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Original Research Article

Extraction, Purification, and Quantification of Artemisinin from Artemisia (*Artemisia annua* L.) using Spectrophotometric Method

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Abstract

Purpose*:* Plasmodium species are the major causative agents of malaria infection. Artemisinin is an effective bioactive compound in *Artemisia annua* used in the treatment of malaria. This study aimed to extract and quantify the amount of artemisinin in different accessions of *Artemisia annua* using different solvents and methods of extraction.
Methods*:* Seventy-nine accessions of *Artemisia annua* were collected at different stages of growth and development, and planted at the orchard and research field of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The extraction of artemisinin from the different accessions was done using ultrasonic-assisted extraction (UAE) and maceration methods with n-hexane and petroleum ether as the extracting solvents. After the extraction, purification, and the derivatization of the artemisinin, the artemisinin content of each accession was determined spectrophotometrically at a wavelength of 253 nm. The yield of artemisinin from each solvent extract and extraction method was calculated. The data collected was analyzed using SAS, and means were separated using Fisher Least Significant Difference (LSD) test.
Results*:* The findings from this study showed that the solvents n-hexane and petroleum ether, as well as UAE and maceration methods of extraction are suitable for the extraction of artemisinin from artemisia annua.
Conclusion: The highest artemisinin content (0.37%) was found in IARART0049 collected at the vegetative stage, and the artemisinin content was constant across the extracting solvents and methods of extraction used. This accession amongst other accessions with similar artemisinin content are promising for the optimal extraction of artemisinin.

Keywords: Artemisinin; Ultrasound-Assisted Extraction; Maceration; n-Hexane; Petroleum ether.

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INTRODUCTION

Artemisia annua is a specie of sweet wormwood native to temperate Asia but distributed worldwide. It is a member of the Asteraceae family.[1] Artemisia is the most extended and varied genus of the Asteraceae family, presenting around 500 species universally distributed.[2] Artemisia species have been utilized in folk medicine to manage conditions such as diabetes, insomnia, depression, and stress, as well as for their antiseptic, purgative, and antispasmodic properties. Artemisinin, a sesquiterpene lactone, was first isolated from *Artemisia annua* by Youyou Tu, contributing meaningfully to antimalarial therapy. Beyond its antimalarial activity, artemisinin and its derivatives exhibit broad pharmacological activities, including anticancer, antiviral, anti-inflammatory, and reno protective properties. Recent research has expanded its therapeutic applications to metabolic syndrome, fibrosis, and as a potential treatment for SARS-CoV-2.[3] These compounds have been shown to be effective against different types of cancers, including leukemia, breast cancer, and colon cancer.[4] Despite its promise, propagation of Artemisia through seeds remains problematic due to their small size and inherent dormancy.[5]

Furthermore, limited knowledge of the crop's reproductive biology, including flowering sequence and fertility, has constrained breeding programs. Developing high-artemisinin-yielding cultivars in combination with the latest extraction and purification methods, is crucial to meeting global market demand.[6] Considering that malaria accounts for nearly one million deaths globally every year, mostly among African children and contributes significantly to maternal mortality, Nigeria could benefit substantially from domesticating and improving this plant. The emergence of drug resistance to chloroquine and sulfadoxine-pyrimethamine makes alternative treatments such as artemisinin combination therapy (ACT) indispensable. Previous studies have shown that artemisinin content obtained from Artemisia varies depending on extraction techniques and solvents.[7,8] Different extraction techniques such as supercritical carbon dioxide (SC-CO2) extraction, subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), and extraction with deep eutectic solvents (DES) have been used for the extraction of artemisinin.[9] Artemisinin from Artemisia annua L. is usually extracted using organic solvents such as propylene glycol methyl ether, butanol, dichloromethane, hexane, petroleum ether, toluene, isopropanol and chloroform.[4] Commercially, solid-liquid extraction using heat and organic solvents remains predominant. Prawang *et al*. (2019)[10] and Nganthoi and Sanatombi (2019)[11] quantified artemisinin content in four Artemisia species from Manipur using UV spectrophotometry validated by HPLC. They observed solvent- and tissue-dependent variations in the artemisinin content, with toluene extracts from *A. vulgaris* flowers giving the highest yield. Their work confirmed UV spectrophotometry as a reliable and affordable quantification technique for artemisinin.

