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Comparative phytochemical evaluation of two leafy vegetables (*Sauropous androgynous and Cnidoscolus aconitifolius*), using chromatography and spectroscopy

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Abstract

This research aimed to investigate the variances in phytochemical composition between two leafy vegetables, *Sauropous androgynous* and *Cnidoscolus aconitifolius*. The study employed Thin Layer Chromatography (TLC) to analyze the phytochemical constituents, revealing distinct band patterns indicative of diverse secondary metabolites. Through comparison of Retention Factor (Rf) values, it was determined that these plants possess differing chemical compositions, with common bands suggesting shared secondary metabolites. Furthermore, the research explored the polyphenol content of both vegetables, with *Cnidoscolus aconitifolius* emerging as the richer source. Polyphenols, known for their antioxidant properties, play a crucial role in mitigating oxidative stress and have been linked to various health benefits. Thus, the findings suggest that *Cnidoscolus aconitifolius* may offer greater health advantages compared to *Sauropous androgynous* due to its higher polyphenol content. This study underscores the importance of phytochemical analysis in understanding the nutritional and medicinal properties of leafy vegetables.

1. Introduction

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fibber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories (Banerjee et al., 2012). Leaf vegetables came from very wide variety of plants and they are plants with edible leaves. Each of us knows lettuce and spinach, as well as mustard, but also early springtime nettles are valuable source of vitamin C. Green leafy vegetables are popularly used for food, being a rich source of β-carotene, ascorbic acid, minerals and dietary fiber (Chang et al., 2013).

Green leafy vegetables are major components of a healthy diet, and their sufficient daily consumption could help prevent major diseases. These vegetables may help to meet daily requirements of these and other essential nutrients, especially in individuals with marginal nutritional

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status (Brouwer-Brolsma et al., 2020). Vegetables can form the cheapest and most readily available sources of important fibers, vitamins, minerals, essential amino acids and substances that help protect you from disease particularly. The type and composition of nutritional components vary among genera and species of different edible leafy vegetables plants (Natesh et al., 2017). The low caloric value of leafy vegetables makes them ideal for weight management and these are a rich source of nutrients, high in dietary fiber, low in lipids, and rich in foliate, vitamin-C (ascorbic acid), vitamin-K (phylloquinone), magnesium, and potassium. Mineral nutrients like iron and calcium are high in leafy vegetables than staple food grains. Also, leafy vegetables are the only natural sources of folic acid, which are considerably high in leaves of spinach, asparagus, lettuce, mustard green, colocasia green leaf and turnip green plants as compared to other leafy and nonleafy vegetables (Kumar et al., 2020). Numerous reports regarding the use of leaf vegetable products for the treatment of many human diseases have been made. Proven medicinal properties include antioxidant, anti-inflammatory, antiallergic, antibacterial, and antiviral effects (Suryawan & Lazarosony, 2021, Koundinya et al., 2019). Most of these properties can be attributed to its high content in bioactive compounds. Bioactive compounds are secondary metabolites of plants, which are extra nutritional constituents that typically occur in small quantities in foods. They are being intensively studied in order to evaluate their effects on health. These compounds may elicit a long range of different effects in man and animals eating the plants dependent on plant species and amount eaten. Plants with potent bioactive compounds are often characterized as both poisonous and medicinal, and their beneficial or adverse result may depend on the amount eaten and the context of intake. For typical food and feed plants with bioactive compounds with less pronounced effects, the intakes are usually regarded as beneficial (Barba et al., 2014, Khoo et al., 2011).

