, 2009) and the condensed type has limited solubility in highly polar organic solvent such as water (Fraga-Corral *et al.* 2020). The low polarity of tannins probably restricted its dissolution in water, which has high polarity compared to the low polar solvents such as ethanol and acetone (Zuo *et al.* 2002).

Downey and Hanlin (2010) found acetone-water mixture more effective than ethanol-water mixture for the extraction of condensed tannins from grape skin. Chavan and Amarowicz (2013) also reported higher quantities of tannins from beach pea with acetone-water mixture than ethanol-water and methanol-water mixtures. Our results agree with the observation by these authors. With the exception of mixtures involving either methanol or ethanol, the solvent mixtures generally improved the stiasny numbers of extracts from *A. indica*. For example, while the alcohols and water mixture (E-M-W) produced the highest yield of tannins ( $98.74 \pm 0.27\%$ ), water only produced the least amount of tannins ( $27.24 \pm 1.61\%$ ). In *M. oleifera*, however, more tannins were extracted by the individual solvents (e.g.  $34.56 \pm 3.4\%$  by ethanol only) than their

various mixtures (e.g.  $3.16 \pm 0.64\%$  by ethanol-water mixture) except water only and, acetone, ethanol and methanol mixture (A-E-M). Therefore, a mixture of solvents will not always produce a synergistic effect on the yields of soluble compounds and tannins from plants.

According to Yazaki and Collins (1994) and Ferreira *et al.* (2008), at least 65% of tannins must be obtained from extracts of plants in order to recommend that plant as a raw material source for commercial wood adhesive production. With the exception of extracts from acetone only and water only in *A. indica*, the quantities of tannins from the other solvent extracts were above 65%, which meets the threshold for adhesive production. Thus, extracts from *A. indica* would be most useful to commercial adhesive producers. In *M. oleifera*, all the solvent extracts did not contain the minimum amount of tannins required for adhesive production. However, based on the recommendation of Ferreira *et al.* (2008), tannins extracted with acetone, methanol, ethanol, Acetone-Ethanol-Methanol and Acetone-Ethanol-Water mixtures can serve as tannin mordants.

### Conclusions

The present work investigated the synergistic effect of ketone, water and alcohols on the yield of soluble compounds and tannins from the barks of two tropical plants, namely, *Azadirachta indica* and *Moringa oleifera*. The conclusions from the work are as follows.

Mixtures of ketone, water and alcohols will not always produce a positive synergistic effect on the yields of soluble compounds and tannins from plants. The extracting efficiencies of the solvents depended mostly on the species. While water extracted the highest quantity of soluble compounds in *M. oleifera*, ethanol-methanol-water mixture extracted the highest quantity of soluble compounds in *A. indica*.

Although water extracted more soluble compounds than all other solvents in *M. oleifera*, a small percentage of these compounds were tannins. The mixture containing ketone (i.e., acetone) and the alcohols (i.e., methanol and ethanol) produced comparatively high tannin yield even though it extracted low quantities of soluble compounds. In *A. indica*, the least yield of soluble compounds was obtained by the ketone and the alcohols mixture (i.e., A-E-M) while mixtures containing either a ketone or the alcohols produced comparatively high tannin yield.

Unlike *M. oleifera* extracts, *A. indica* extracts from all the solvents except acetone only and water only would be most useful to commercial adhesive producers since the quantities of tannins obtained from them were higher than the minimum threshold (65%) required by the adhesive industry.