Given that artemisinin combination therapy (ACT) remains the most effective malaria treatment,[12,13] research on optimizing the extraction and quantification of artemisinin is critical. Various accessions of *A. annua* have been collected by the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, but not characterized for artemisinin content. This study, therefore, aimed to evaluate the extraction efficiency of different solvents and methods for extracting artemisinin, quantify artemisinin content using UV spectrophotometry, and identify promising accessions for potential large-scale cultivation.

MATERIALS AND METHODS

Chemicals and standards used

The chemicals used in this study were methanol (Oxford, India), n-hexane (Oxford, India), petroleum ether (Scharlau, Spain), sodium hydroxide (Scharlau, Spain), (Oxford, India), hydrochloric acid (Oxford, India), and activated charcoal. The chemicals were of analytical grade and used without further purification. The Artemisinin standard (C15H22O5 – CAS 63968-64-9, 98%) used was supplied by Sigma Aldrich (St. Louis, MO, USA) through Bristol Scientific, Lagos, Nigeria.

Experimental sites

The planting of *Artemisia annua* was carried out at the orchard and field of the Institute for Agricultural Research, while extraction and quantification were done at the Multi-Users Laboratory, Department of Chemistry, Ahmadu Bello University, Samaru–Zaria. Samaru-Zaria is located in the northern Guinea savanna ecological zone of Nigeria (11° 11' N, 07° 38', 680 m above sea level). It has a mean annual rainfall of about 1029.52 mm; rainfall is well distributed throughout the growing season between May and October. The mean annual temperature is between 26°C and 37°C.

Planting materials

The bulked seeds of seventy-nine (79) accessions of *Artemisia annua* collected by the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Kaduna State, Nigeria were planted at the Orchard and Research field of the Institute during the rainy season in 2024. The individual plant obtained was tagged and characterized, and collections were made from about 79 stands of Artemisia plants. These samples were collected at different stages of growth and development. Eight samples were collected at the vegetative stage, thirty-five at the flowering stage, and thirty-six at the maturity stage.

Preparation of artemisinin calibration curve
Artemisinin calibration curve was prepared using different concentrations of the artemisinin standard (0, 500, 1000, 1500, and 2000 mg/L).

Preparation and extraction of samples using the maceration method
Fresh leaves of *Artemisia annua* were collected from the field, dried at room temperature, and ground to a fine powder. The powdered leaves were stored in airtight Ziploc bags and subsequently refrigerated at 4°C until further use for extraction. To investigate the effect of solvents used for extraction on artemisinin content, 5 g of each powdered leaf sample was macerated separately with 250 mL of petroleum ether and n-hexane at room temperature for 48 h. The extract was then washed with an equal volume of NaOH (2% w/v), followed by 4–5 washes with distilled water. Furthermore, activated charcoal (3%) was added to the extract to remove coloured pigments. The filtrate was allowed to dry at room temperature, and the residue was dissolved in 5 mL of methanol and stored at 4°C for further use.

Ultrasound-Assisted Extraction (UAE) and purification of the extract
Powdered leaf samples of *Artemisia annua* (5 g) were weighed and extracted separately with 250 mL of n-hexane and petroleum ether using an ultrasonic bath (model no: PS-40A) with an ultrasonic power of 240 W and an ultrasonic frequency of 40 KHz. After the extraction, the n-hexane and petroleum ether phases were washed with 2% NaOH solution in a separating funnel to remove impurity. The lower alkali solution was discarded, and the upper solution was washed with distilled water several times until it was neutralized. Activated charcoal (3%) was added to the extract to remove coloured pigments. The extract obtained after evaporation was dissolved in methanol and then filtered into a 250 mL measuring flask. The extract was stored at 4°C until further analysis.[11]
The yield of n-hexane and petroleum ether extracts was calculated using the following equation (1).

$$w = \frac{ax100\%}{b} \quad \text{..... (Eq. 1)}$$

Where w is the yield of n-hexane or petroleum ether extract, a is the weight of n-hexane or petroleum ether extract, and b is the weight of the plant sample used.

Derivatisation of artemisinin
Standard artemisinin was dissolved in methanol to prepare stock solutions (1000 µg/mL). Due to its non-chromogenic property, artemisinin was converted to a UV-sensitive compound for quantification by UV spectrophotometry and RP-HPLC techniques. For this, 1 mL of filtered methanol solution of standard artemisinin or plant extract was treated with 4 mL of 0.2% NaOH (w/v) and incubated at 50°C for 30 min. After cooling to room temperature, 0.2 N HCl was added to the solution to make up the final volume to 10 mL for quantification.

