



## Phytochemical, Phenolic and Flavonoid Constituents of the Extract of *Trichilia emetica* Seeds

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### ABSTRACT

*Trichilia emetica* seeds crude extracts (aqueous, methanol, hexane and chloroform) were screened for their secondary metabolites. Phytochemicals such as alkaloids, glycosides, terpenoids, steroids were present in all the crude extracts, while saponin, phenols, flavonoids, tannins and anthraquinones were only present in methanol and water extracts, and resins was only present in hexane and chloroform extracts. The yield obtained for 20 g of dry plant materials for each of the extracts were  $1.71 \pm 0.014$ g in aqueous,  $2.11 \pm .092$ g in methanol,  $2.41 \pm 0.043$ g in hexane and  $3.03 \pm 0.032$ g in chloroform. The total phenolics and flavonoids contents were quantified in the aqueous plant extracts as 42.66 mg of Gallic acid equivalents /g and 59.10 mg of Rutin equivalents /g, respectively.

**Keywords:** *Trichilia emetica*, phytochemicals, flavonoids, phenolics, extract

### INTRODUCTION

Plants have always been an indispensable companion of mankind by providing the basic necessities of life such as food, shelter, clothing and fuel. Furthermore, after fulfilling these basic needs they also serve humanity in the cure or prevention of different diseases (Gasparetto et al., 2011). The use of plants to cure diseases and relieve physical suffering predates written human history (Hill, 1989). Different plant parts such as roots, stems, barks, leaves, flowers, fruits, seeds, exudates and modified plant organs are believed to possess varied medicinal properties that produce a definite physiological action on the human body (Sumathi and Gowthami, 2014; Alorkpa et al., 2016). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Sumathi and Gowthami, 2014).

*Trichilia emetica* belong to *Meliaceae* family and genus *trichilia* (Komane et al., 2011). The phytochemical studies conducted on the leaves, root and stem bark of this plant revealed the presence of resins, tannin, pectin, sesquiterpenoid and a mixture of triterpenes (calcidrin) Diallo et al., 2003; komane et al., 2011). And these compounds have demonstrated antimicrobial, antioxidant, and anti-insect properties and also DNA damage repairs (Kubo and Klocke, 1982; Germano et

al., 2005 and 2006). Recent studies, on the aqueous extract of *T. emetica* whole seeds has resulted in the isolation of several flavonoids (Usman et al., 2018). Flavonoids most especially flavanol occur widely in plant derived foods such as red wine, green tea, cocoa-derived products and many other fruits consumed globally (Teresa et al., 2000; Schroeter et al., 2006). An epidemiological investigation into consumption of flavanol-rich foods and the risk of cardiovascular diseases reveals an inverse relationship and this assumption has remained vague because of limited information regarding their metabolism and distribution after consumption (Lewis and Ausubel, 2006). *T. emetica* has a long history of use in Africa's traditional medicine (Diallo et al., 2003; Komane et al., 2011) it is in view of this that the phytochemical content of the seeds were analysed.

### METHODOLOGY

#### Sample Collection and Preparation

*T. emetica* seeds was collected from Kumasi, Ghana, in February and identified by botanist Mr. Martin A. Arkoh of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A voucher specimen TBG-2014-1 was deposited at the herbarium of Treborth Botanical Garden Bangor, UK.

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The freshly collected sample was shade dried for seven days until all the water molecules evaporated. The air dried samples were pulverize into powder form by the use of mortar and pestle and transferred into airtight containers with proper labelling for future use. The dried powdered sample was separately extracted using cold extraction method with distilled water, methanol, n hexane and chloroform. These was carried out by suspending 20 grams of the finely ground sample in 100ml of distilled water, methanol, n hexane or chloroform for 48 hours. The mixtures were regularly stirred using separate glass rod every 6 hours. After 48 hours, each mixture was filtered through Whatmann No. 1 filter paper and the filtrates were rotary evaporated at 40 °C to obtain a greasy extracts. Each of the extract was transferred to sterile screw capped bottles, properly labelled and stored under refrigerated condition till use.

