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Quantitative evaluation of plant metabolites and dye extraction from leaves of *Ampelocissus latifolia* (Roxb.) planch

Deep Chhavi Anand, Preeti Mishra and Vidya Patni

Abstract
The focus on environmental concerns is increasingly causing textile industries to look for natural dyes as compared to synthetic dyes. The plant *Ampelocissus latifolia* (Roxb.) Planch. belonging to the family Vitaceae, is an example of ethnomedicinal plant species which is the source of dye and a range of traditional medicines that cure various diseases. The aim of the current study was quantitatively assess primary metabolites and secondary metabolites in in vivo and in vitro extracts and extraction of natural dyes from leaves of *Ampelocissus latifolia.* Primary metabolites like total soluble carbohydrates, proteins, ascorbic acid and secondary metabolites such as flavonoids and total phenols were estimated and it was found that leaves contain maximum amount of phytochemicals. Extraction procedure for natural dye was done by extracting dyes from fixed quantity of dried and crushed leaves. The dyed materials were evaluated by observing the shades of colour taken by the cloth fabrics.

Keywords: *Ampelocissus latifolia*, extraction, ethnomedicinal, mordant, dye, textile

Introduction
Plants has been a source of medicine all over the world for thousands of years. Traditional herbal medicine is based on the promise that plants contain natural substances that can promote health and alleviate illness [1, 2]. Use of plants as a source of medicine has been practiced from ancient times and in India, it is an important component of the health care system. Herbal medicines are currently in demand and their uses and popularity are increasing day by day.

Plants synthesise thousands of chemical compounds for performing various functions including defence against insects, fungi, diseases, and herbivorous animals. A huge number of phytochemicals with potential biological activity have been identified in plants. These phytochemicals can be derived from barks, leaves, flowers, roots, fruits, seeds [3]. Knowledge about the chemical constituents of plants is necessary because such information will be valuable for synthesis of complex chemical substances. The chemicals present in plants are grouped into two main categories, namely primary metabolites which include common sugars, proteins, chlorophyll etc., and secondary metabolites consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc. [4, 5]. Majority of phytochemicals like flavonoids, phenols, have been known for their valuable therapeutic activities such as insecticidal, antimicrobial, antioxidant activities [6, 7] etc. Thus, quantitative analysis is very essential for identifying the compounds present in the medicinal plants and they find their medicinal value due to respective phytochemical constituents present in them.

Ampelocissus latifolia (Roxb.) Planch. belonging to the family Vitaceae, is an example of ethnomedicinal plant species which is a source of dye and a range of traditional medicines that cure various diseases [8]. The common name of the plant is "Wild grape". There are more than 500 dye yielding plants [9] and among them *Ampelocissus latifolia* (Roxb.) Planch. is used widely for dyeing as natural green dye is obtained by boiling its leaves [10].

Since antient times natural or plant based dyes were used to color fabrics, ropes and foodstuffs. Natural dyes are colorants derived from plant sources like roots, fruits, bark, leaves, etc. More consumers have become concerned about the health and environmental impact of synthetic dyes as they can impose toxic and allergic reactions and now there is a growing demand for products made up of natural dyes as so many plants yield them.

Natural dyes are considered eco-friendly and biodegradable, on the other side, synthetic dyes are supposed to release hazardous chemicals that are allergic, carcinogenic and toxic to human health. Thus, the aim of the study is to extract dye from leaves of Ampelocissus latifolia (Roxb.) Planch. for textile dyeing and quantitative estimation of primary and seconday metabolites.

Materials and Methods

Survey and collection of plant

The plant was collected from Baghdhara Nature Park, Udaipur, Rajasthan. The plant was identified and verified from the Herbarium, Department of Botany, University of Rajasthan, Jaipur.

Leaves of *Ampelocissus latifolia*

Preparation of plant extract

The leaves were washed thoroughly with water to remove dirt. They were dried in shade and grinded into powder with the help of a grinder. The dried and fresh, both materials were taken for further investigation.