Quantification of artemisinin
Artemisinin content of the different extracts for the different extraction methods was quantified according to the method described by Nganthoi and Sanatombi (2019).[11] An Agilent Cary 300 UV/VIS spectrophotometer with a 1 cm matched quartz cuvette was used to quantify the artemisinin content in the extracts. The wavelength of maximum absorption (λmax) of standard artemisinin was determined by scanning at 200 - 350 nm wavelengths. A calibration curve of standard artemisinin was then prepared at the λmax using five different concentrations of the standard. The absorbance value of each sample was taken in duplicate, and the concentration of artemisinin was determined from the equation obtained from the calibration curve as shown below.

Abs = 0.00164* Conc + 0.04543 …….....…(Eq. 2)

Data collection and analysis
Data were collected on the following parameters: Absorbance, artemisinin concentration, and the yield of the extracts. The data collected were analyzed using SAS version 9.0. Copyright ©2002 by SAS Institute Inc., Cary, NC, USA.

RESULTS AND DISCUSSION

Artemisinin content of the different extracts
The derivatization method of artemisinin was based on the reaction of artemisinin with sodium hydroxide solution, which resulted in the formation of an unstable intermediate product, followed by the reaction of the intermediate with hydrochloric acid to form a more stable final product. This was in line with what was reported by Nganthoi and Sanatombi (2019).[11] The maximum absorption of the completely derivatized artemisinin was obtained at 253 nm for the UV spectrophotometric study. The absorption intensity of artemisinin at 253 nm increased with an increase in the concentration of artemisinin. This agrees with the findings of Nganthoi and Sanatombi (2019),[11] who also observed that the absorption intensity of artemisinin at 263 nm increased with an increase in concentration. The regression equation of the standard curve was obtained as follows: a = 0.00164b + 0.04543 (where 'a' is the absorbance and 'b' is the concentration in mg/L), while y = 0.021× – 0.015 ± 0.036 (where 'y' is the absorbance and 'x' is the concentration in µg/mL) was obtained by Nganthoi and Sanatombi (2019).[11] The calibration curve obtained from using the different concentrations of the standard artemisinin is shown in Figure 1.

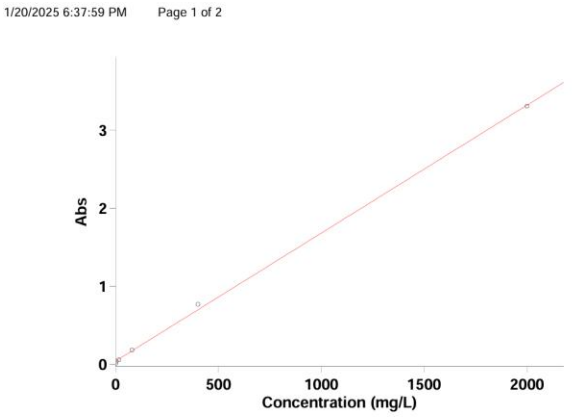


Figure 1: Atesinin calibration curve

Effect of extracting solvent and method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the vegetative stage

Using the UAE method, the highest content of artemisinin was obtained from IARART0049 (0.37%), which was significantly different from what was obtained from the other accessions. A negative value was recorded for IARART004 using petroleum ether for the extraction of samples collected at the vegetative stage (Figure 2).

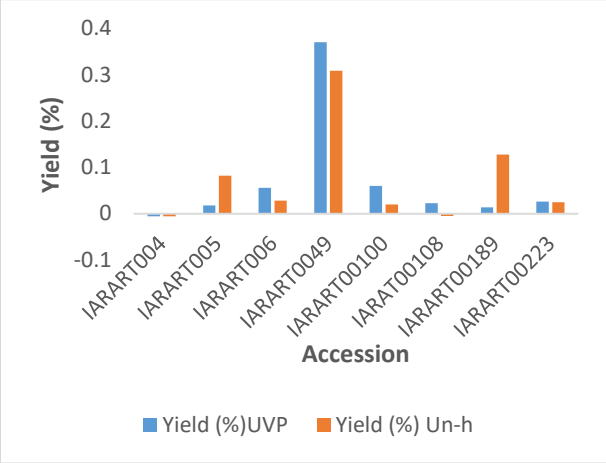


Figure 2: Effect of extracting solvent and UAE method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at vegetative stage. UVP: Ultrasonic Assisted method of extraction using petroleum ether as solvent, Un-h: Ultrasonic Assisted Method of Extraction using n-hexane as solvent.

