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PRELIMINARY STUDIES ON *ARTEMISIA ANNUA* L. (ASTERACEAE) GROWN IN GHANA: MORPHOLOGY, MICROSCOPY AND ARTEMISININ YIELD

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ABSTRACT: *Artemisia annua* L. is an herbaceous plant from which the antimalarial sesquiterpene lactone, Artemisinin, is obtained. In Ghana, Artemisinin-Combination therapies are used as first-line treatment for managing malaria. The present study, the first of its kind in Ghana, sought to carry out the preliminary morphological and phytochemical evaluation and investigate the artemisinin content of *Artemisia annua* planted in Ghana. Seeds of *A. annua* were planted, and the dimensions of the shoots of the germinated seedlings were taken from week one till maturity. Qualitative phytochemical analysis was performed on the powdered plant parts following standard techniques. The artemisinin contents of the leaves and stem were determined using Ultra-High-Performance Liquid Chromatography coupled with a triple Quadrupole Mass Spectrometry (UPLC-QqQ-MS) using Multiple Reaction Monitoring (MRM) modes. Morphological observation showed that the Ghanaian grown *A. Annua* attained an average height of 36.94 cm at maturity. The leaves are lobed and have a strong aromatic odour. The ethanol and petroleum ether extracts of leaves, stem and roots s tested positive for secondary metabolites such as phenols and alkaloids. The artemisinin content was highest in the powdered leaf (0.2% - 0.24%), while the ethanol extracts of the leaves, stem, and roots contained no artemisinin. All these findings serve as preliminary data for further studies on Ghanaian-grown *A. annua*.

INTRODUCTION: Malaria remains the most prevalent life-threatening infectious disease in the tropics [1]. *Plasmodium falciparum* accounts for approximately 400,000 deaths per year, the majority of which occur in children under five years [2].

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Nonetheless, the fight against malaria has seen successes over the past two decades [3]. This has been partly but massively due to Artemisinin-based combination therapies [3]. Artemisinin is a sesquiterpene lactone obtained from the Chinese medicinal herb *Artemisia annua* L. (Asteraceae).

Artemisinin-Combination Therapies (ACTs) act as the first-line treatment for multi-drug resistant *Plasmodium falciparum* malaria. Major commercial production regions for *Artemisia annua* include China, the United Republic of Tanzania, Kenya, and Vietnam [4]. The majority of the challenges faced by the commercial production

of *A. annua* include lack of available high quality and affordable seed and low artemisinin yields in plants [5]. Due to these shortcomings, uncertainty over future demand discourages both farmers and extractors from making long-term investments in the cultivation of medicinal plants. There is, therefore, the need to explore the feasibility of large-scale cultivation and production of *Artemisia annua* in Ghana, where malaria is endemic and has ravaging effects. Owing to this, Key Laboratory of Beijing for Identification and Safety Evaluation of Chinese Medicine, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing-China, and the Department of Pharmacognosy and Herbal Medicine of the University of Ghana School of Pharmacy sought out to pilot the cultivation of *A. annua* in Ghana hence promoting the supply of artemisinin for the reduction of malaria pandemic in Africa and globally. This paper seeks to report on the preliminary assessment of morphological, microscopic and phytochemical characteristics and the artemisinin yield of *A. annua* grown in Ghana for the first time.

MATERIALS AND METHODS:

Plant Collection and Preparation: The seeds of *Artemisia annua* supplied by Key Laboratory of Beijing for Identification and Safety Evaluation of Chinese Medicine, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, in Beijing-China, were planted at the University of Ghana Botanical Gardens, Legon, Ghana. The seeds were planted in seedling bags and transplanted unto raised beds three weeks after germination. Black soil was used for cultivation. The plants were eventually harvested after fifteen weeks when flowering was observed. Harvesting was done by gently uprooting from the roots. The fresh samples were cleared of debris, the roots cut off, leaves plucked, and the stem cut into smaller pieces of approximately 10 cm in length. The samples (leaves, stem, roots) were air-dried until no moisture was detected. It was weighed and milled into a semi-coarse powder. Part of the plant materials were kept whole for macro and micro-morphological examinations.