Euphorbiaceae, the spurge family, is a large family of flowering plants with 300 genera and around 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. Some are succulent and resemble cacti. This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America. A large variety occurs in tropical Africa, but they are not as abundant or varied as in these two other tropical regions (Davis et al., 2007). Some species of Euphorbiaceae have economic significance, such as cassava (*Manihot esculenta*), castor oil plant (*Ricinus communis*), Barbados nut (*Jatropha curcas*), and the Pará rubber tree (*Hevea brasiliensis*). Many are grown as ornamental plants, such as poinsettia (*Euphorbia pulcherrima*) or garden croton (*Codiaeum variegatum*). Leafy spurge (*Euphorbia esula*) and Chinese tallow (Triadicasebifera) are invasive weeds in North America (Davis et al., 2007). Wild edible plants have been apart of human life since time immemorial. They played an important role in the livelihood of the rural communities in many developing countries. In India, most of the rural communities depend on wild resources including wild edible plants to meet their food requirements in period of food crisis as well as for additional food supplements. India has a tribal population of 42 million, of which 60 percent live in forest areas and depend on forests for various edible products (Puri & Kumar, 2018). Euphorbiaceae is one such family which contributed more on this aspect. *Sauropous androgynous* and *Cnidoscolus aconitifolius* are two common leafy vegetables (Perron & Brumaghim, 2009).

Knowledge on different phytochemical presence and its content in vegetables is important for an appropriate choice of products according to the physiological needs. Phytochemicals are a large group of plant derived compounds, the plant`s way of protecting itself. In addition they appear to have significant physiological effects in the human body. There are more than thousand known phytochemicals. They are acting as antioxidants, stimulating enzymes, interfering with DNA replication, destroying bacteria, as well as they seem to act to reduce the onset of diseases such as cancer and heart diseases (Krishnaswamy& Raghuramulu, 1998). Understanding the various phytochemicals present and how much of them are present in vegetables can help you choose items that will meet your demands physiologically. So the present study is focussed on the identification of the variation of phytoconstituents in *Sauropous androgynous* and Cnidoscolus aconitifolius using thin layer chromatography (TLC) and identification of the best leafy vegetable in terms of polyphenolic content.

2. Materials and Methods

2.1 Collection, identification and drying of Plant Materials

The plants used for the present investigation is Sauropous androgynous and Cnidoscolus aconitifolius. Leaf of the plants were collected in fresh condition from natural habitats of Kozhikode district, Kerala and was identified and authenticated at Department of Botany, St. Joseph's College, Devagiri. The voucher specimens of the same is deposited at DEV herbarium for future reference and studies.

The plant material was washed thoroughly with water. The plant material was cut into small pieces and were shade dried until the chopped parts became dried for grinding. After drying, the plant materials were grinded using mechanical blender into fine powder and transferred into airtight containers at ambient temperature with proper labelling for future use.

2.2 Preparation of extracts

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The extracts of the leaf plants were prepared using Methanol. The extraction was done using Soxhlet. Dried plant material was weighed into 5gm and used for the extraction. 150 mL of methanol was taken in the

Round Bottom (RB) flask. The Setup at boiling temperature was kept for about three hours.The extracts thus obtained were filtered and concentrated to 30 ml in water bath and used for further study.

2.3 Thin Layer Chromatography (TLC) studies

Complex extracts of plant constituents often require very effective separation techniques to allow the identification of different compounds. The TLC separation of plant extracts is described as a method of analysis in different pharmacopoeias. It can provide a chromatographic fingerprint of a plant extract, which is very useful for identification purposes.

2.4 Stationary phase: Aluminium backed pre-coated Merck silica gel plate 60 F254 plate (20×10 cm).

2.5 Solvent system: Mobile phase standardized using suitable solvents in different combination and the following one was fixed as the solvent system.

Toluene: Ethyl acetate: Methanol= 7: 3: 1, and few drops of Acetic acid.

2.6 Procedure: Samples were applied on the plate using a capillary tube. Applied test solutions on a pre-coated Silica gel 60 F254 TLC plate (Merck India) of uniform thickness of 0.2 mm plate in the form of bands with width 8 mm. Develop the plate using the solvent system in a twin trough chamber to a distance of 9 cm.

