

Pharmacognostic Studies and Artemisinin Content of *Artemisia Annu* L. Grown in Togo

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ABSTRACT

Objective: *Artemisia annua* grown in Togo is used as an antimalaria drug. The present study shows a detailed analysis of pharmacognostic evaluation of leaf powder and root that will be used for the purpose of identification, authentication, and consequent standardization. **Materials and Methods:** Both the leaf and root were evaluated for their macroscopic and microscopic features. The physicochemical parameters of the leaf powder and its phytochemical screening were done based on its total phenols and flavonoid content. Artemisinin content was also performed using weigh method after extraction. **Results:** Physicochemical evaluation yielded water, alcohol, acetone, methanol, chloroform, and petroleum ether soluble extractive values which are 2.25%, 1.25%, 4.22%, 8.12% and 3.77% (w/w), respectively. Fluorescence analysis imparted characteristic colors to the leaf powder when observed under visible, UV light 254 and 365 nm. Phytochemical screening of leaf powder showed the presence of alkaloids, flavonoid, and anthracene derivatives. Total phenols and flavonoid content were 32.5 ± 0.67 mEq Gallic Acid/100 mg and 11.3 ± 1.52 mgEq Quercetin/100 mg, respectively. Artemisinin content value was 0.009% (w/w). **Conclusion:** Various pharmacognostic parameters which were evaluated assisted in identification and standardization of *A. annua* leaf in powder and crude form.

Key words: *Artemisia*, Pharmacognostic, Artemisinin, Total phenols, Flavonoid.

INTRODUCTION

According to World Health Organization (WHO) chapter of standardization and quality control of herbal drugs, standardization involves the physicochemical evaluation of crude drug covering aspects such as selection and handling of crude material. Most attention is usually given to quality indices including macro and microscopic examination, ash values, moisture content, extractive values, crude fiber, qualitative and quantitative chemical evaluation, and chromatographic examination.^{1,2}

Wild plants form a major source of raw drugs for local communities and herbal drug industry. The raw materials are often adulterated when purchased from the market.³

Across the globe, herbal industries and local communities generally face the problems of adulteration and substitution at the raw material stage.⁴ In this study, the herbal drug *Artemisia annua* was selected as a case study for methods of authenticating it based on taxonomic and pharmacognostic analysis.

Artemisia L. is a genus of small herbs and shrubs, belonging to the family of Compositae (Asteraceae), found in northern temperate regions commonly known as wormwood. *A. annua* is a herbaceous, scented plant with alternate leaves, pinnate, and native to China. It has been widely used in traditional medicine for centuries and

is recommended in the treatment of malaria and other parasitic diseases.⁵ Nowadays, artemisinin is extracted from this plant, which is considered to be the most potent molecule against Plasmodium spp. Due to its proven effectiveness, it has gained commercial value, especially in herbal medicine and in the pharmaceutical industry. *Artemisia annua* plant is native to temperate countries (China) and its adaptation to our tropical countries such as Togo can alter its quality or its composition in active principle.

In view of its medicinal importance and the fact that no pharmacognostical work is available on the cultured species in Togo, the present investigations were undertaken. This will help in evaluating or assuring the quality of raw this drug.

Artemisia annua

Artemisia annua is an annual herbaceous plant of the Asteraceae family (Compositae), 30 to 150 centimetres high. It is sometimes up to 250 centimetres in optimal growing conditions, and it is hairless and very fragrant.

Taxonomy

Reign: Plantae

Branch: Angiosperms

Class: Dicotyledones

Subclass: Campanulids

Order: Asterales

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Family: Asteraceae

Species: *Annuua* L.

MATERIALS AND METHODS

Materials

Reagents and chemicals of analytical grade were purchased from VWR. A microscope fitted with camera DBL124 (Italy) was used for all the microscopy. Fluorescence analysis was performed with UVGL58 UV lamp (Cambridge, UK).

Plant collection and authentication

Samples of *A. annua* leaves were collected during October 2018 from Achanvé, a Tsévié village in Togo. The samples were authenticated at Department of Botany, Sciences Faculty, University of Lomé, Togo where voucher specimens were deposited.