Macroscopic and Microscopic Observations: The aerial parts of *A. annua* were macroscopically examined one week after transplanting and also at fifteen weeks to obtain parameters such as the plant height, leaf shape and arrangement, and flower characteristics. Also, sections of *A. annua* leaves were cut and cleared in 80% w/v choral hydrate. The cleared leaves were then investigated for their microscopic features.

Plant Extract Preparation: Fifty (50) grams each of the powdered plant parts (leaf, stem, and root) of *A. annua* was subjected to Soxhlet extraction using 2L of petroleum ether and 60% ethanol separately for 24 hours. The liquid extracts were evaporated *in vacuo* at 40 °C and air-dried. The extracts were kept in a desiccator until needed for use. The percentage yield of each extract was determined.

Phytochemical Investigation: The petroleum ether and ethanol extracts of leaves, stem and roots of *A. annua* were each investigated for the presence of secondary metabolites following standard methods [6, 7].

Determination of Artemisinin Yield: The content of artemisinin, the main antimalarial compound in *A. annua* was determined using standard methods.

Experimental Conditions: Ultra- High-Performance Liquid Chromatography coupled with a triple Quadrupole Mass Spectrometry (UPLC-QqQ-MS) using Multiple Reaction Monitoring (MRM) mode was employed for the determination of the artemisinin content of the samples. Column: Eclipse Plus C18 (2.1 x 50 mm, 1.8 μm); column temperature: 35 ℃, injection volume: 1 μL, flow rate: 0.2 mL·min^{-1}. Ion source: electrospray ion source (ESI source), dry gas (N_2) temperature: 300 ℃, dry gas flow rate: 5 L·min^{-1}, atomising gas pressure: 45 psi, sheath gas temperature: 250 ℃, sheath gas flow rate: 11 L·min^{-1}, capillary voltage: 3500 V. Positive ion mode: mobile phase: water (A) ~ acetonitrile (B), gradient elution time: 0~3 min, 50%~100% B; 3~5 min, 100% B; after 2 min. Mother ion: 283.2, daughter ion: 265.1, 247.1, voltage: 135.0V, energy: 5.0. The standard external method calculates the concentration.

Preparation of Sample Solution: An amount of 0.1 g of the powdered leaf of *A. annua* L. was weighed and placed in a 50 ml centrifuge tube. Ten (10) mL of methanol was added. Ultrasonic extraction was performed for 30 minutes at room temperature. The sample was filtered using a 0.2

µm micro-porous membrane to obtain the test solution (P_l). The procedure was repeated on the powdered stem, petroleum ether leaf, petroleum ether stem, petroleum ether root, ethanol leaf, ethanol stem and ethanol root extracts of *A. annua* L to obtain the test solutions P_s, E_{pl}, E_{ps}, E_{pr}, E_{el}, E_{es} and E_{er} respectively.

Preparation of Reference Solution: A reference stock solution of artemisinin 50 µg·mL^{-1} was prepared using methanol. It was further diluted to produce reference solutions with concentrations of 2500, 1250, 625, 312.5, 156.25, 78.1, 39.1 ng·mL^{-1}. The reference solutions were then filtered through a 0.2 µm microporous membrane and 1 µL of each solution was taken for the determination.

RESULTS:

Determination of Macroscopic and Microscopic Characteristics: Initial measurement taken one week after transplanting the seedlings revealed an average plant height of 5.3 cm, average leaf length and width of 6.09 cm and 3.16 cm, respectively, and an average petiole length of 1.8 cm.

At fifteen weeks, the mature *A. annua* plant had attained an average height of 36.94 cm, leaf length and width of 7.42 cm and 4.14 cm, respectively and petiole length of 2.66 cm. Morphologically, *A. annua* was observed to be an erect, herbaceous plant with a vase-like appearance. Leaves are pinnately lobed and alternately arranged **Fig. 1**.