When n-hexane was used as the extracting solvent, the highest artemisinin content of 0.31% was also obtained from IARART0049, which was significantly different from values obtained from other accessions. Negative values were obtained for IARART00108 (-0.005) and IARART004 (-0.006). The artemisinin content obtained from IARART0049 (0.37%) was higher than the artemisinin content reported for the flower of *A. vulgaris* L (0.091% per gram dry weight) and leaves of *A. parviflora* (0.087%) when toluene was used as one of the extracting solvents.[11] Their result showed the variable efficiency of different organic solvents in extracting artemisinin from plant samples, and toluene was observed to be a better artemisinin-extracting solvent compared to petroleum ether and n-hexane. It was also found that leaves and flowers were more suitable parts from which artemisinin is extracted in good yield.[11]

Using the maceration method, the highest yield of artemisinin was obtained from IARART0049 (0.17%); this was significantly higher compared to values obtained from other accessions. Negative values were obtained for IARART004 and IARART005 (-0.01) using petroleum ether as extracting solvent. When n-hexane was used, the highest artemisinin content was also recorded for IARART0049 (0.10%), and this was significantly higher than the values obtained from other accessions. IARART00100 gave a negative value (-0.01%) for artemisinin content (Figure 3).

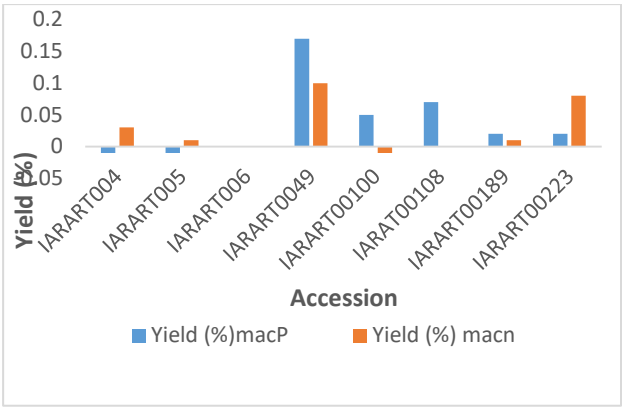


Figure 3: Effect of extracting solvent and maceration method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the vegetative stage. macP: Maceration method of extraction using petroleum ether as solvent. macn: Maceration method of extraction using n-hexane as solvent.

The results of this study showed that there was significant variation in artemisinin content among different accessions of Artemisia annua collected at the vegetative stage and this can be attributed to differences in the genetic make-up of each accession. Differences in artemisinin content based on genotype has previously been reported.[14] The similarity in artemisinin content across the different solvents and methods of extraction was observed in IARART0049, while some accessions had varied artemisinin content across the solvents and methods of extraction.

Effect of extracting solvent and method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the flowering stage

The highest artemisinin content of 0.24% was obtained from IARART0060, followed by IARART0054, with artemisinin content of 0.22% using petroleum ether as extracting solvent for the samples collected at the

flowering stage (Figure 4). These values were significantly different from what was obtained from other accessions.