Quantitative determination of primary metabolites

Primary metabolites directly involved in growth and development and are of prime importance and essentially required for growth of plants. Primary metabolites include total carbohydrates, proteins, chlorophyll, ascorbic acid, etc.

1. Determination of total soluble carbohydrates

Phenol Sulphuric acid method was used to estimate the total carbohydrate present in plant material. 1 ml of leaf sample was mixed with 1 ml of 5% phenol and then 5 ml of 96% sulphuric acid. Incubated in water bath (30^0C) for about 20 minutes after which the absorbance was read at 490 nm against a blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

2. Determination of proteins

Protein content was determined according to the method of Lowry *et al*. [11]. 1 ml of leaf sample was mixed with 0.5 ml of 0.1 N Sodium hydroxide and 5 ml of alkaline copper reagent, then, incubated at room temperature for 30 minutes. Added 0.5 ml of Folin–Ciocalteau reagent and incubated again for 10 minutes at room temperature. Absorbance was read at 660 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

3. Determination of Ascorbic acid

Ascorbic acid was determined using the protocol described by Chinoy [12]. 1 ml of leaf sample was mixed with 2 ml of 5% Meta-phosphoric acid and kept for 30 mins at room temperature, then 5ml of n-amyl alcohol and 3.2ml of 2,4-dichlorophenol indophenol(5mg/100ml) were added. Shaken vigorously and upper layer was taken for estimation of ascorbic acid at wavelength 546nm.

Quantitative determination of secondary metabolites

Like primary metabolites, secondary metabolites are not involved directly and they have been found to work as biocatalysts. They are produced by the plants under stressed conditions. Some important secondary metabolites in plants are flavonoids, steroids, alkaloids, phenols, etc.

1. Determination of total phenols

Total Phenolic Content was determined by using Folin-Ciocalteu method [13] with Catechol as standard. 1 ml of leaf sample was diluted and 1ml of Folin-Ciocalteu reagent was added. After 3 min, 2ml of 20% sodium carbonate was added and then the contents were mixed thoroughly. The total volume was made upto 20 ml. The colour was developed and absorbance measured at 650nm. The concentrations of phenol was calculated from the calibration plot and expressed as mg catechol equivalent/g of sample. The analysis was performed in triplicates and the results were expressed as mg/g sample.

2. Determination of total flavonoids

The aluminium chloride method was used for flavonoids determination [14]. Aliquots of extract solutions (0.5 ml) were taken and made up to volume 2ml with methanol. Then 0.1ml AlCl3 (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken and allowed to stand for 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin (0.1mg to 1.0mg/ml). The concentrations of flavonoid was calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample. The analysis was performed in triplicates and the results were expressed as mg/g sample.

Statistical analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation.

Dye extraction

The leaves were washed thoroughly with water to remove dirt. They were dried under direct sunlight and ground into powder with the help of a grinder. The dried material was strained using a fine strainer, and finally, weight was taken.

The color component was extracted from the leaves in aqueous extraction process [15]. Extraction was carried out with fixed quantity of crushed leaves (10 gram) with a liquor ratio of 1:10 (Weight of crushed leaves in gram; amount of water in millilitre) at 98 °C for 60 min to optimize extraction medium. In the process of extraction, the mixture was cooled down and finally the dye extracts obtained through above

mentioned method was filtered and used for dyeing different types of cloth and yarns like cotton, silk, wool etc.

Dyeing procedure

The extract obtained through aqueous extraction was cooled, filtered and then used for dyeing. Cloth fabric used for dyeing was boiled in sodium hydroxide solution (10%) for 15 minutes to remove starch from the cloth, then washed thoroughly with cold water. The cloth was then treated with mordant (Alum) for 30 minutes and dye bath for one hour. The shades of dye were also observed without using mordant. The cloth was finally treated with tepol to fix the colour and then dried in sunlight. After aqueous extraction of dye different types of cloth ($5cm^2$) and yarns (5cm) were experimented for dyeing like cotton, silk, wool etc. (Figure 2) Different shades of colour were observed in different textile fabrics after dyeing. (Table 2) Dyeing was carried out with the optimized dye extract on mordanted and unmordanted cloth fabrics.