Flower heads are pale yellow in colour and occur as racemous inflorescence. They are also characterised by a strong aromatic odour. Microscopic investigation of the cleared leaves revealed wavy epidermal cells, glandular trichomes, and anomocytic stomata **Fig. 2.**

FIG. 1: AERIAL PARTS OF YOUNG *A. ANNUA* PLANT

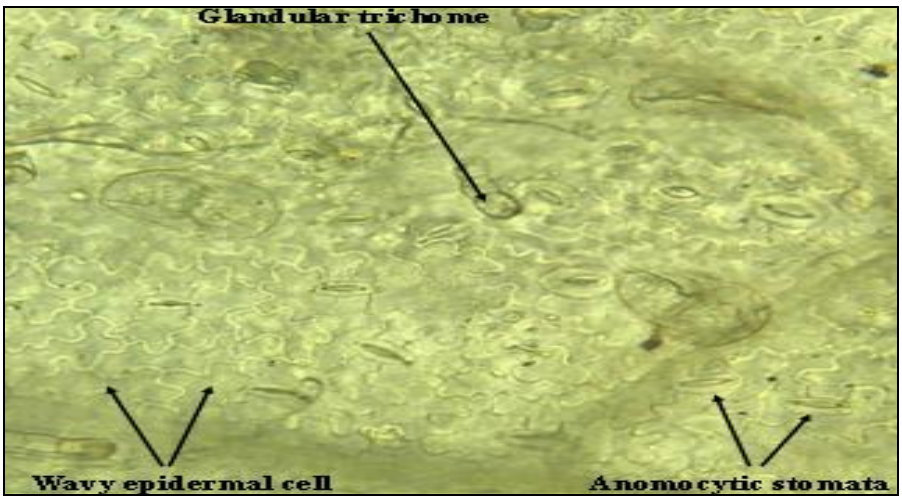

FIG. 2: MICROSCOPY OF THE LEAF SURFACE SHOWING GLANDULAR TRICHOMES, ANOMOCYTIC STOMATA AND WAVY EPIDERMAL CELLS

Determination of Extractive Values: The leaves of *A. annua* recorded a higher yield when extracted with both non-polar and polar solvents as compared with the stem and the roots **Table 1.**

TABLE 1: PETROLEUM ETHER AND ETHANOL EXTRACTIVE YIELDS OF *A. ANNUA*

Extract	Petroleum ether	60% ethanol
Leaf extract	22.5	27.03
Stem extract	2.80	3.78
Root extract	2.11	3.85

Phytochemical Analysis: Preliminary phytochemical analysis was performed to ascertain the classes of phytoconstituents present in each extract.

Alkaloids, saponins, phenols, flavonoids and tannins were detected in the various plant materials. Alkaloids were, however, found to be present in all the extracts. **Table 2** gives a summary of the secondary metabolites.

TABLE 2: RESULT OF PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extract	Alkaloids	Glycosides	Saponins	Phenols	Flavonoids	Tannins
Ethanol stem	+	+	+	+	+	-
Ethanol leaf	+	-	+	+	+	+
Ethanol root	+	+	+	+	-	-
Pet ether stem	+	-	-	-	-	-
Pet ether leaf	+	-	+	-	+	-
Pet ether root	+	-	-	-	+	+

+ = detected; - = not detected

Determination of Artemisinin Content: The artemisinin content was quantified using a linear calibration graph. The linearity of the artemisinin calibration curve was assessed on five (5)-point scale by spotting increasing amounts of the artemisinin working standard solution of 50 μg.mL^{-1}, starting from 39.1 to 2500 ng.mL^{-1}. Good linearity was obtained with a correlation coefficient R^2= 0.998.

The linear equation of the line was obtained as y = 37.119x + 3966.6 **Fig. 3.** Different yields of artemisinin were found in the powdered materials and extracts, as represented in **Table 3**. There was also a significant difference between the powdered samples and extracts, with a considerably increased artemisinin content in the leaf powder (0.24 % content).