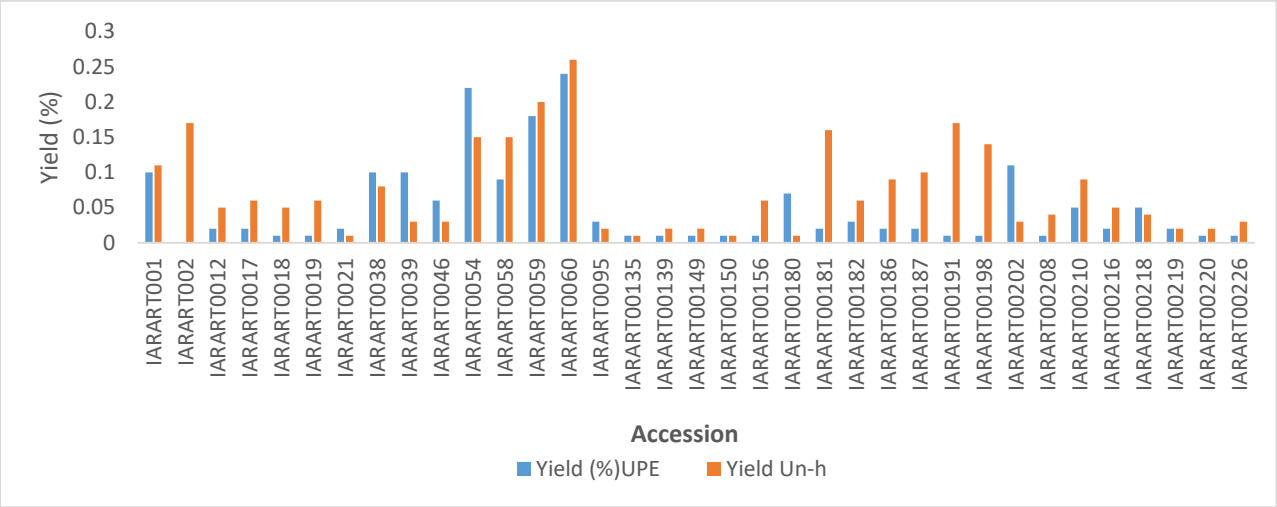


Figure 4: Effect of extracting solvent and UAE method of extraction on artemisinin content on different accessions of Artemisia annua collected at the flowering stage. UPE: Ultrasonic Assisted method of extraction using petroleum ether as solvent, Un-h: Ultrasonic Assisted Method of Extraction using n-hexane as solvent.

The lowest artemisinin content was obtained from IARART00135 while no artemisinin was extracted from IARART002. When n-hexane was used for extraction, the highest content of artemisinin (0.26%) was obtained from the same accession (IARART0060), followed by IARART0059, with artemisinin content of 0.20%. The lowest artemisinin content was extracted from IARART0021 and IARART00135, both having artemisinin content of 0.01% (Figure 4).

Using the maceration method, the highest artemisinin content was obtained from IARART0012 (0.11%), followed by IARART00218 (0.10%) and IARART0058 (0.09%) using petroleum ether as extracting solvent. When n-hexane was used as the extracting solvent, the highest yield of artemisinin (0.19%) was obtained from IARART0058, followed by IARART0059, with artemisinin yield of 0.11%. No artemisinin was extracted from IARART156 (-0.01%) using the maceration method and n-hexane as the extracting solvent (Figure 5).

Significant variation in artemisinin content among different accessions was also observed for the sample collected at the flowering stage, and this can also be attributed to differences in the genetic makeup of each accession. This also agrees with the findings of Ma et al. (2024)[14] who reported differences in artemisinin content of two different genotypes used for the extraction. The similarity in artemisinin content using petroleum ether, n-hexane, and UAE as methods of extraction was observed in IARART0060, IARAR0058, and IARART0059, that is, artemisinin content was similar across the solvent used and method of extraction, while variation was observed for some accessions used.

Effect of extracting solvent and method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the maturity stage

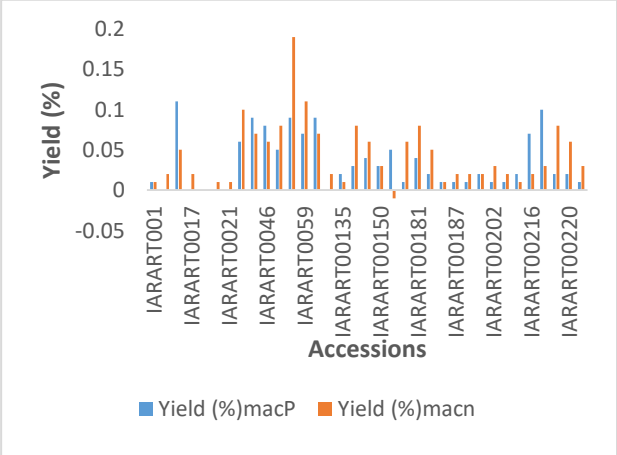


Figure 5: Effect of extracting solvent and maceration method of extraction on artemisinin content on different accessions of *Artemisia annua* collected at the flowering stage. macP: Maceration method of extraction using petroleum ether as solvent. macn: Maceration method of extraction using n-hexane as solvent

A highly significant variation in artemisinin content was observed among the samples collected at the maturity stage. The highest yield of artemisinin was obtained from IARART0057 (0.18%), followed by IARART00178, and the lowest artemisinin content was obtained from IARART0010, IARART00141, and IARART00148 with 0.01% of artemisinin when petroleum ether was used as the extracting solvent and UAE as the method of extraction. When n-hexane was used as solvent for extraction, the

highest yield of artemisinin was obtained from the same accession, IARART0057 (0.19%), followed by IARART00193, which yielded 0.17% artemisinin, the lowest artemisinin yield was obtained from IARART0090 and IARART00101 with 0.0% of artemisinin (Figure 6).

