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RESEARCH PAPER

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Effect of Plant age in Relation to Artemisinin Content in *Artemisia annua* Special Reference to Assam

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ABSTRACT

Around 1.5 million people die every year of malaria; every 30 seconds a child dies due to this preventable and curable disease. Most of the affordable antimalaria drugs have become ineffective because Plasmodium falciparum – the malarial parasite responsible for the most severe malaria cases and deaths - has developed resistance to them. According to the World Health Organization (WHO) and other agencies, artemisinin based combination therapy (ACT), derived from the plant Artemisia annua, is the most promising anti-malarial drug for tackling this problem. Supply does not yet meet demand. The production and supply chain needs to grow and significant public and private interventions are required to make an effective and affordable anti-malaria drug available to Indian patients. The most important is artemisinin and its derivatives, which are used as a remedy against malaria. The specific research objectives were the determination of exact Artemisinin content in the plant of Artemisia annua in Assam condition which may be more effective properties of anti-malarial drugs.

Key words: Artemisia annua L., Artemisinin, Antimalarial, Variation and Artemisinin production Assam.

INTRODUCTION

A. annua is an annual herb endemic to Asia, most probably China. It occurs naturally as part of steppe vegetation in the northern parts of Chahar and Suiyuan provinces at 1000 to 1500 m above sea level. *A. annua* is cultivated as an annual crop in China and Vietnam as a source of artemisinin, and in Romania and Bulgaria for its essential oils. It is also cultivated on a small scale in the United States as a source of material for aromatic wreaths (Janick, 2002). The best plants are found in the wild only in certain parts of China, such as Guangxi and Hunan, which (along with Vietnam) produce most of the world's supply. Chinese scientists started to domesticate the wild species of *A. annua* after the curative effect of artemisinin was discovered in 1972. In 2004, China claimed that it was growing a total acreage of 2000 ha of *A. annua*. Production has also started in West Africa, notably Ghana and Gambia, and an extraction plant is planned for Senegal. In Brazil, the production of *A. annua* is being promoted, while the crop has been grown in the United States and Australia on an experimental scale. *A. annua* is also collected in Eastern Europe for the extraction of essential oils for the perfume industry. The plant is increasingly grown for this purpose in such countries as Romania and Bulgaria. In India it is being cultivated on an experimental scale in temperate as well as sub-tropical condition. (Jain et al., 2000. Domestication of *Artemisia annua* plant and development of new anti-malarial drug arteether in India)

MATERIALS AND METHODS

The seeds of *A. annua* (cv. CIM-Arogya) were obtained from the Central Institute for Medicinal and Aromatic Plants (CIMAP), Lucknow and sown in the nursery for the field experiments. Seedlings having 20 cm height were transplanted in the field with spacing 50 cm between rows and 30 cm between plants and grown using standard agronomic procedures. Changes in artemisinin accumulation and biomass formation through plant development of 4 breeding lines of *Artemisia annua* has been taken under the field conditions of Assam. In first phase all leaves and then all leaves and flowers have been collected from the plants in every 2 weeks. Then in 4 lines the maximum artemisinin accumulation has been determined in different harvesting stage like leaf growth stage, flowering stages, floral bud stages in connection with specific day length and photoperiod. Experimental cultivation of *Artemisia annua* has cover plant research, cultivation and selection followed by laboratory tests to assess the level of artemisinin produced in the plants by following the below mentioned procedure.

a) Experimental Plot (Plantation Time)

Experimental plot confined to pre-monsoon period (March –April) and post-monsoon period (October-November). Each experimental plot was maintained in a plot size of 5x 5 m Standard and uniform cultivation method for maintaining all the germplasms standard and uniform cultivation method for maintaining all the germplasms.

b) Evaluation of germplasms

The collected materials further multiplied and raised in polybags for planting in experimental plots of 5 x 5 m size of uniform cultivation condition with four replications (considering two growth seasons in a year) in the three locations of Goalpara, Nagaon and Kamrup district. Weeds were controlled mechanically (between rows) and manually (on rows) after 30 and 60 days from transplanting. No major infection of pest or disease occurred. During the growing period the phenological stage and the plant height were surveyed weekly on the same plants (three plants per each plot).

c) Chemo-profiling for Artemisinin

Starting from the 45th day after transplanting, plants were weekly sampled in order to follow the variation of artemisinin content within each crop season. Samples were obtained from each elementary plot by collecting 100 grams of plant from the upper layer of canopy. The samples were air-dried and then the artemisinin content was measured. (Gupta et al., 1996. A rapid analytical method for the estimation of artemisinin in *Artemisia annua*). For the analysis of artemisinin content, 0.1 g shade-dried leaves were ground, boiled in hexane, filtered and evaporated. The residue was used for artemisinin analysis through HPLC and TLC (Figure 1-4). All samplings for the artemisinin content analysis were carried out in triplicate for analysis. (Charles et al., 1990. Germplasm variation in artemisinin content of *Artemisia annua* using an alternative method of artemisinin analysis from crude plant extracts)

RESULT AND DISCUSSION

Experiment was carried out to study the variation of artemisinin content in mature (6-month-old) field grown *A. annua*. The aerial portion of the plant was demarcated into upper (top about 30 cm), middle (about 30 cm), lower (between middle and leafless region, about 30 cm) and leafless (upto 45 cm height from the ground) regions. Leaf samples were collected from different experimental plots of Assam (Nagaon, Goalpara and Kamrup).