### Acknowledgement

We are grateful to Mr. Douglas Amoah (Technician at the Chemical Laboratory of the Department of Wood Science and Technology, KNUST) for his guidance during the laboratory activities. We also thank Mr. Daniel Quarcoe (Daniemma Medichem Ventures) for supplying the reagents used for the work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### References

- Akowuah, G.A.; Z. Ismail; I. Norhayati; A. Sadikun, A. 2005. The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chemistry* 93(2): 311-317.
- Antwi-Boasiako, C.; S.O. Animapauh. 2012. Effect of Solvent Type on Tannin Extractability from Three *Tetrapleura tetraptera* (Schum. & Thonn.) Taubert Positions for Wood Composite Adhesive Formulation. *Journal of Emerging Trends in Engineering and Applied Sciences* 3(3): 517-525.
- Arranz, S.; F. Saura-Calixto; S. Shaha; P.A. Kroon. 2009. High contents of nonextractable polyphenols in fruits suggest that polyphenol content of plant foods have been underestimated. *J. Agri. Food Chem.* 57: 7298-7303.
- Azwandia, N.N. 2015. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation' *Medicinal and Aromatic Plants* 4(3): 1-6.
- Boadu K.B; C. Antwi-Boasiako; L. Ofosuhene. 2018. Solvent extraction of inhibitory substances from three hardwoods of different densities and their compatibility with cement in composite production. *Journal of the Indian Academy of Wood Science* 15(2): 140-148.
- Chavan, U.D.; R. Amarowicz; F. Shahidi. 2013. Effects of various solvents systems on extraction of phenolics, tannins and sugars from beach pea (*Lathyrus maritimus* L). *International food research Journal* 20(3): 1139-1144.
- Cuong, D. X.; N.X. Hoan; D.H. Dong; L.T.M. Thuy; N.V. Thanh; H.T. Ha; D.T.T. Tuyen; D.X. Chinh. 2020. Tannins: Extraction from Plants. In: Alfredo Aires (Eds.), *Tannins - Structural Properties, Biological Properties and Current Knowledge. IntechOpen*. <https://doi.org/10.5772/intechopen.86040> (Accessed on April 04, 2020).
- Dailey, A.; V.Q. Vuong. 2015. Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. *Cogent Food & Agriculture* 1(1): 1115646, DOI: 10.1080/23311932.2015.1115646.
- Darkwa, N.A.; F.K. Jetuah. 1996. Distribution of Tannins in Three Ecotypes of *Rhizophora* spp. along the Coastal Belt of Ghana. *Ghana Journal of Forestry* 2: 25-28.