#### Determination of total phenolic content

The amount of total phenolic compounds present in aqueous seed extract was determined by

the Folin-Ciocalteu reagent using gallic acid as standard. Gallic acid was dissolved in deionised water (0.01 g/100 mL) to prepare triplicate standard stock solutions. The stock was diluted to form gallic acid concentrations for a 7-point calibration curve Table 1. Samples extract and standards (0.1 mL) were placed directly into a cuvettes; Folin & Ciocalteu's Phenol reagent (0.1 mL), 15% w/v Na<sub>2</sub>CO<sub>3</sub> (0.3 mL) and H<sub>2</sub>O (0.5 mL) were added. The mixture was incubated at room temperature in the dark for 1hour and were analysed at wavelengths ( $\lambda$ ) of 725, using the AT1 UNICAM UV/VIS spectrometer UV4 coupled to Vision V3.40 computer software. Results are given as Gallic Acid Equivalents (GAE).

Table 1: Stock solution for 7-points calibration curve.

C (ppm)	0	10	20	30	40	50	60
V <sub>1</sub> (mL)	0	1	2	3	4	5	6
V <sub>2</sub> (mL)	10	9	8	7	6	5	4

C: Concentration of Gallic acid

V<sub>1</sub>: Volume of stock

V<sub>2</sub>: Volume of solvent

#### Determination of flavonoid content

The level of flavonoid content in the seed extract was determined spectrophotometrically using rutin as standard. Rutin stock solution 0.05 mg/mL was prepared in methanol and six, five-fold serial dilutions was made to obtain a concentration of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/L. 1 mL of 2% AlCl<sub>3</sub> in methanol was separately added to methanol solution of the extract (1 mL of 0.05 mg/mL) and stock solution 1 mL. The mixture was incubated at room temperature for 1 hour. The absorbance was measured at 415 nm, using the AT1 UNICAM UV/VIS spectrometer UV4 coupled to Vision V3.40 computer software. The sample, blank (methanol) and standards were prepared in triplicate for each analysis and the mean value of absorbance was obtained and express in terms of rutin equivalents (mg of RU/g of extract).

#### Phytochemical screening

The seed extract of *Trichillia emetica* was screened to detect secondary plant metabolites such as alkaloid, glycosides, saponins, terpenoid, steroids, phenols, flavonoids, tannins and anthraquinone using standard methods (Evans and Trease, 1996; Harbone, 1998).

#### Alkaloids (Dragendorff's and Wagners test)

0.2g of the sample extract was boiled with 5 mL of 2% hydrochloric acid on a steam bath for 10 minutes. The mixture was allowed to cool and filtered. 2ml portion of the filtrate was measured into two 25 ml vials and 1 ml each of Dragendoffs which is a mixture of A [(Bismut nitrate (0.17) in glacia acetic acid (2mL) and water (8 mL)] with B[ Potassium iodide (4g) in glacial acetic acid (10 mL) and water (20 mL)] dilute to 100 mL with water.

Wagner's reagent [Mix Iodine (2g) and Potassium Iodide 6g in 100 mL of water] was added to separate portion of the filtrate. A red precipitate or reddish-brown precipitate indicates the presence of alkaloid

#### Glycosides

0.2g of extract was dissolved in 3ml of deionised water and 3ml of 10% sodium hydroxide was added. The formation of a yellow colour indicates the presence of glucosides.

#### Saponins

0.1g of extract was boiled with 5ml distilled water for 5 minutes and the mixture was filtered while still hot. 1 ml of the filtrate was collected into 25 ml vials and 3 drop of olive oil added. The mixture was shaken for two minutes; the formation of an emulsion is an indication of presence of saponins.

#### **Terpenoids**

0.01g of the sample extract was added 2ml of chloroform and 2 drops of concentrated sulphuric acid. The formation of reddish-brown colour indicates the presence of terpenoids.