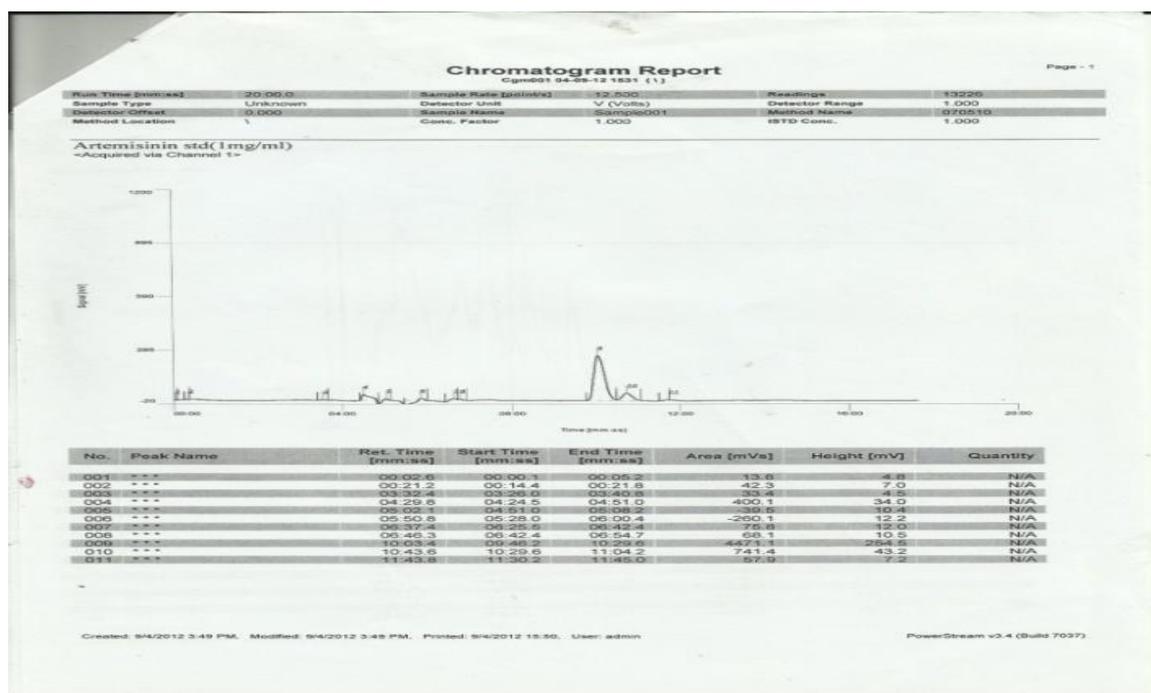


Figure 1. Chromatogram Report showing Artemisinin content.

Artemisinin content was always found to be optimum in the young leaves at upper levels of secondary branches as compared to the leaves, whereas it was undetected in the roots of the plant. Starting from 6-day-old seedling stage monthly sampling of leaves was carried out. Leaf artemisinin content was found to increase from 2 month old plant stage, reaching the maximum at the pre-flowering stage (6-month-old plant/August) and declining thereafter.

