Preliminary Phytochemical and Physicochemical Analysis of Selected Mistletoes from Ethiopia

Abraham Yirgu (abrahamyirguw@gmail.com)
Ethiopian Environment and Research Institute  https://orcid.org/0000-0001-7621-8733

Yalemtehay Mekonnen
Addis Ababa University Faculty of Science: Addis Ababa University College of Natural Sciences

Research note

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Abstract

Objectives: Mistletoes have been used for the treatment of human and animal health ailments. This study investigated the physicochemical and phytochemical constituents of *Englerina woodfordioides*, *Phragmanthera regularis*, *Tapinanthus globiferus*, and *Viscum tuberculatum* from central Ethiopia.

Results: The four plant species collected from 11 host trees grown in six distinct locations revealed the presence of alkaloids, phenols, saponins, cardiac glycosides, steroids, terpenes, tannins, quinines, and coumarins. In contrast, no flavonoids, anthraquinones and phlobatannins were detected. The total ash value of *E. woodfordioides* and *P. regularis* ranges from 6.04 to 13.23% and 5.62 to 15.22%, respectively. Comparable total ash content was found in *T. globiferus* and *V. tuberculatum*. However, no significant difference was obtained in the mean percent moisture content of the study species.

Introduction

Mistletoes are hemi-parasitic parasitic flowering plants found attached to the branches of other plants [1]. They are known as complementary and alternative medicine in the treatment and management of various diseases around the world [2–5]. The biological activities of mistletoe rely on the presence of secondary metabolites [6]. The phytochemical composition of African mistletoes is largely dependent on a few species of *Tapinanthus* and *Phragmanthera* species grown in West African countries. The phytochemical composition of these mistletoes varied depending on the time of harvest, host plants and the manufacturing process [7–8].

Ethiopia has a long history and deep-rooted culture in using mistletoe to treat various health problems. Accordingly, *Englerina woodfordioides* use to treat diarrhea and eye disease [9], syphilis [10], otorrhoea, and scabies [11]. Similarly, *T. globiferus* were reported in the treatment of spider poisoning, urinary problem, cough, blotting [12], and dyspepsia and impotence [13]. Despite these importances, to our knowledge, the study on the phytochemical composition of species of mistletoes achieved less attention in the past. Therefore, the aim of this study was to investigate the phytochemical and physicochemical properties of *Phragmanthera regularis*, *Englerina woodfordioides*, *Tapinanthus globiferus* and *Viscum tuberculatum* in Ethiopia.

Materials And Methods

Plant collection and authentication

Mistletoes were collected from branches of trees grown in the roadside, homesteads, and natural forests in six localities in Ethiopia in April 2019. These specimens were identified by Melaku Wondafrash, plant taxonomist, at the National Herbarium (ETH) of the College of Natural Sciences, Addis Ababa University. Specimens were deposited at ETH.

Preparation of plant crude extracts

Fresh and healthy leaves of mistletoes were washed several times with running tap water to remove soil and debris. Air-dried leaves were fine powdered using an electrical grinder mill.

Plant crude extraction

Twenty grams leaf powder were separately macerated with 200 ml of chloroform (99.8% AR), ethyl acetate (99.8% AR), methanol (99% AR), and distilled water. Extracts were kept in a glass jar for seven days with occasional shaking at room temperature. These extracts were filtered using Whatman no. 1 filter paper. The marc was re-macerated with the same solvent until the extraction was exhausted. The first three solvents extracts were concentrated to dryness using a Rotary evaporator. The resulting aqueous filtrate was lyophilized.

Determination of solvent extraction yield

The crude extracting values of mistletoes were tested using the above-mentioned solvents. The percentage yield of extraction was determined as follows [14].

\[
\text{Extractive value} = \frac{\text{Mass obtained after extraction}}{\text{total mass taken for extraction}} \
\]

Determination of phytochemical constituents of plant extracts

Test for alkaloids

Wagner’s test

Crude extract powder was dissolved in de-ionized distilled water and filtered using Whatman no1 filter paper. The filtrate was acidified with hydrochloric acid (HCl) [15]. To this solution, Wagner’s reagent was dissolved in water and gently added to the test tube [16–17]. The formation of reddish-brown precipitate indicates the presence of alkaloids [15].