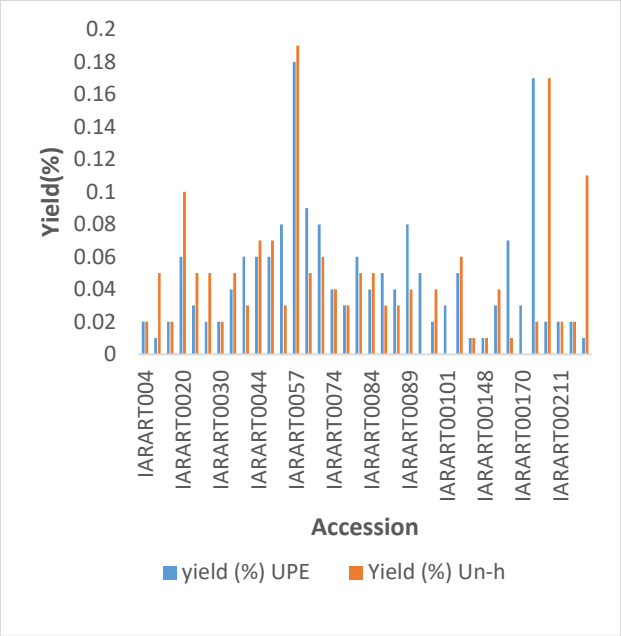


Figure 6: Effect of extracting solvent and UAE method of extraction on artemisinin content on different accessions of *Artemisia annua* collected at the maturity stage. UPE: Ultrasonic Assisted method of extraction using petroleum ether as solvent, Un-h: Ultrasonic Assisted Method of Extraction using n-hexane as solvent

For the maceration method using petroleum ether as an extracting solvent, the highest yield of artemisinin was obtained from IARART0080 (0.20%), followed by IARART0044 with an artemisinin content of 0.15%. Some samples produced no artemisinin. When n-hexane was used as solvent for extraction, the highest artemisinin content was obtained from IARART00154 (0.31%), followed by IARART0073 (0.15%), while no artemisinin was observed in some samples (Figure 7). Similarity in artemisinin content was also observed for IARART0057 when petroleum ether and n-hexane were used as extracting solvents and UAE as a method of extraction. This was similar to what was observed for some accessions for both samples collected at the vegetative and flowering stages.

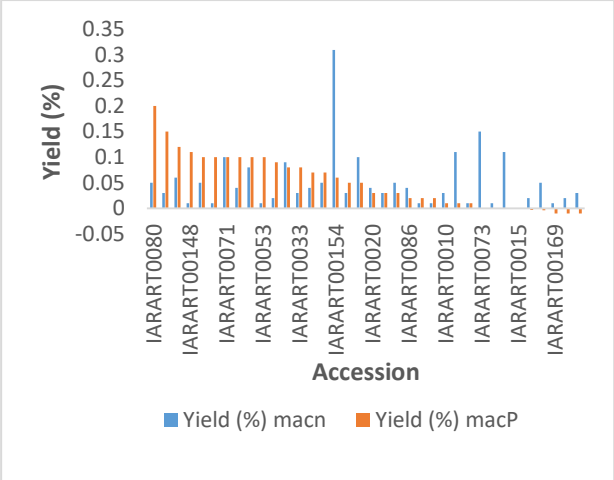


Figure 7: Effect of extracting solvent and maceration method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the maturity stage. macP: Maceration method of extraction using petroleum ether as solvent. macn: Maceration method of extraction using n-hexane as solvent

For the combined analysis of the samples collected at the vegetative stage, the highest yield of artemisinin was obtained from IARART0049, which was significantly different from what was observed in other accessions. The lowest artemisinin content was obtained from IARART004 (Figure 8).