2.7 Visualization: Visualize the plate at UV 254, 366 nm and in visible light (550 nm).

2.8 Phenolic Content Estimation

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity**.** phenolics content were determined by using the Folin-Ciocalteu assay. An aliquot (1 ml) of extracts were taken in two different test tubes containing 9 ml of distilled water. A reagent blank using distilled water was prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 ml of 7% Na 2 CO 3 solution was added to the mixture. The volume was then made up to 25 mL. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with a Colorimeter. Beer–Lambert law was used to interpret the result.

3. Results and Discussion

3.1TLC Profiling

TLC profiles were developed for the two plant materials with solvent methanol. For the separation of components in the plant materials Toluene: Ethyl acetate: Methanol= 7: 3: 1, and few drops of acetic acid was used. Disparities were observed in terms of number of bands and band intensity of the TLC profiles developed for of Sauropous androgynous and Cnidoscolus aconitifolius. Comparison of Rf values observed at different wavelength of light were given in the **Table 1,2 & 3** and the plate observed was also given **Figure 1.**

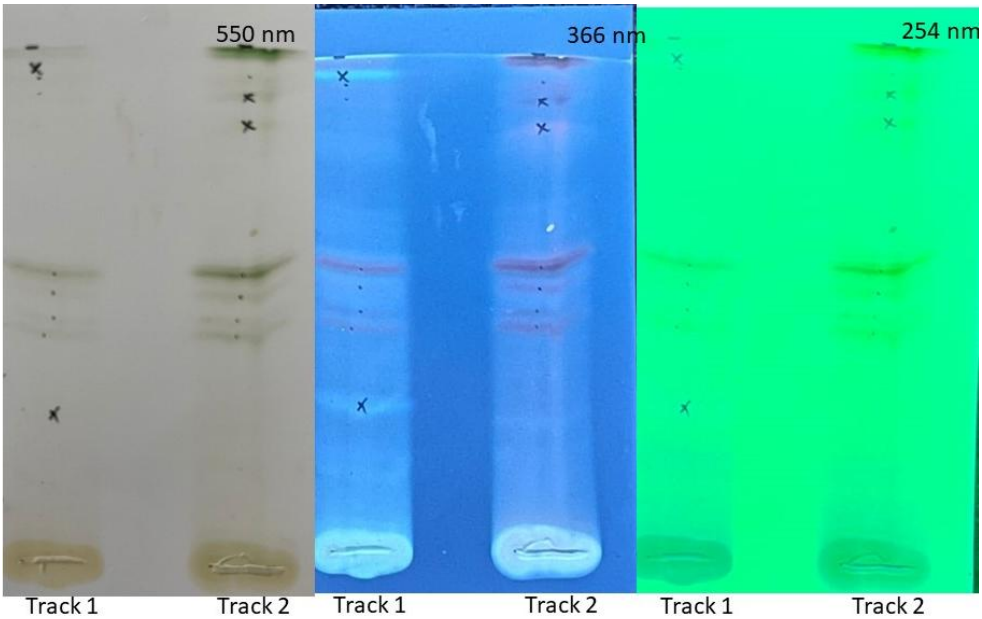

Fig. 1: Visualization of TLC plates at three different wavelengths. Track 1- *Cnidoscolus aconitifolius* and Track 2- *Sauropous androgynous*

Table 1: Comparison of Rf values at 550 nm

Rf values	*S. androgynous*	*C. aconitifolius*
.46	_	+
.47	+	
.48	_	+
.50	+	_
.53	_	+
.55	+	_
.58	+	+
.92	_	+
.97	+	_

+ indicates the presence of band, _ indicates the absence of band

Table 2: Comparison of Rf values at 366 nm

Rf values	*S. androgynous*	*C. aconitifolius*
.30	_	+
.46	_	+
.47	+	_
.48	_	+
.50	+	_
.53	_	+
.55	+	
.58	+	+
.87	+	_