Test for anthraquinones
**Borntrager's test**

Crude extract was dissolved in chloroform, shaken for 5 min [18], and ammonia solution was added. The solution was filtered using Whatman no 1 filter paper [19]. The control test was done by adding ammonia solution in chloroform [20]. The formation of bright pink coloration in the upper aqueous layer indicates the presence of anthraquinones [20].

**Test for cardiac glycosides**

**Keller-Killani test**

Crude extract was diluted in distilled water [21]. Two milliliters of plant extract [22] were mixed with glacial acetic acid followed by drop wise addition of ferric chloride (FeCl$_3$) solution. Then, the mixture was poured into another test tube containing concentrated sulfuric acid (H$_2$SO$_4$). A brown ring formed at the interface indicates the presence of cardiac glycosides [21, 23].

**Test for coumarins**

**Sodium hydroxide (NaOH) test**

Crude extract was dissolved in distilled water. Sodium hydroxide solution was added to the aqueous plant extract. The appearance of yellow color indicates the presence of coumarins [24–25].

**Test for flavonoids**

**NaOH or Alkaline Reagent test**

Crude extract was dissolved in water and filtered using Whatman no 1 filter paper. The plant extract was treated with aqueous NaOH solution [22, 25]. The formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids [23, 26–28].

**Lead acetate test**

Crude extract was dissolved in water and filtered using Whatman no 1 filter paper. To the extract, few drops of basic lead acetate solution were added. The formation of reddish-brown precipitate indicates the presence of flavonoids [17].

**FeCl$_3$ test**

Crude extract was dissolved in water and filtered using Whatman no 1 filter paper. Few drops of neutral FeCl$_3$ solution were added to the extract. The deposition of blackish-red precipitate indicates the presence of flavonoids [17].

**Test for phenols**

**FeCl$_3$ test**

Crude extract was mixed with distilled water [28]. The extract was treated with an aqueous FeCl$_3$ solution [29]. The formation of bluish-black color indicates the presence of phenols.

**Test for phlobotannins**

**Precipitate test**

Two milliliters of aqueous extract of the plant sample were boiled with HCl acid. The deposition of a red precipitate indicates the presence of phlobotannins [24–25].

**Test for quinones**

Two milliliters of plant extract were treated with HCl acid. The formation of a yellow-colored precipitate indicates the presence of quinones [30].
Test for saponins

Foam test

Plant extract was shaken vigorously with water. The appearance of foam indicates the presence of saponins [31–32].

Test for steroid

Liebermann-Burchard test

A few drops of acetic anhydride solution were added to the extract. To this solution, a few drops of concentrated $\text{H}_2\text{SO}_4$ were added carefully along the side of the test tube. Formation of reddish-brown ring at the junction of the two layers indicates the presence of steroids [17].

Test for tannins

FeCl$_3$ test: Plant extract was dissolved in distilled water, and then filtrated. Two milliliters FeCl$_3$ was added to the filtrate. The appearance of blue-black, greenish-black or dark blue color indicates the existence of tannins in the test samples [19].

Test for terpenoids

Salkowski’s test

Plant extract was added to 2 ml of chloroform. Three milliliters of concentrated $\text{H}_2\text{SO}_4$ were carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids [21].

Test for reducing sugar

Benedict test

Plant extract was dissolved in distilled water and filtered separately. One milliliter of filtrate and 4 ml of Benedict’s reagent were mixed, and heated gently in a boiling water bath for 5 min. The appearance of green, red, or yellow coloration indicates the presence of reducing sugar in the plant extract [33].

Test for protein

Biuret test

Plant extract put in a test tube was treated with an equal volume of NaOH. Then, a few drops of CuSO$_4$ were added. Pink or purple color indicates the presence of proteins [34].

Determination of physicochemical analysis of plant materials

Total ash values

The total ash content of the plants was determined as stated in Idris et al [35] with some modifications. A dry heat-resistant porcelain crucible was weighed (W$_1$). Thereafter, 2 g of leaf powder was weighed along with crucible (W$_2$), and ignited gradually in an electrical muffle furnace, increasing the heat to 600°C until the plant materials were white that indicate the absence of carbon. The crucible was removed, and allowed to cool in a desiccator and then re-weighed (W$_3$).