#### **Steroids**

3 ml of chloroform and 3 drop of concentrated sulphuric acid was carefully added to 0.4g of the extract. Reddish-brown colours indicate the presence of steroid.

#### **Phenols (*Ferric chloride test*)**

2 ml distilled water and 1 ml 10% Ferric chloride was added to 1 ml of the extract. The formation of blue or green colour indicates the presence of phenols.

#### **Flavonoids**

0.2g of extract was heated with 10 ml of ethyl acetate in water bath for 3 minutes. The mixture was allowed to cool and filtered. 4 ml of the filtrate was collected and 1 ml of 1% NH<sub>3</sub> was added, the mixture was shaken and allowed to stand for the lower layer to separate out. Yellow colouration at the ammonia layer indicates the presence of flavonoids.

#### **Tannins**

0.2g of the extract was boiled with 5 ml of 45% ethanol for 5 minutes. The mixture was cooled and filtered. 1 ml of the filtrate was diluted with deionised water and 1 ml of ferric chloride added. A transient greenish to black colour indicates the presence of tannins.

#### **Anthraquinones (*Ammonia test*)**

0.4g of the seed extract was boiled in 20 ml of 10% ethanol for 5 minutes and filtered. 5.0 ml of the filtrate was collected and equal volume of chloroform added and shaken vigorously for 1 minute. Organic layer was collected and 5 ml of distilled water added, this was then extracted with 5.0 ml of ammonia solution. Red colour in the ammonia upper phase indicates the presence of anthraquinones.

#### **Resins**

10 ml of 95% ethanol was added to 0.2g of the extract, the mixture shaken thoroughly and allowed to stand for 5 minutes and filtered. The filtrate was poured into 10 ml of distilled water in 50 ml beaker. The occurrence of a precipitate shows the presence of resins.

#### **Statistical analysis**

All the experiments were performed in triplicate and the results were expressed as mean $\pm$ SD

(Standard Error). Statistical analysis was performed using MS-Excel 2007.

### **RESULT AND DISCUSSION**

Methanol, water, hexane and chloroform extracts were prepared and screened for secondary metabolites. The yield obtained from 20 g of dry plant material was measured for each extract (Table 2).

Table 2: The yield of solid residue after extractions and evaporation from 20 g dried sample<sup>a</sup>

Extracts	Yield (g)
Chloroform	3.03 $\pm$ 0.032
Hexane	2.41 $\pm$ 0.043
Methanol	2.11 $\pm$ 0.092
Aqueous	1.71 $\pm$ 0.01

Value  $\pm$  STD (Standard Deviation) of three determination

The total phenolic and flavonoid content in the aqueous seed extracts of this sample is as shown in Fig. 1. The studies conducted on the aqueous root extract of this plant revealed a high phenolic content (169.152 mg/g) (Germano *et al.*, 2006) which is higher than 42.86 mg/g reported in this studies. Previous studies on the root and stem bark have also revealed the presence of tannin (Burkill, 1997). The concentration of flavonoids in the aqueous extract was 59.10 mg/g which is comparable to 62.7 mg/g reported for lotus leaves (Lin *et al.*, 2009).

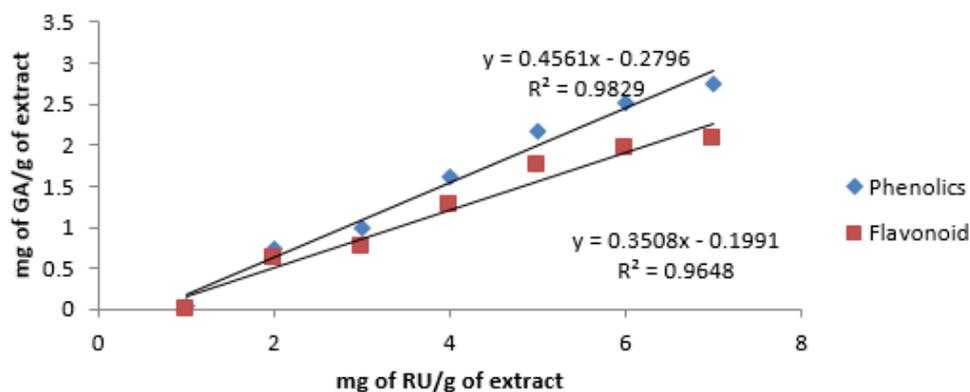


Figure 1: Calibration curve for total phenolic and flavonoid content in the aqueous seed extracts.