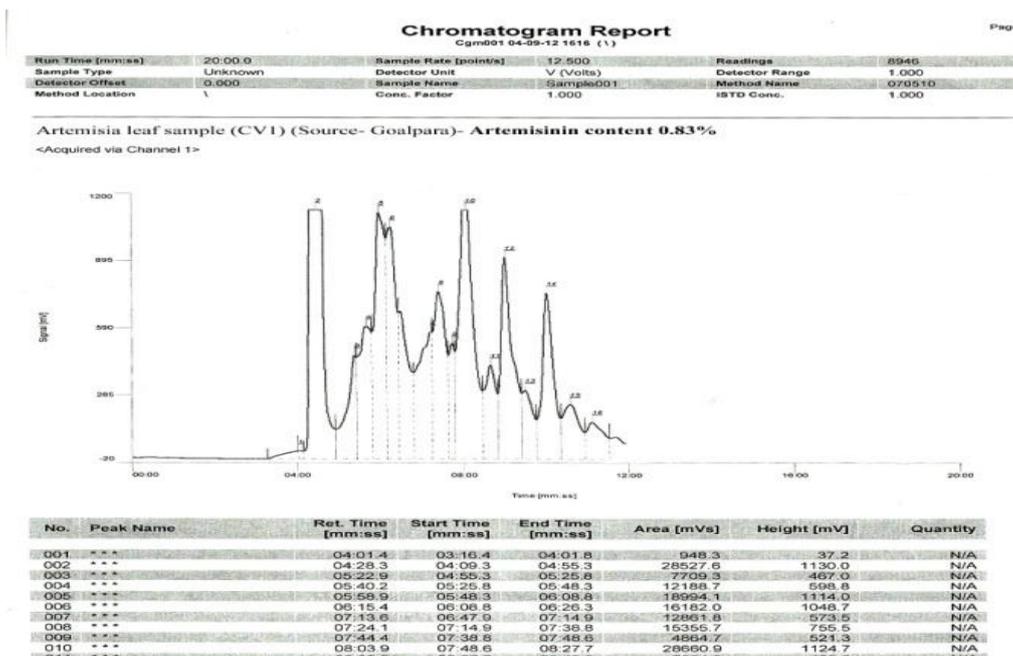


Figure 2. Chromatogram Report showing (immature leaves artemisinin content (mature leaves)).

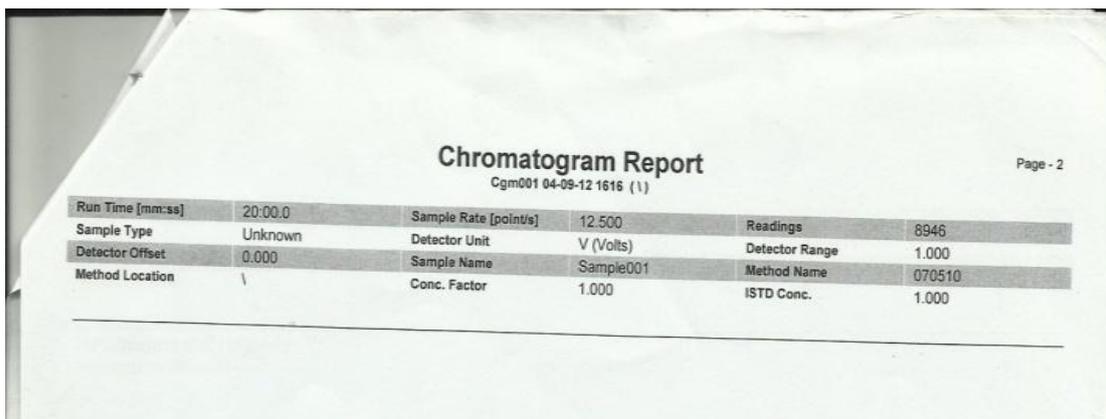


Figure 3. Chromatogram Report showing artemisinin content.

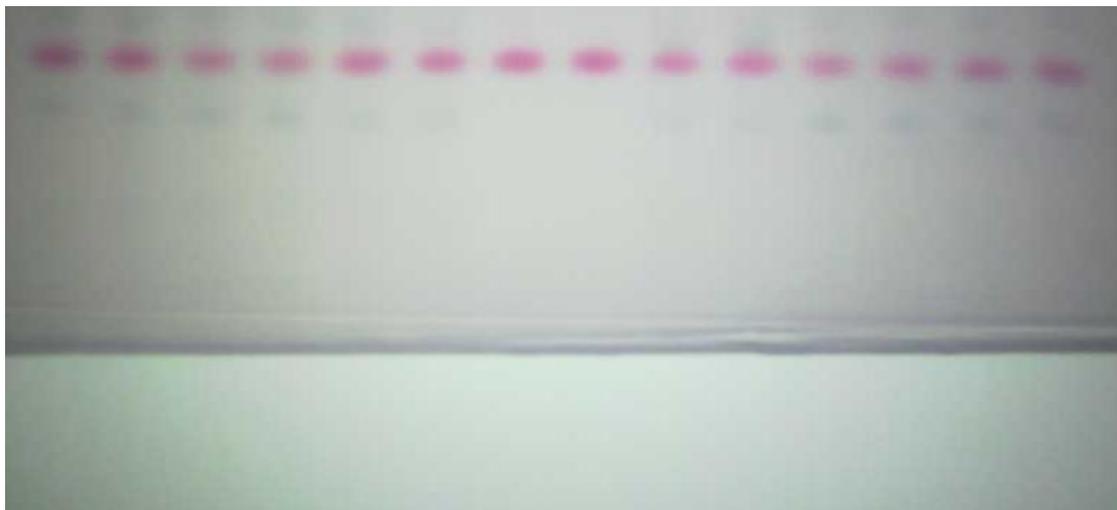


Figure 4. TLC image commercial analysis for artemisinin determination.

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