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THE COMPARISON OF GC-MS DATA OF LEAVES OF ALOE VERA (*A. BARBADENSIS* MILL) PLANT GROWN IN DIFFERENT SOIL COMBINATIONS WITH CHEMICAL LAB WASTES

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Abstract

Purpose of the study: The main purpose of this study is to do the comparison of the chemical constituents present in the leaves of Aloe Vera (*A. barbadensis* Mill) plant grown in different soil combinations using Gas Chromatography- Mass Spectrometry (GC-MS) data analysis. Plants were grown in two different combinations of soil, one in normal soil and the other in the combination of normal soil and solid chemical waste obtained from the chemistry laboratory of an educational institute.

Methodology: One Aloe Vera (*A. barbadensis* Mill) plant was grown in normal soil without adding any compost or any fertilizer to the soil. The other plant was grown in soil, which was combined with the solid chemical waste collected from the chemistry laboratory. After observing the morphological characters of the plants, plants were studied for the chemical constituents present in them by using GC-MS data analysis technique.

Main Findings: Growth of plants depends on the soil composition, physical characters and the surrounding environment. How the variation in chemical composition of soil affects the chemical constituents of plant leaves, has been discussed in this paper. According to morphological characters the Aloe Vera (*A. barbadensis* Mill) plant grown with soil combined with solid chemical waste has shown better results compared with the one grown in normal soil. GC-MS results also indicated variation in the chemical constitution of plant leaves taken for the research experiment.

Applications of this study: This study has helped to understand that the soil environment and soil nutrients are largely responsible for the changes in chemical constituents of plants. This study can be applied to the other plants as well.

Novelty/Originality of this study: In place of fertilizers, solid chemical waste from the laboratory was used for the research purpose. The method is useful and if implemented on a large scale, will help to curb pollution caused by educational institutes to some extent. This kind of research is not done previously by any other researcher.

Keywords: *A. Barbadensis Mill, GC-MS, Soil Combination, Chemical Constituents, Chemical Wastes.*

INTRODUCTION

Aloe Vera (*A. barbadensis* Mill) is a succulent, xerophytic plant, can withstand harsh environments for its survival with lots of therapeutic and medicinal properties. Aloe Vera gel provides soothing and cooling effects on burns, rashes and helps in healing wounds and scars. The leaves of the plants contain many bioactive compounds such as carbohydrates, proteins, lipids, and 18 essential amino acids, vitamins (E.g., A, C, E, vitamin B12, folic acid), minerals, glycoprotein, C-glucosyl chromone, anthraquinones etc. (Chowdhary et al. (2018). Secondary metabolites such as alkaloids, aloins, Glucomannan, lectins, lignin, phenolic compounds, saponins, and tannins are also found to be present in the Aloe Vera plant. (Chowdhury et al. (2018), Kar et al. (2018)

Aloe Vera plant possesses various medicinal and biological properties such as antiviral, antibacterial, antimicrobial, antifungal, anti-inflammatory, anti-cancer, antiulcer, antidiabetic, antioxidant, analgesic, antiproliferative, hepatoprotective, neuroprotective, radioprotective and wound healing properties (Kar et al. (2018). Non-bitter Aloe Vera is also cultivated in some parts of the country, that can be used for vegetable and health drink purpose (Chowdhury et al.(2018), Kar et al. (2018). Aloe Vera possesses anti-aging properties and used in many herbal cosmetic and cosmetic products Kar et al. (2018). Research shows that same plant, grown in different soil, or different geographical region, changes in climatic conditions, pH, temperature, etc. causes changes in the morphology of the plants, changes in physical and chemical properties of soil, as well as chemical properties of plants by making some changes in the structure of the molecules or increasing or decreasing the percentage of the chemical components of essential oils or in different parts of the plants Farouk et al. (2016) (Salinas et al. (2016), Chowdhury et al. (2018).