$$\text{Total ash (\\%)} = \frac{(W_3 - W_2)}{(W_2 - W_1)} \times 100$$

Acid-insoluble and water-soluble ash

The acid-insoluble and water-soluble ashes were separately prepared by adding 25 ml of HCl into total ash content obtained in 2.6.1. The solutions were gently boiled for 5 min covered with a watch glass. The insoluble matters were filtered using Whatman no 40 Ashless filter paper. The filtrate was washed with...
hot water and then ignited for 15 minutes at 450 °C. The filter paper containing the insoluble matter were transferred to the original crucible, dried on a hot plate and ignited to 450°C to constant weight (W₄). The residue was cooled in desiccator and weighed.

\[
\text{Acid insoluble ash (\%)(W₄=W₃-W₂) x 100}
\]

\[
\text{Water-soluble ash (\%) = \frac{W₃-W₄}{W₃-W₂} x 100}
\]

**Determination of moisture content**

The moisture content (loss on drying) of the fresh leaves was determined as stated in Danso-Boateng et al [36]. The air-dried samples (3 g) were weighed and kept at 105 ± 3°C for 24 h. Samples were then removed from the oven, cooled in a desiccator, and weighed.

\[
\text{Moisture content (\%) = \frac{\text{Wet leaf with crucible} - \text{dry leaf with crucible}}{\text{Wet leaf with crucible}} x 100}
\]

**Results**

**Plant collection and authentication**

In this investigation, four mistletoes were collected from 11 host plants [Table 1].

| Plants | Aerial parasitic plant | Host plant (Family name) | Location of collection | Voucher no |
|--------|------------------------|--------------------------|------------------------|------------|
| EWSM   | Englerinawoodfordioides (Schweinf) M. Gilbert | Shinus molle (Anacardiaceae) | Shashemene | AY2 |
| EWEG   | Englerinawoodfordioides (Schweinf) M. Gilbert | Eucalyptus globulus (Myrtaceae) | Menagesha | AY4 |
| EWDP   | Englerinawoodfordioides (Schweinf) M. Gilbert | Discopodium pumilinervum (Solanaceae) | Gambo | AY19 |
| EWAA   | Englerinawoodfordioides (Schweinf) M. Gilbert | Acacia abyssinica (Fabaceae) | Gambo | AY34 |
| PROEM  | Phragmanthera regularis (Sprague) M. Gilbert | Olea europaea ssp. cuspidate (Oleaceae) | Menagesha | AY7 |
| PROEC  | Phragmanthera regularis (Sprague) M. Gilbert | Olea europaea ssp. cuspidate (Oleaceae) | Chilimo | AY9 |
| PRFS   | Phragmanthera regularis (Sprague) M. Gilbert | Ficus sur (Moraceae) | Menagesha | AY11 |
| PRAM   | Phragmanthera regularis (Sprague) M. Gilbert | Acacia melanoxylon (Fabaceae) | Menagesha | AY16 |
| PRRA   | Phragmanthera regularis (Sprague) M. Gilbert | Rosa abyssinica (Rosaceae) | Menagesha | AY18 |
| PRSM   | Phragmanthera regularis (Sprague) M. Gilbert | Shinus molle (Anacardiaceae) | Debre Libanose | AY20 |
| PRFV   | Phragmanthera regularis (Sprague) M. Gilbert | Ficus vasta (Moraceae) | Chilimo | AY25 |
| VTTN   | Viscum tuberculatum A. Rich | Tecteolobis (Rutaceae) | Gambo | AY24 |
| TGES   | Tapinanthus globiferus (A. Rich) Tieghem | Eucalyptus saligna (Myrtaceae) | Mekkassa | AY15 |

**Solvent extraction yield**

Methanol extraction provided the highest percent yield for *P. regularis* and aqueous solvents for *E. woodfordioides*, *T. globiferus*, and *V. tuberculatum*. In contrast, the lowest yield of extraction was obtained by ethyl acetate for *E. woodfordioides* and *P. regularis*, and chloroform for *T. globiferus*, and *V. tuberculatum*. There was also variation in solvent extraction yields between *E. woodfordioides* and *P. regularis* collected from *S. molle* and *O. europaeae*.