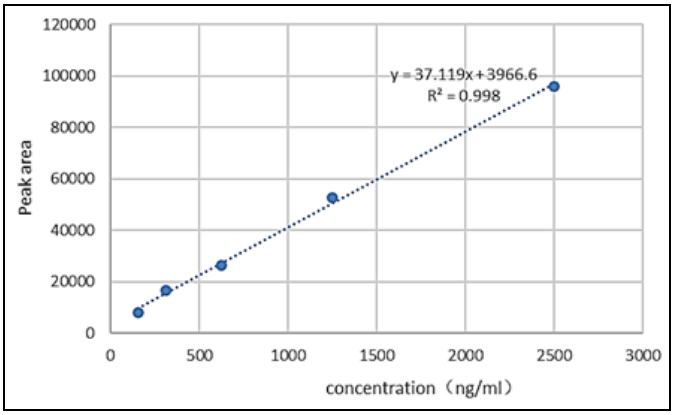

FIG. 3: A LINEAR CURVE OF PEAK AREA AGAINST CONCENTRATION IN ng.mL^{-1}

TABLE 3: PERCENTAGE ARTEMISININ CONTENT IN DIFFERENT PREPARATIONS OF *ARTEMISIA ANNUA*

Sample	Sample name	Artemisinin Content (%)
Plant	Artemisia leaf powder 1 (P_l)	0.24
	Artemisia leaf powder 2 (P_l)	0.20
	Artemisia stem powder 1 (P_s)	0.01
	Artemisia stem powder 2 (P_s)	0.01
Extract	Petroleum ether leaf extract (E_{pl})	0.08
	Petroleum ether stem extract (E_{ps})	0.18
	Petroleum ether root extract (E_{pr})	0.06
	Ethanol leaf extract (E_{el})	0.00
	Ethanol stem extract (E_{es})	0.00
	Ethanol root extract (E_{er})	0.00

DISCUSSION: *A. annua* is a robust annual shrub that is able to grow in a wide range of subtropical and temperate environments. In China, the most suitable areas for planting *A. annua* with high artemisinin content (the content of artemisinin >0.8%) are in the south of the country [8]. These regions have climates akin to the tropics. Presently, sub-Saharan Africa is responsible for the highest

malaria burden [1]. Due to this, some African countries have taken to the cultivation of *A. annua* [4-9]. Subsequently, this study investigated the morphological and microscopic features, the presence of different secondary metabolites, and the artemisinin content in *Artemisia annua* grown in Ghana for the first time. The morphological and microscopic features of the Ghanaian grown *A. annua* were similar to those in previous reports [10, 11]. It is important to know the height attained by *A. annua* as works by [12], have shown that the herb yield and artemisinin content is positively correlated to the plant height [12].

The determination of the extractive yield gives a possible indication of the amount of constituent that may be obtained when a particular solvent is used for the extraction of a given plant part [13]. Published reports have shown that most secondary metabolites from the plants were obtained from the leaves [14]. In our study, the artemisinin content was highest in the leaf powder. Artemisinin has been reported to occur within the trichomes of the leaves and floral parts [15-17]. Trichomes may, however, decline in number when leaf development is halted [11,] and this may affect artemisinin yield.

There are many other factors that determine artemisinin content. These include varieties, climatic conditions, soil nutrients and harvesting times [18-20]. It has been observed that wild *A. annua* varieties have highly variable artemisinin content, most of which fall below 0.6%. However, some varieties with reasonably high artemisinin content have been identified after decades of breeding. Such is a hybrid variety with 1.0 to 1.5% artemisinin yield introduced to some East African countries [4].

In this study, a new variety that was developed through mixed breeding methods was used for cultivation. Previous investigations have shown an average artemisinin content as high as 2.11±0.38% when planted in South East of China [18]. However, the artemisinin content of this same variety was much lower when planted in Ghana for the first time. Some factors that may have accounted for this result have been identified. One possible reason could be the different sowing times of the seeds in the two countries. In Ghana, planting was done in the month of May, whiles it is usually planted in February or March in China. Again, the difference in rainfall pattern could contribute to the high difference in artemisinin yield. The most suitable annual rainfall for this particular variety is 987 to 1688 mm. The lower annual rainfall for Accra, Ghana (730 mm) may have resulted in moisture stress in the young plant, thus resulting in a reduced yield of artemisinin. Finally, the difference in ecological environments could be attributable to the low yield in Ghana as temperature, light, and moisture are all significantly related to artemisinin yield [19, 20].

CONCLUSION: This study has reported the potential of *Artemisia annua* as a viable plant on Ghanaian soil for the first time. The antimalarial compound artemisinin occurred mostly in the leaf. Different solvent types may be used for extraction to determine the possibility of higher artemisinin yield. The work has given evidence on the cultivation possibility of *A. annua* in Ghana and has served as a basis for more research to improve the artemisinin content of the plant. Further research to improve planting conditions is ongoing.

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CONFLICTS OF INTEREST: Authors do not have any conflict of interests to declare.

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