.92	_	+
.93	+	_
.97	+	_

+ indicates the presence of band, _ indicates the absence of band

Table 3: Comparison of Rf values at 254 nm

Rf values	***S. androgynous***	***C. aconitifolius***
.46	_	+
.47	+	_
.48	_	+
.50	+	_
.53	_	+
.55	+	_
.58	+	+
.92	_	+
.97	+	_

+ indicates the presence of band, _ indicates the absence of band

3.2 Phenolic content

The Beer-Lambert law states that: for a given material sample path length and concentration of the sample are directly proportional to the absorbance of the light. Hence considering the absorbance **(Table 4, Fig. 2)** of two different samples such as, *Sauropous androgynous* and *Cnidoscolus aconitifolius,* the later one shows more absorbance. As a result the phenolic content in the *Cnidoscolus aconitifolius* is more compared to other.

Table 4: Absorbance of samples *S. androgynous* and *C. aconitifolius* measured using colorimeter at 550 nm

Sample	**Absorbance at 550 nm**
S. androgynous	1.20
C. aconitifolius	2.36

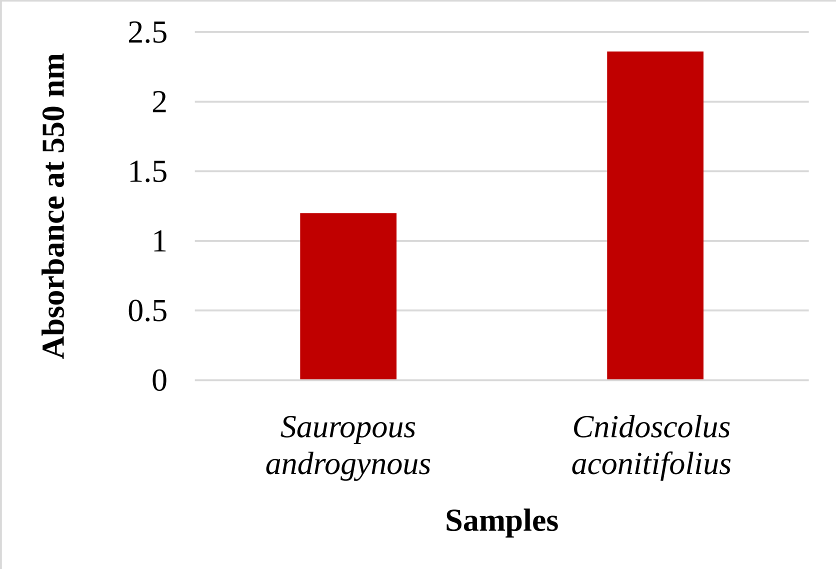

Fig. 2: Absorbance of samples *S. androgynous* and *C. aconitifolius* measured using colorimeter at 550 nm.

4. Conclusion

The present work attempts to determine the differential expression of phytochemicals on commonly used leafy vegetables such as *Sauropous androgynous* and *Cnidoscolus aconitifolius.* The result of the current work proved that the phytochemical constituents vary in two different plants even though it is from the same family. The TLC plates of the two species show a difference in their band pattern. Each band is a representation of each phytochemical. The bands on a TLC plate can vary in size, and they can be used to identify specific compounds based on their Rf values. Compounds with similar Rf values may have similar chemical structures or properties. By comparing the Rf values of two different vegetables, we can identify if they contain similar or different compounds and if any specific compounds are present in one vegetable but not the other. So, the difference in the Rf values explains that the compounds or secondary metabolites of two plants are different. The bands common in both plants represent the common secondary metabolites present in them.

Polyphenols have been associated with several potential health benefits, including reduced risk of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders. By comparing the polyphenol content of two different vegetables, we can gain a better understanding of their potential health benefits. Obtained results showed that the richest source of Polyphenol is *C. aconitifolius*. Polyphenols are potent antioxidants, that complement and add to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress caused by excess reactive oxygen species. Hence, we can say that *Cnidoscolusaconitifolius* is more beneficial for health than *Sauropous androgynous*.

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