**Phytochemical constituents of plant extracts**

The phytochemical test showed that there were alkaloids, phenols, saponins, cardiac glycosides, steroids, terpenoids, tannins, quinines, and coumarins in one or the other four mistletoes. No flavonoids, anthraquinone and phlobatannin were detected. *E. woodfordioides* and *P. regularis* have similar results in the contents of flavonoids, cardiac glycosides, steroids, anthraquinones and phlobatannins. Tannins were found in all *E. woodfordioides*. Similar to this finding there was saponins to all *P. regularis* collected from different host plants. In contrast, the phytochemical composition of *V. tuberculatum* was much similar to *E. woodfordioides* collected from *E. globules* than the others [Table 2]. Meanwhile, a trace amount of reducing sugar was found in *E. woodfordioides*, *T. globiferus* and *V. tuberculatum*. In contrary, no protein was detected in all the samples.
### Table 2
Phytochemical screening of aqueous extracts of aerial mistletoes

| Active principles | Test method       | Plant extracts |
|-------------------|-------------------|----------------|
|                   | EWSM  | EWEG  | EWDP  | EWAA  | PROEM | PROEC | PRFS  | PRAM  | PRRA  | PRSM  | PRFV  | VTTN | TGES  |
| Alkaloids         | Wagner | +     | -     | -     | +     | +     | -     | -     | -     | +     | -     | -     |       |
| Anthraquinones    | Borntrager | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |       |
| Cardiac glycosides| Keller Killani | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | +     |
| Coumarins         | NaOH   | -     | -     | -     | +     | +     | -     | -     | -     | -     | -     | -     |       |
| Flavonoids        | NaOH   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |       |
|                   | Lead acetate | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |       |
|                   | FeCl$_3$ | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |       |
| Phenols           | FeCl$_3$ | -     | +     | -     | +     | -     | -     | -     | +     | -     | -     | -     | +     |
| Phlobotannins     | Precipitate test | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |       |
| Quinones          | -      | +     | -     | -     | +     | +     | -     | -     | +     | -     | -     | -     | +     |
| Saponins          | Foam test | +     | -     | +     | -     | +     | +     | +     | +     | +     | +     | -     | +     |
| Steroids          | Liebermann-Burchard | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Tannins           | FeCl$_3$ | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Terpenoids        | Salkowski | +     | +     | +     | -     | -     | -     | +     | -     | +     | +     | +     | +     |

*(-) indicate the presence and (+) absence of the tested biochemical.

### Physicochemical analysis of plant materials

There was significant difference in the percent of total ash values between and within species of mistletoes. The total ash value of *E. woodfordiodes* and *P. regularis* ranges from 6.04 to 13.23% and 5.62 to 15.22%, respectively. Comparable total ash content was found in *T. globiferus* and *V. tuberculatum* [Table 3]. Similarly, the results on the acid-insoluble ash and water-soluble ash revealed variation between and within species of mistletoes based on the host plants. No significant difference was obtained in the mean percent moisture content of the study species.
### Table 3
Pair wise comparison of total ash content of mistletoes (mean ± SEM, n = 3, p < 0.05)

| Plants  | Mean ± SEM | Plants  | Mean ± SEM |
|---------|------------|---------|------------|
|          | EWSM       | EWEG    | PROEM      | PROEC      | PRFS      | TGES      | PRAM      | PRRA      | EWDP      | PRSM      | VTTN      | EWAA      |
| EWSM    | 13.226 ± 0.157 | 7.1843  | 5.036      | 3.933      | 0.548      | 7.170     | 7.604     | 4.450     | 3.586     | 5.792     | 5.820     |
| EWEG    | 6.041 ± 0.157  |        |            |            |            |          |           |           |           |           |           |
| PROEM   | 8.190 ± 0.157  | 2.149   | 2.135      | 2.568      | 0.757      | 0.784     | 1.859     | 1.886     |           |           |           |
| PROEC   | 9.292 ± 0.157  | 3.251   | 1.102      | 3.237      | 3.670      | 0.517     |           |           |           |           |           |
| PRFS    | 12.677 ± 0.157 | 6.636   | 4.487      | 3.385      | 6.622      | 7.055     | 3.902     | 3.038     | 5.244     | 5.271     |           |
| TGES    | 6.055 ± 0.157  |        |            |            |            |          |           |           |           |           |           |
| PRAM    | 5.622 ± 0.157  |        |            |            |            |          |           |           |           |           |           |
| PRRA    | 8.776 ± 0.157  | 2.734   | 0.586      | 2.720      | 3.154      | 1.342     | 1.370     |           |           |           |           |
| EWDP    | 13.950 ± 0.157 | 0.724   | 7.908      | 5.760      | 4.657      | 1.272     | 7.894     | 8.328     | 5.174     | 4.310     | 6.516     | 6.544     |
| PRSM    | 9.639 ± 0.157  | 3.598   | 1.449      | 3.584a     | 4.017      | 0.864     | 2.206     | 2.233     |           |           |           |           |
| VTTN    | 7.433 ± 0.157  | 1.392   |            | 1.378      | 1.811      |           |           |           |           |           |           |
| PRFV    | 15.224 ± 0.157 | 1.999   | 9.183      | 7.034      | 5.932      | 2.547     | 9.169     | 6.449     | 1.275     | 5.585     | 7.791     | 7.818     |
| EWAA    | 7.406 ± 0.157  | 1.365   |            | 1.351      | 1.784      |           |           |           |           |           |           |