The purpose of the study is to compare the GC-MS data analysis of the same plants grown in two different soil environments. Plant growth varies according to the soil environment, fertilizers and other biotic and abiotic factors. For this research, chemical constituents of plants grown in laboratory waste mixed with soil were compared with the chemical constituents of the plants grown in the controlled one. Educational institutions' laboratory waste is harming the environment as an added pollutant. This research can be utilized for decreasing the environmental pollution. This will open the new aspects of research as well as help to reduce the harmful chemical pollution caused by a number of chemical laboratories existing in the cities.

Chowdhury (2018), showed how Aloe Vera plants grown in different soil types of Bangladesh with reference to growth and yield of the plants. Acidic, calcareous, non-calcareous, Charland, saline1, saline2, peat soil and acid sulfate soil were used for Aloe Vera cultivation. The plants were observed for their Physico-chemical properties such as colour, texture, bulk density, particle density, field capacity, pH, electrical conductivity, etc. The best results for the plants were shown by the one grown in calcareous soil than acidic soil followed by the rest of the soil taken for the research. (Chowdhury et al. (2018)

Kar (2018) had discussed the multifunctional properties of Phytochemical constituents of Aloe Vera. Almost 200 bioactive chemical constituents are present in the Aloe Vera plant, according to different research done previously. Anti-inflammatory, antiseptic, antidiabetic, antibacterial, antiviral, antimicrobial and other medicinal and use of Aloe Vera in cosmetics and other properties are discussed in this paper (Kar et al. (2018).

In 2018, Nejatzadeh conducted the experiment on Aloe Vera plants, by treating them with four different levels of nitrogen fertilizer and four different levels of vermicompost. The better results were shown by plants grown with higher concentration of nitrogen fertilizer and vermicompost considering the plant height and number of leaves and other Physico-chemical factors. Combining nitrogen fertilizer and vermicompost can also be used for the study that was not used in this experiment. (Nejatzadeh (2018)

Quipse, in 2018 compared chemical composition and antioxidant activity of four different plant parts of Aloe Vera i.e. peel, flowers, gel, and roots using UHPLC-Q/Orbitrap/MS/MS method for analysis grown in Tarapacá, Chile. 25 Phenolic components were identified from the extracts of different parts after analysis. Aloe Vera gel has always been given so much importance that other parts of the plants are overlooked. This study showed that peels of the Aloe Vera plant exhibited highest antioxidant activity. (Quipse et al. (2018)

In 2017, the research was carried out on three different cultivars of Basil grown in Armenia by Avetisyan to compare the chemical composition and biological activities. The oil of *O. basilicum var. purpureum* showed the highest % of methyl-chavicol (estragole), *O. basilicum var. thyrsiflora* showed the highest % of linalool and *O. citriodorum* showed the highest % of nerol and citral. The biological activities exhibited by the plants were also found to be different from each other. Highest antioxidant activity and highest tyrosinase inhibition level were exhibited by *O. basilicum var. thyrsiflora* and highest antimicrobial activity was exhibited by *O. citriodorum.* (Avetisyan et al. (2017)

Kumar (2017), has collected the Aloe Vera plant extract from different states of India for the research purpose. The difference in phytochemicals and antioxidant properties have been observed due to difference in the climatic conditions of different states. Phytochemicals were analyzed by FTIR spectroscopy, showing that cold climate is responsible for a higher percentage of phytochemicals in Aloe Vera. Aloe Vera grown in the Northern part of India had shown higher antioxidant properties compared with Aloe Vera grown in southern India. (Kumar et al. (2017)

Aziz (2016), conducted experiments on *Catharanthus roseus* to compare the elemental composition present in leaves and flowers of the plants. For quantitative elemental analysis, an atomic absorption spectrophotometer was used. Total 13 elements were analyzed for the experiment and concluded that maximum elements were present in leaves with higher concentration in comparison with flowers elemental analysis. Results revealed that different parts of the plants also show variation in elemental composition and other chemical constituents. (Aziz et al. (2016)