Macroscopic and microscopic evaluation

Macroscopic characteristics such as size, color, and other visible properties were noted. Transverse sections and ground powders were observed under a microscope to determine the anatomical and histological characteristics.⁶⁻⁸

Physicochemical studies

Determination of loss on drying; water, alcohol, acetone, methanol, chloroform and petroleum ether soluble extractive values and water content⁹ were evaluated to establish the pharmacognostic specification of *A. annua* leaves.⁶ The details of the methodology followed WHO guidance.¹⁰ Briefly, the loss on drying test was performed by drying the sample at 105°C until it achieves a constant weight. The total ashes left after burning at 300°C for 10 hours were determined for inorganic components. In addition, the moisture content was quantitated using the azeotropic distillation method. Impurities were determined using standard method.¹¹ All sampling was carried out in triplicate.

UV Fluorescence analysis

About 0.5 gms of plant powder was put into clean and dried test tubes. On each tube, 5 ml of different organic solvents like distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, sulphuric acid, hydrochloric acid 5% , and NaOH were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible day light and UV light of short wavelength (254 nm) and UV light of long wavelength (365 nm) for their characteristic color.¹²

Preliminary phytochemical screening

Preliminary phytochemical studies were carried out for the leaf powder as per standard procedures for the presence of various phyto constituents like alkaloids, flavonoid and anthracene derivatives.¹³

Total phenols content

Total phenolic content was analyzed using the Folin-Ciocalteu colorimetric method. An aliquot of 0.3 mL of leaf powder were mixed with Folin-Ciocalteu phenol reagent (2.25 mL). After 5 min, 6% sodium carbonate (2.25 mL) was added and the mixture was allowed to stand at room temperature for 90 min. The absorbance of the mixture was measured at 725 nm. Standard calibration curve for gallic acid in the range of 0-200 g/ml was prepared in the same manner and results were expressed as mg gallic acid equivalent (GA) per gram of extract.¹⁴

Total flavonoids content (TF)

Total flavonoids content was analyzed using the aluminum chloride colorimetric method with some modifications. In this method,

quercetin was used to make a standard calibration curve in the range of 0-100 g/ml. In different test tubes, sample (1 mL) and standard solutions (1 mL) were placed into it. After then, 2% aluminum chloride (1 mL) and 5% sodium acetate (3 mL) were added and mixed well. The mixture was then centrifuged at 3000 rpm for 20 min to get a clear solution. The absorbance of the standard solution and sample were taken at 440 nm. Results were expressed as mg quercetin equivalent (QE) per gram of extract.¹⁵

Extraction of artemisinin

A. annua powder (400 g) was macerated for one hour in dichloromethane. The solution was then filtered to separate the filtrate from the residue of the powder. To the residue was added again dichloromethane. It was left to macerate for one hour and filtered. This operation was repeated a third time. The filtrates obtained were combined and evaporated to reduce the amount of filtrate to a small volume that is easy to handle. The viscous solution obtained constitutes the crude filtrate. The crude filtrate was treated with a mixture of water and acetone (8:10 v/v). The solution was filtered using a filter that can retain all the heavy magma. The filtrate which contains artemisinin is treated with an excess of water and a white precipitate appears. This solution was maintained at a low temperature, which aims to accelerate the formation of the precipitate. The solution is filtered and the residue is dried. This dried residue constitutes crude artemisinin.¹⁶ Artemisinin was highlighted in UV examination after spraying an ethanol solution of sulphuric acid and heating it at 105°C.¹⁷

RESULTS

Macroscopic appearance

A. annua powder has brown green color, aromatic pleasant odor with bitter taste, and a smooth texture. *A. annua* is an annual herbaceous plant, very fragrant, and a tall erect stalk which is more than 2 m tall in optimal growing conditions. *A. annua* has alternates and pennants leaves. Its stem is cylindrical, erect, and glabrous with achenes and ovoid fruits (Figure 1).

Microscopy

The stem of *A. annua* has a circular shape. The epidermis is monolayer, the medulla occupies about 2/3 of the radius of the stem, the medulla is central and relatively broad, and the vascular tissue is formed of vascular bundles arranged in a circle surrounding the medulla (Figure 2).

The root of *A. annua* has a circular shape. The epidermis is monolayer with many epidermal hairs. The pericycle delimits the end of the vascular bundle, while the vascular bundle is inside the pericycle. Within the center, the medulla occupies 1/3 of the radius of the root (Figure 3).