*the Standard error of the mean of total ash content is 0.222

### Discussion

There is an uneven distribution of mistletoes in Africa. Species such as *E. woodfordiodes*, *P. regularis*, and *V. tuberculatum* are limited to East Africa, and *T. globiferus* widely distributed from East to West Africa [37].

The results on the phytochemical content of *T. globiferus* agreed with that reported for the same species by Abubakar et al [14], Bassey [39] and Umarudeen and Chika [40] on anthraquinones, and tannins. In contrast, it partly agrees with results in other phytochemicals to Abubakar et al [14], Bassey [39] and Umarudeen and Chika [40]. On the other hand, the results obtained on alkaloids, quinone and terpenoids in *E. woodfordiodes* agreed to Ngbolua et al [41]. Similarly, the results obtained in *P. regularis* partly agrees with *P. incana* in tannins and anthraquinone [42], tannins, saponin and anthraquinone to *P. capitata* [43], alkaloid and tannins to *P australarobica* [38]. In general, it is necessary to consider the detection of preliminary phytochemical composition of mistletoe with respect to host plants, extraction solvents [8] and the test methods.

The determination of higher moisture content in this study indicated the more probability of microbial attack and enzymatic hydrolysis of the plant materials [44, 45–47]. The higher extraction yield of mistletoes using water and methanol in this study might be associated to the higher polarity of the extraction materials, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration [48–49].

### Conclusions

This study revealed the presence of secondary metabolites that have potential bioactive activities. Therefore, future studies need to investigate the potential use of these plants for their bioactive compounds using *in vitro* and *in vivo* models.

### Limitations

This preliminary phytochemical and physicochemical study on mistletoes did not account quantification of bioactive compounds and limited to certain part of the country.

### List Of Abbreviations

- EWSM- *Englerina woodfordioides* (Schweinf) M.Gilbert collected from *Shinus molle*
- EWEG- *Englerina woodfordioides* (Schweinf) M.Gilbert collected from *Eucalyptus globulus*
- EWDP- *Englerina woodfordioides* (Schweinf) M.Gilbert collected from *Discopodium penninervum*
- EWAA- *Englerina woodfordioides* (Schweinf) M.Gilbert collected from *Acacia abyssinica*
- PROEM- *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Olea europea* ssp. *cuspidate* at Menagesha
PROEC - *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Olea europea* ssp. *cuspidata* at Chilimo

PRFS - *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Ficus sur*

PRAM - *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Acacia melanoxylon*

PRRA - *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Rosa abyssinica*

PRSM - *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Shinus molle*

PRFV - *Phragmanthera regularis* (Sprague) M.Gilbert collected from *Ficus vasta*

VTTN - *Viscum tuberculatum* A. Rich collected from *Teclea nobilis*

TGES - *Tapinanthus globiferus* (A.Rich) Tieghem collected from *Eucalyptus saligna*

**Declarations**

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**Authors’ contributions**

AY investigated, analyzed the data, and drafted the manuscript. YM supervised this study and edited the manuscript. Both authors read and approved the final manuscript.

**Authors’ details**

1. College of Natural and Computational Sciences, Addis Ababa University. P.O.Box 1176, Addis Ababa, Ethiopia.
   2. Central Ethiopia Environment and Forest Research Center, Addis Ababa. P.O.Box 33042, Addis Ababa, Ethiopia

**Competing interests**

The authors declared no competing interests.

**Availability of data and materials**

Raw data from this study will be available for researcher upon request from the first author.

**Consent for publication**

Not applicable

**Ethics approval and consent to participate**

Not applicable

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