Farouk (2016) compared the antioxidant activity and chemical composition of essential oils of *Ocimum basilicum* L. grown in Egypt and Al Madinah Al Munawara, Saudi Arabia. The result showed the presence of 56 components in Egyptian chemotype and 65 components in Madinah chemotype after GC-MS analysis. Methyl chavicol and linalool were found with maximum percentage in Egyptian chemotype and Eugenol and linalool were found with maximum percentage in Madinah chemotype. Essential oils of Egyptian chemotype of *Ocimum basilicum L.* have shown higher antioxidant activity compared to Madinah chemotype and synthetic antioxidant, TBHQ. (Farouk et al. (2016)

Salinas (2016), studied the Fructans structural modification on the Aloe Vera plant due to water deficiency. Different irrigation regimes of 100%, 75%, 50% and 25% field capacity (FC) were used for the plants. On GC-MS analysis, plants irrigated with 50% and 25% FC have shown changes in the structure of Fructans. This showed that changes in any factors for the plant's growth, leads to change in their morphological, chemical and structural characteristics. (Salinas et al. (2016)

Hashemi (2015), has discussed the healing properties of Aloe Vera plant used for treating cutaneous wounds. Soothing and cooling properties of Aloe Vera are well known and used commonly by people as an instant home remedy. (Hashemi et al. (2015)

In 2015, Aloe Vera cultivated in Chile was studied by Mun˜ oz for extraction, properties and characterization of Aloe Vera gel, in fresh and freeze-dried form. Comparison between the two were done by taking pH, acidity, colour, antioxidant property, moisture content, swelling and other factors into consideration. Fresh and freeze-dried Aloe Vera gel had shown different results in comparison. (Munoz et al. (2015)

Souguir (2015), did the 14-month research on the Aloe Vera plant cultivated in normal water and two different concentrations of saline water. Change in growth of plants, hydrogen peroxide content, lipid peroxidation and phenolic

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compounds of the Aloe Vera plants were compared at the time of harvest. The results showed the increase in hydrogen peroxide content and phenolic compounds and lipid peroxidation. (Souguir et al. (2015)

METHODOLOGY

Collection and Identification of plant Material

Fresh leaves of *A. barbadensis* Mill were collected in June 2019 from the plants being used for the research purpose. The leaves were washed thoroughly with running tap water, followed by distilled water and submitted for GC-MS analysis at Ross Life Science Private Limited, Pune, Maharashtra.

Chemicals and Apparatus

Distilled water, methanol, ethanol, acetone, n-hexane, ethyl acetate, pastel and mortar, conical flask, spatula, measuring cylinder.

Preparation of Ethyl acetate extract

Fresh leaves of *A. barbadensis* Mill were crushed into pulp with the help of pastel and mortar. The 200 g was transferred into a conical flask and dissolved in 500 ml of ethyl acetate. The conical flask was kept aside for the night at room temperature and filtered. The filtrate was collected in a clean flask and kept for drying using rotavapor to obtain the ethyl acetate extract of the leaves of *A. barbadensis* Mill. The extract obtained was used for further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

The purpose of using GC-MS was to find out different chemical constituents present in the same plant grown in two different soil media. For GC-MS analysis the Shimadzu model of GC-MS was used. Column temperature was kept 80^0C and injection temperature was 120^0C at a pressure of 81.1 kPa. The column was run for approximately 20 minutes.

Data Analysis and Identification of Compounds

Compounds were identified from the NIST14 library for qualitative and quantitative analysis of compounds and displayed with the help of Chromatogram percentage and tables for two *A. barbadensis* Mill plants.