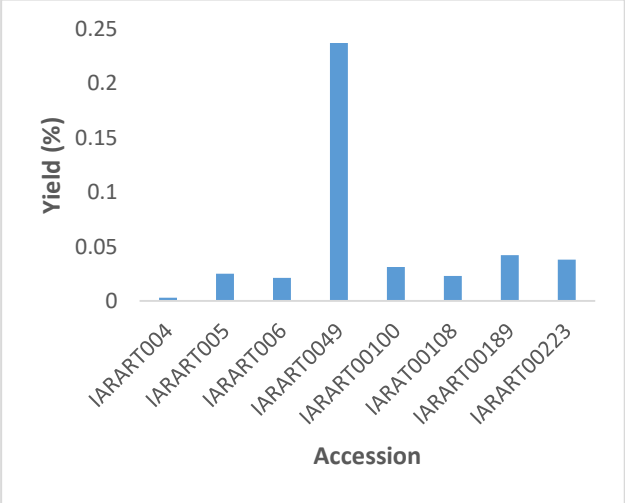


Figure 8: Combined effect of extracting solvent and method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the vegetative stage

For the solvent used, more artemisinin content was obtained from using petroleum ether as the extracting solvent, but not significantly different from what was recorded when n-hexane was used (Table 1). This implies that both solvents are suitable for the extraction of artemisinin. Heat treatment with organic solvents, such as chloroform, ethyl acetate, n-hexane, and petroleum ether,

has been reported as the most preferred and effective commercial solid-liquid extraction method for artemisinin

Table 1: Combined effect of extracting solvent on artemisinin content of different accessions of *Artemisia annua* collected at different stages of growth and development

	Yield (%)		
Solvent Used	Vegetative stage	Flowering stage	Maturity stage
Petroleum ether	0.06	0.04	0.049
n-Hexane	0.05	0.06	0.047
Mean	0.05	0.05	0.048
SEM	0.01	0.00	0.00

extraction.[10,15] For the extraction method, the highest artemisinin yield was obtained from using UAE, which was significantly higher than the artemisinin content obtained from the maceration method for the sample collected at the vegetative stage (Table 2).

Table 2: Combined effect of method of extraction on Artemisinin content of different accessions of *Artemisia annua* collected at different stages of growth and development

	Yield (%)		
Method of Extraction	Vegetative stage	Flowering stage	Maturity stage
Maceration	0.03	0.06	0.051
UAE	0.07	0.04	0.046
Mean	0.05	0.05	0.05
SEM	0.01	0.00	0.00

This implies that the UAE method extracted artemisinin better compared to maceration for the samples collected at the growth stage. Therefore, the UAE is evidently very effective in the extraction of artemisinin from sweet wormwood. During UAE, sonication creates cavitation bubbles, which have the potential to collapse asymmetrically and produce microjets.[16] The result of this study agrees with findings of Dogan *et al*. (2021)[15] who reported the highest artemisinin content from Ultrasonic-Assisted Extraction of *Artemisia annua* using n-hexane as extracting solvent. Ultrasonic-Assisted Extraction is an environmentally friendly and highly effective extraction technology.[17] It has the potential to reduce or eliminate the need for organic solvents, thereby reducing its effect on the environment.[15] Furthermore, UAE has been found to boost the extraction of target bioactive components, making it an attractive method in the industry.

For the combined analysis of the samples collected at the flowering stage, the highest yield was recorded for IARART0060 (0.16%), followed by IARART0059 (0.14%), IARART0058 (0.14%), and IARART0054 (0.13%), while the lowest artemisinin content was obtained from IARART0021 (0.01%) (Figure 9).

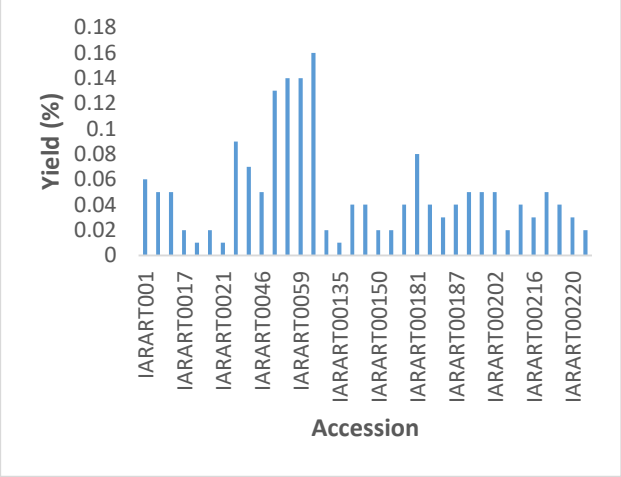


Figure 9: Combined effect of extracting solvent and method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the flowering stage