The obtained extracts (chloroform, hexane, methanol and aqueous) were screened for their phytochemical constituents (Table 2). The study revealed the presence of alkaloids, glycosides, terpenoids, steroids in all the extracts while phenols, flavonoids, tannins, anthraquinones, saponin were in methanol and Aqueous extracts. Resins is present in chloroform and hexane extracts. Studies has shown that alkaloids, terpenoids and phenolic compounds are common secondary metabolites found in most medicinal plant (Charistima and Thiruvengadarajan, 2013). The result shows that aqueous and ethanol extracts contains more phytoconstituents than the chloroform and hexane extracts, implying that most of the phytoconstituents were polar and are readily extracted with polar solvents (Alorkpa et al., 2016; Charistima and Thiruvengadarajan, 2013).

An alkaloid, quinine and artemisinin isolated from *Cinchona ledgeriana* and *Artemisia annua* are known to have anti-malarial activity (Arts and Hollman, 2005), while Phenolic phytochemicals such as flavonoids and tannins have been demonstrated to possess antioxidative, anticarcinogenic, antidiabetic, antimicrobial, anti-allergic, antimutagenic and anti-inflammatory agents (Sermakkani and Thangapandian, 2010). The anti-allergic function of flavonoids is particularly advantageous since it may help in the treatment of immune system disorders that are responsible for 5–10% of recurrent

miscarriages (Akamene, 2008; Mohapatra, 2011). Since flavonoids prevent platelet stickiness (platelet aggregation), they are

probably wonderful remedies for the treatment of all types of miscarriages (Njoku et al., 2011; Ifeoma, 2008).

Table 2: Phytochemical screening of *Trichillia emetica* seed extracts.

Phytochemical constituents	Chloroform	Hexane	Methanol	Water
<b>Alkaloids</b>				
Dragendorff's reagent	+	+	+	+
Wagner's reagent	+	+	+	+
<b>Glycosides</b>				
Sodium hydroxide test	+	+	+	+
<b>Saponin</b>				
Emulsion test	-	-	+	+
<b>Terpenoids</b>				
Conc. H <sub>2</sub> SO <sub>4</sub> test	+	+	+	+
<b>Steroids</b>				
Conc. H <sub>2</sub> SO <sub>4</sub> test	+	+	+	+
<b>Phenols</b>				
FeCl <sub>3</sub> test	-	-	+	+
<b>Flavonoids</b>				
Ammonium test	-	-	+	+
<b>Tannins</b>				
FeCl <sub>3</sub> test	-	-	-	-
<b>Anthraquinones</b>				
Ammonium test	-	-	+	+
<b>Resins</b>				
Precipitate test	+	+	-	-

Key: (-) = Absent, (+) = Present

Saponins are promoted commercially as dietary supplements and nutraceuticals; they are also use as adjuvants in the production of vaccines while toxicity related to sterol complexation by saponins remains a major concern. Steroids are related to sex hormones and they also interact with androgen receptors to increase muscle and bone synthesis (Trease and Evans, 1996).

### Conclusion

This study has shown the phytochemicals, phenolic and flavonoid contents of *T. emetica* whole seeds extract. Phytochemicals such as alkaloids, glycosides, terpenoids, steroids were present in all the crude extracts while resins was only present in hexane and chloroform

extracts. The total phenolic and flavonoids constituent of the aqueous seeds extract was also reported to be 42.86 and 59.10 mg/g.

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