RESULT AND DISCUSSION

GC-MS analysis of leaves of Aloe Vera or *A. barbadensis* Mill shows the presence of fatty acids, fatty acid derivatives, hydrocarbons, alcohols, ester derivatives, and Dienes. 7 chemical compounds are identified from A2 plant (a plant grown with soil combined with solid chemical waste) figure 1. With highest percentage of Oleoyl chloride (RT = 21.772 & 22.344, Peak Area = 30.17 & 11.63%, respectively, MF = $C_{18}H_{33}ClO$), (Z)-Decyl icos-9-enoate (RT = 21.911 & 22.221, Peak Area = 9.86 & 8.3%, respectively, MF = $C_{30}H_{58}O_2$), E-8-Methyl-9-tetradecen-1-ol acetate (RT = 22.519, Peak Area = 25.59%, MF = $C_{17}H_{32}O_2$) and Hexacontane (RT = 23.074, Peak Area = 10.96%, MF = $C_{60}H_{122}$), low percentage of 2-(2-Hydroxyethoxy)ethyl acetate (RT = 4.762, Peak Area = 0.84%, MF = $C_6H_{12}O_4$), and traces of Tetradecane (RT = 8.674, Peak Area = 0.21%, MF = $CH_3(CH_2)_{12}CH_3$), and Phytol (RT = 14.013, Peak Area = 0.29%, MF = $C_{20}H_{40}O$) are identified from GC-MS analysis. Oleoyl chloride, (Z)-Decyl icos-9-enoate, E-8-Methyl-9-tetradecen-1-ol acetate and Hexacontane, Phytol and Tetradecane are not present in A7 plants.