Physicochemical parameters

The values of physicochemical parameters are displayed in Table 1. Water content, impurity content, and total ash were 11.6 ± 0.18 , 0.05 ± 0.003 , 13.16 ± 0.14 , 6.82 ± 0.09 % of dried weight, respectively. Water, alcohol, acetone, methanol, chloroform, and petroleum ether soluble extractive values were 2.25%, 1.25%, 4.22%, 8.12% and 3.77 % (w/w), respectively.

Fluorescence analysis

Fluorescence analysis is also an important tool for the determination of constituents in herbal drugs, and it provides an idea about their chemical nature. The powder drug analysis was performed when treated with various chemical reagents and observations were made in visible light and UV light of short and long wavelengths (Table 2).



Figure 1: *Artemisia annua* L. whole plant.

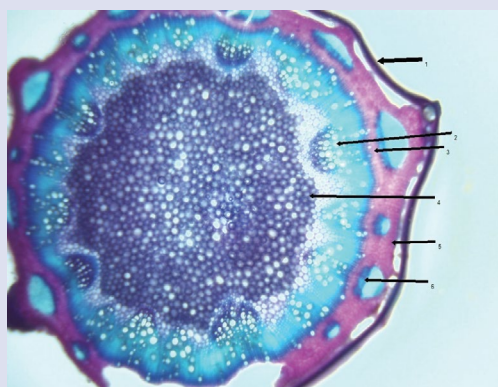


Figure 2: Microscopic characteristics of the transverse section of the *A. annua* stem.

1 = epidermis, 2 = xylem, 3 = phloem, 4 = medulla, 5 = parenchyma, 6 = sclerenchyma.

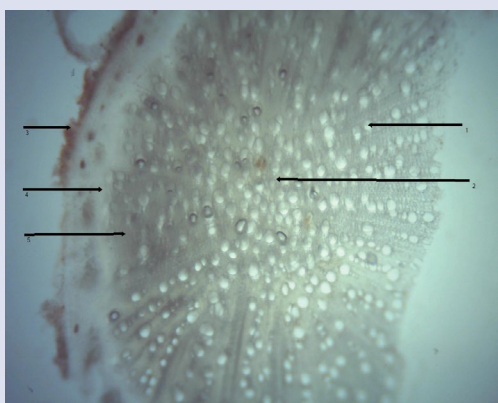


Figure 3: Microscopic characteristics of the transverse section of the root of *A. annua*.

1 = xylem, 2 = medulla, 3 = horn, 4 = pericycle, 5 = phloem.

Table 1: Physicochemical parameters for the leaf powder.

Parameters	Result (%)
Alcohol soluble extractive (%w/w)	2.25 ± 0.05
Acetone soluble extractive (%w/w)	1.125 ± 0.075
Methanol soluble extractive (%w/w)	4.225 ± 0.275
Chloroform soluble extractive (%w/w)	8.125 ± 0.625
Petroleum ether soluble extractive (%w/w)	3.775 ± 0.925
Water soluble extractive (%w/w)	20.85 ± 0.7
Water content (%w/w)	11.6 ± 0.5
Impurity rate (%w/w)	0.05
Crude artemisinin content (%w/w)	0.009
Total ash (%w/w)	11.9 ± 0.67

Table 2: Fluorescence behavior of *A. annua* powder with different chemical reagents.

Leaf powdered Drug	Visible/day light	Ultraviolet (254 nm)	Ultraviolet (365 nm)
1 Powder as such	Pale green	No fluorescence	No fluorescence
2 Powder+Water	Pale green	Brown	Blue
3 Powder+Petroleum ether	Pale green	Brown	Reddish
4 Powder+Conc.H ₂ SO ₄	Black	Brown	Light blue
5 Powder+50% H ₂ SO ₄	Pale green	No fluorescence	Blue
6 Powder+Chloroforme	Pale green	Yellow	Pink
7 Powder+Methanol	Green	Brown	Violet
8 Powder+Acetone	Pale green	No fluorescence	reddish
9 Powder+KOH (0.1N)	yellow	No fluorescence	Green fluorescence
10 Powder+HCl (0.1 N)	Black	Brown	Bleu
11 Powder+Ethanol	Light green	Brown	Violet

Table 3: Flavonoïds and total phenols of *A. annua* powder.