- Dhawan, D.; J. Gupta. 2017. Comparison of Different Solvents for Phytochemical Extraction Potential from *Datura metel* Plant leaves. *International Journal Biological Chemistry* 11(1): 17-22.
- Downey, M.O.; R.L. Hanlin. 2010. Comparison of Ethanol and Acetone Mixtures for Extraction of Condensed Tannin from Grape Skin. *South African Journal of Enology and Viticulture* 31(2): 154-159.
- Ferreira, da S.E.; C.C.R. Leis; O. de E. Brito; S. Iwakiri. 2008. Use of Tannins from *Pinus Oocarpa* Bark for Manufacture of Plywood. Proceedings of the 51st International Convention of Society of Wood Science and Technology. November, 10-12, 2008; Concepción, Chile.
- Fraga-Corral, M.; P. García-Oliveira; A.G. Pereira; C. Lourenço-Lopes; C. Jimenez-Lopez; M.A. Prieto; J. Simal-Gandara. 2020. Review: Technological Application of Tannin-Based Extracts. *Molecules* 25: 614.
- Hagerman, A.E. 2002. Tannin Handbook. Available at [www.academia.edu](http://www.academia.edu) (Accessed 3rd December 2018).
- Jetuah, F.; E. Quayson; D. Sekyere. 2001. Partial Replacement of Phenol in Phenol-Formaldehyde Resin with Tannins from *Acacia nilotica*. *Ghana Journal of Forestry* 10: 53-56.
- Lysyuk, R.M.; V.O. Antonyuk. 2011. A Textbook of Pharmacognosy. Available at [www.pharmacognosy.org.ua](http://www.pharmacognosy.org.ua) (Accessed 4th December, 2018).
- Nolan, T.M.; A. Twumasi. 1992. Forest management unit 17 working plan (Asukese forest reserve and Amama shelterbelt forest reserve) (pp. 29). Accra, Ghana: Forestry Department Ghana.
- Pandey, B.K.; I.S. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity* 2(5): 270-278.
- Paridah, M.T.; O.C. Musgrave; Z. Ashaari. 2002. Determination of polyphenolic content of bark extracts for wood adhesives. *Holzforschung International Journal of Biological Chemistry* 56(56): 267-272.
- Prakash, G.; S.S. Bhojwani; A.K. Srivastava. 2002. Production of Azadirachtin from Plant Tissue Culture: State of the Art and Future Prospects. *Biotechnology and Bioprocess Engineering* 7: 185-193.
- Readel, K.; D. Seigler; K. Hwang; J. Keesy; S. Seilheimer. 2001. Tannins from Mimosoid Legumes of Texas and Mexico. *Economic Botany* 55(2): 212-222.
- Sarhan, A.A.O.; C. Bolm. 2009. Iron (III) chloride in oxidative C-C coupling reactions. *The Royal Society of Chemistry* 38(9): 2730-2744.
- Shi, Y.; X. Liang; L. Chi; Y. Chen; L. Liang; J. Zhao; Y. Luo; W. Zhang; Q. Cai; X. Wu; Z. Tan; L. Zhang. 2021. Ethanol extracts from twelve *Curcuma* species rhizomes in China: Antimicrobial, antioxidative and anti-inflammatory activities. *South African Journal of Botany* 140: 167-172. doi.org/10.1016/j.sajb.2021.04.003.
- Simon, de F.B.; J. Perez-Illarbe; T. Hernandez; C. Gomez-Cordoves; I. Estrella. 1992. Importance of phenolic compounds for the characterization of fruit juices. *Journal of Agricultural and Food Chemistry* 40(9): 1531-1535.
- Singh, M., Jha, A., Kumar, A., Hettiarachchy, N., Rai, A. K., Sharma, D. 2014. Influence of the solvents on the extraction of major phenolic compounds (punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril. *Journal of Food Science and Technology* 51(9), 2070-2077. <https://doi.org/10.1007/s13197-014-1267-0>
- Standard Colour Chart. Available at [www.AXISGEAR.ca](http://www.AXISGEAR.ca)
- Stefanelli, D.; S. Brady; T. Plozza; C. Trenerry; R. Jones. 2013. The Effect of Freezing and Extraction Solvent on the Total Polyphenol Content Measurement in Whole 'Royal Gala' Apple Fruit. *Acta horticulturae* 1012(1012): 1459-1466
- Tan, K.W.: J.M. Kassim. 2011. A correlation study on the phenolic profiles and corrosion inhibition properties of mangrove tannins (*Rhizophora apiculata*) as affected by extraction solvents. *Corrosion science* 53(2): 569-574.
- Tian, F.; B. Li; B. Ji; J. Yang; G. Zhang; Y. Chen; Y. Luo. 2009. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity affects the bioactivities. *Food Chemistry* 113(1): 173-179.
- Ugovsek, A.; M. Kariz; M. Sernek. 2010. Bonding of Wood with Adhesive Mixtures made of Liquefied Wood Combined with Tannin or Phenolic Resin. "Future with Forests" - First Serbian Forestry Congress, Beograd 2010.
- Wissing, A. 1955. Utilisation of Bark II. Investigation of the Stiasny reaction for the precipitation of polyphenols in pine bark extractives. *Svensk. Paperstidn* 207: 45-50.
- Yazaki, Y.; P.J. Collins. 1994. Wood Adhesives Based on Tannin Extracts from Barks of some Pine and Spruce spp. *European Journal of Wood and Wood Products* 52(5): 689-700.
- Zuo, Y.; H. Chen; Y. Deng. 2002. Simultaneous determination of catechins, phenolic acids and caffeine in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. *Talanta* 57(2): 307-316.
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