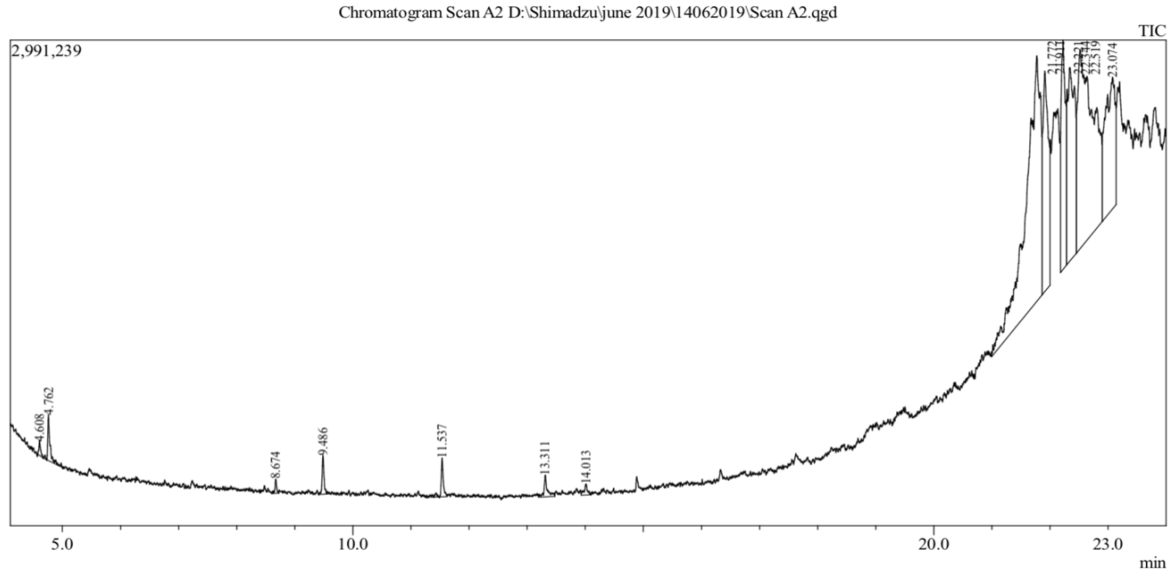

Figure 1: A2 Plant grown in soil combined with Solid Chemical Waste

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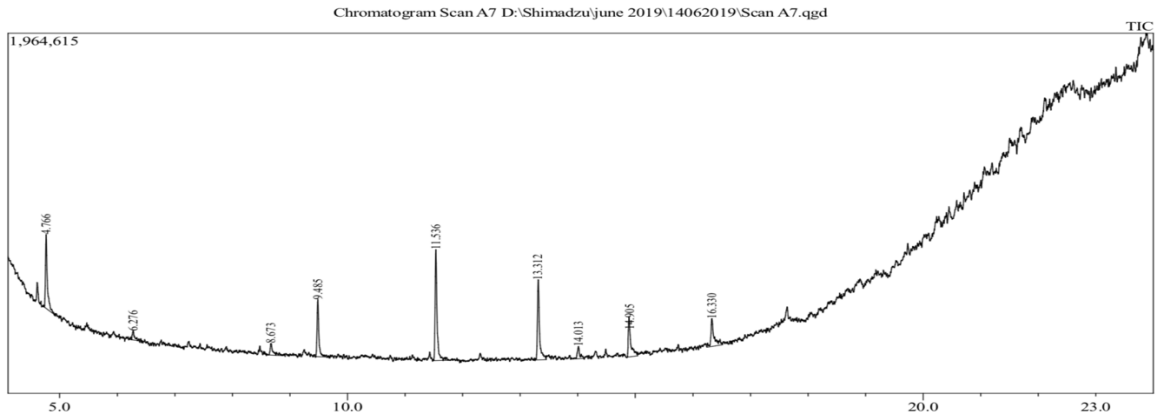

Figure 2: A7 Plant grown in Normal Soil

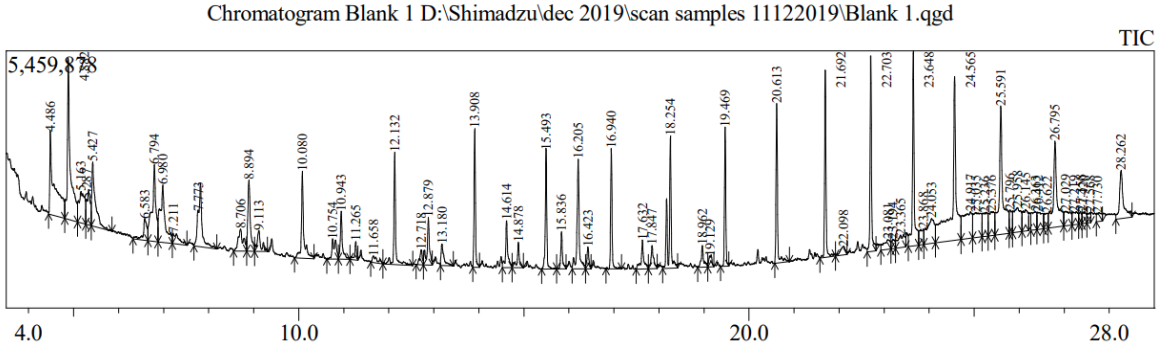

Figure 3: Blank GC-MS of Ethyl Acetate (For comparison of impurities or traces of solvent)

4 compounds are identified from A7 plant (plant grown with normal soil) figure 2. Maximum percentage of 2-(2-Hydroxyethoxy)ethyl acetate (RT = 4.766, Peak Area = 14.9%, MF = $C_6H_{12}O_4$) (less in A2), and low percentage of Ethanol, 1-[2-[2-(1-methylethoxy)ethoxy]ethoxy]- (RT = 6.276, Peak Area = 2.66%, MF = $CH_3(CH_2)_{12}CH_3$), Heptadecane, 2,6,10,150-tetramethyl- (RT = 8.673, Peak Area = 3.95%, MF = $C_{21}H_{44}$), Neophytadiene (RT = 14.013, Peak Area = 3.42%, MF = $C_{20}H_{38}$) are identified from GC-MS analysis of the A7 plant. Neophytadiene, Heptadecane, 2,6,10,150-tetramethyl-, Ethanol, 1-[2-[2-(1-methylethoxy)ethoxy]ethoxy]-, are absent in A2 plant. 2-(2-Hydroxyethoxy)ethyl acetate present in A7 is more than its presence in A2 plant. Different cyclosiloxanes peaks are present in figure 1 & 2, on observing the Figure 3 (Blank Ethyl acetate), these different cyclosiloxanes are from the ethyl acetate impurity.