	Total phenols (mgEq AG / 100 mg)	Total Flavonoïds (mgEq Q / 100 mg)
<i>A. annua</i>	32.5 ± 0.067	11.3 ± 0.152

value represents the mean ± E.S.M, n = 3.

Total phenols and flavonoïds content in the powder

Total phenols and flavonoïd content were respectively 32.5 ± 0.67 milligram Equivalent Gallic Acid/100 mg and 11.3 ± 1.52 milligram Equivalent Quercetin/100 mg (Table 3).

DISCUSSION

This study is an attempt to establish the diagnostic characteristics of the growth of *Artemisia annua* in Togo. These results can be employed as suitable quality control measures to ensure the quality, safety, and efficacy of this herbal drug material. The parameters studied are useful to identify and authenticate the traditionally important medicinal plant *A. annua*. Thus, this will prove helpful in the preparation of herbal monographs and pharmacopoeial standards as emphasized by WHO.

Since the safety and efficacy are the ultimate goals to ensure the reproducible quality of the herbal drugs, the exact identification and quality assurance of the raw material is essential.¹⁸

The herbal materials supplied mostly to the market is shrunken, twisted, rolled and deformed, and is without trade name and proper identification. So, such drugs can easily be adulterated or substituted.

The application of pharmacognostic protocols such as macromorphology, micromorphology, organoleptic tests, ash value, physicochemical studies, and UV fluorescence study will help in identifying genuine drugs because it serves as tests result for particular drugs. Microscopy also plays an important role in drug identification. The importance of epidermal characters, in general, is widely recognized in taxonomic considerations. In many cases, these were successfully used in the identification of taxa at genus as well as species levels.¹⁹ Similarly, studies in stomata have a great taxonomic as well as pharmacognostic value in proper identification of medicinal plants.²⁰

The physical parameters are almost constant for a plant; therefore, these are helpful in setting standards for a crude drug. Various physicochemical parameters were evaluated for the leaf powder drug, as mentioned in WHO guidelines. These parameters are important for detection of drug adulteration or improper handling of raw materials.⁵ One of such parameter is ash value, which gives an idea of inorganic composition and other impurities in a plant drug. The total ash value is also important for detection of metal, salts, and silica.²¹ Water content value makes it possible to ensure the good preservation of the drug. Low impurity found in *A. annua* powder means that there is no foreign object mixed with the powder. There are also chances of microbial growth when the crude drug is stored for a longer period of time and the moisture content of crude drug is directly related to its stability and consequently with the shelf life of the crude drug. The lower the moisture content, the higher will be the stability of that drug and chance of microbial growth will be less and *vice versa*.²² Identification of the different classes of phytochemical constituents of the plant is an important parameter, which gives an indication of the pharmacological active metabolites present in the plant.

Quantification of the phenolic and flavonoid contents has been evaluated in terms of gallic acid and quercetin equivalent using colorimetric assays. It can be said that the polyphenolics and flavonoid present in the powder can be combined with other phytochemicals present in *Artemisia annua* so as to make it a very important drug.

The relatively low artemisinin content (0.009%) in the powder and very variable in the plant can lead to resistance to plasmodium. In fact, the yield of artemisinin from the aerial part in the People's Republic of China was 0.01% to 0.5%²³, and that of the leaves must be at least 0.7%¹¹, although the yield of artemisinin varies according to the environment.²⁴ The data generated from this study would help in the authentication of various parts of *A. annua*, a very important herbal drug. Thus, this may lead to easier authentication.

CONCLUSION

Scientists are making efforts to evaluate many plant drugs used in traditional system of medicine. Empirical knowledge about medicinal plants plays a vital role in primary health care, and it has great potential for the discovery of new herbal drugs. The results from this present research provided useful information for the identification and maintenance of the quality of *A. annua*. Thus, the standardization of *A. annua* will reduce adulteration in raw materials and would prove beneficial for the preparation of Togolese Herbal Pharmacopoeia monograph from this plant.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest regarding the publication of this article.

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GRAPHICAL ABSTRACT



- **Macroscopic and microscopic evaluation**
- **Physico-chemical parameters**
- **Artemisinin extraction**

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