For the solvent used, the highest artemisinin content was obtained from n-hexane (0.06%). This agrees with the findings from a previous study in which hexane, 95% ethanol,

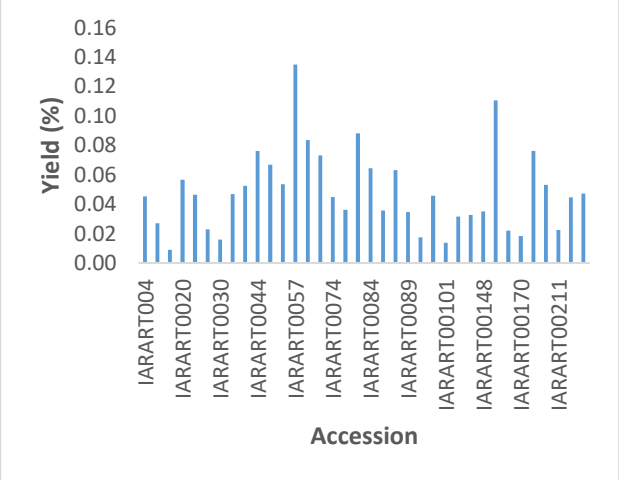


Figure 10: Combined effect of extracting solvent and method of extraction on artemisinin content of different accessions of *Artemisia annua* collected at the maturity stage

and isopropanol were found to be the most effective solvents for the extraction of artemisinin, resulting in the highest content of artemisinin in the range of 0.062 – 0.066%.[15] However, in the study of Babacan *et al*. (2022)[18], the highest artemisinin content was extracted using ethanol the extracting solvent. For the method of

extraction, maceration produced the highest artemisinin yield. This implies that maceration extracted artemisinin better for the samples collected at the flowering stage (Table 2). This is in agreement with the findings from the work of Anyinkeng *et al*. (2023)[19] where maceration was reported as the solvent for artemisinin extraction from *Artemisia annua*.

For the combined analysis of the samples collected at the maturity stage, the highest yield of artemisinin was obtained from IARART0057, followed by IARART00154, and IARART0080. For the solvent used, the highest artemisinin yield was obtained from petroleum ether (0.05%) (Figure 10). This implies that petroleum ether was suitable for the extraction of artemisinin from *Artemisia annua* accessions collected at the maturity stage while for the extraction method, the highest artemisinin yield was obtained from maceration. This implies that maceration extracted artemisinin better from the samples collected at the maturity stage (Table 2). This is in line with the findings from the study of Chandrakant *et a*l. (2013)[20] who reported the highest yield of artemisinin from *Artemisia annua* using maceration method. The maceration is a simple and cheap extraction method that does not require special equipment, has limited use of solvent, and can also give good and selective extraction.[20]

The suitability of both the maceration and UAE methods for the extraction of artemisinin from *Artemisia annua* has been established in this study. This agrees with the findings of Soktoeva et al. (2013)[21] who reported no significant difference in the artemisinin content of *Artemisia annua* extracted by ultrasonic-assisted extraction and maceration using different solvents (hexane and ethanol).

Generally, the UAE method proved to be a better extraction method of artemisinin from *Artemisia annua* samples collected at the vegetative stage, while maceration method is better for samples collected at the flowering and maturity stages.

CONCLUSION

The suitability of both solvents (petroleum ether and n-hexane) and methods of extraction (UAR and maceration) for the extraction of artemisinin from *Artemisia annua* was observed in this study. It was observed that the amount of artemisinin obtained depends on the accession used and the stage of sample collection. IARART0049 collected at the vegetative stage, recorded the highest (0.37%) artemisinin content when compared with other accessions collected at other stages of growth and development. IARART0049 yielded constant artemisinin content across the solvents and method of extraction used. The accessions IARART0057, IARART0058, IARART0059, and IARART0060 produced similar artemisinin content across the extracting solvents and the methods of extraction for the samples collected at flowering stage, while IARART0057 and IARART0080 produced similar artemisinin content for the samples collected at the maturity stage. Therefore, these accessions are promising for the optimal extraction of artemisinin.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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