The observed difference is probably due to the presence of different nutrients in the soil, environmental, chemical and other factors. Nutrients present in soil react differently inside the plant changing their chemical composition and growth of plants. The difference of chemical constituents present in leaves of *A. barbadensis* Mill A2 and A7 plants is clearly seen in Figure 1 & 2.

Research done by Avetisyan (2017), showed that two different varieties of *O. basilicum* var. purpureum and *O. basilicum* var. thyrsiflora had a different composition of chemical constituents in their essential oils (Avetisyan et al. (2017). In 2016, experiments conducted by Aziz showed that elemental composition variation in the leaves and roots of *Catharanthus roseus* plant grown in Bangladesh. This shows that different parts of the same plant also have different composition of chemical components after their chemical analysis (Aziz et al. (2016). Biswas (2015) conducted the experiment on the Aloe Vera plant, using Arsenic (As) as a stress factor in the form of disodium hydrogen arsenate [Na2HAsO4.7H2O] in different concentrations in soil. Stress caused by Arsenic (As), showed shoot biomass and growth reduction in plants at some concentrations compared to others. There was difference seen in trichome structure and GC-MS analysis showed variation in chemical composition of essential oils. Linalool percentage increased in the plants treated by higher concentration of Arsenic (As), whereas 1,8-cineol and methyl eugenol percentage decreased and camphor was absent in all Arsenic (As) treatment. Methyl cinnamate was found to be present at lower concentrations of Arsenic (As) treatment but absent at higher concentration (Biswas et al. (2015). Aloe Vera had shown differences in growth of plants grown in different types of soil in Bangladesh as research conducted by Chowdhury (2018). Morphological differences in growth and changes in Physico-chemical characteristics of plants were also seen in the experiment and acidic and calcareous soil found to be better than other soil for Aloe Vera growth in that region (Chowdhury et al. (2018). Farouk (2016), Showed in the experiment of the GC-MS analysis that of *Ocimum sanctum* Linn, grown in Saudi Arabia and Egypt also has variation in their chemical composition (Farouk et al. (2016).

From all the research conducted by researchers mentioned above, it shows that all plants vary in their chemical composition. Same plants grown with different soil types, grown in different climatic region shows variation in their morphological characteristics and changes in chemical components. Different plants grown in the same soil changes the Physico-chemical properties of the soil as well.

CONCLUSION

This research concludes that the same plants grown in different soil having different nutritive value and different chemical composition, affect the chemical composition of plants. In A2 plant Oleoyl chloride is present in maximum amount along with E-8-Methyl-9-tetradecen-1-ol acetate, (Z)-Decyl icos-9-enoate and Hexacontane whereas in A7 plant 2-(2-Hydroxyethoxy)ethyl acetate is present in maximum amount which is clearly seen from the given Chromatograms and tables. As we all know that *A. barbadensis* Mill is an herbal medicinal plant and used for many therapeutic purposes. By changing soil composition, essential oil composition or chemical constituents, phytochemicals of plants can be changed and used in a more useful way.

LIMITATIONS AND STUDY FORWARD

The present research was conducted to find out the impact of laboratory waste on chemical constituents of Aloe Vera plants grown in two different soils. This can be done with other plants as well, different composition of soil and waste can be prepared and different solvents can be used for GC-MS analysis. Working with many plants at the same time is tedious and time consuming. All plants cannot be grown in the soil combined with laboratory chemical waste.

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