Results and Discussion

Phytochemical evaluation

The present study revealed the quanitative estimation of phytochemicals in *Ampelocissus latifolia*. The preliminary phytochemical studies of *Ampelocissus latifolia* showed the presence of carbohydrates, glycosides, tannins, alkaloids, saponins, flavonoids, steroids, phenols, proteins, hexose sugars, mucilages & gums [16]. Estimation of plant metabolites is required to ensure the therapeutic efficacy to be utilised for further higher biochemical studies. The plant *Ampelocissus latifolia* showed good amount of primary and secondary metabolites like total carbohydrates, protein, ascorbic acid, total phenol and flavonoid content. (Table 1)

The current study provides the estimated amounts of the primary metabolites (total soluble carbohydrates and total proteins) and secondary metabolites (total flavonoids and total phenols) present in the plant extracts. Further the quantitative phytochemical screening may aid in the detection of the bioactive elements that can be further evaluated. Carbohydrates and Proteins are one of the main components of living things. Plant sugars can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding [17]. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [18]. Phenols and Flavonoids are plant secondary metabolites, and they have an important role as defence compounds. They have been reported to exert multiple biological properties including antimicrobial, cytotoxicity, anti-inflammatory, antibacterial, antiviral, antiallergic [19-21], antitumor and cytotoxic, gastroprotective, treatment of neurodegenerative diseases, vasodilatory action [22, 23]. Thus, quantitative evaluation of the plant metabolites may be useful in the analysis of the compounds present in the plant to primarily access therapeutic properties present in them.

Table 1: Quantitative analysis of primary and secondary metabolites (mg/gm.) in *in vivo* and *in vitro* plant parts of *Ampelocissus latifolia*.

Metabolites	Leaves	Stem	Callus
Total Carbohydrates	22.32±0.13	13.04 ±0.02	3.63 ±0.07
Total Proteins	14.92 ±0.02	8.84 ±0.17	4.12 ±0.32
Ascorbic acid	1.55 ±0.28	1.14 ±0.18	0.57 ±0.06
Total Phenols	19.26 ±0.23	6.08 ± 0.14	4.21 ±0.22
Total Flavonoids	18.63 ±0.01	10.83 ±0.21	5.65 ±0.08

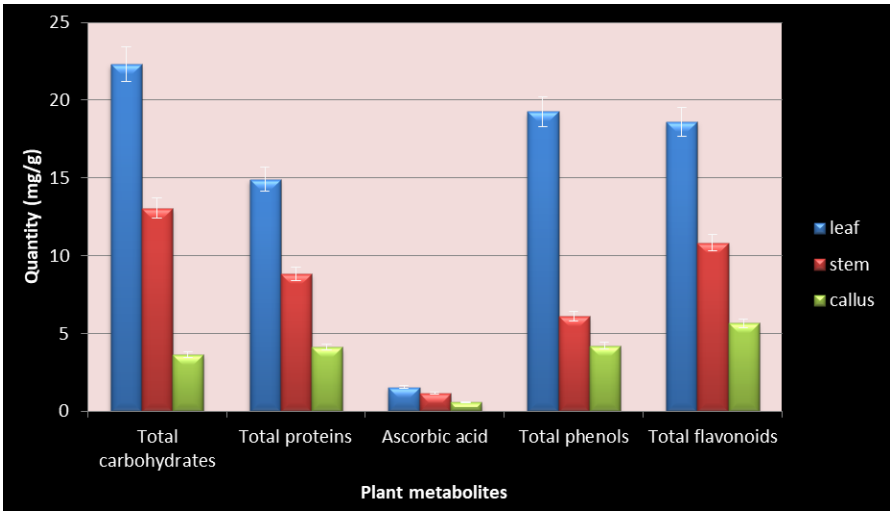

Fig 1: Quantitative estimation of primary and secondary metabolites in *Ampelocissus latifolia*

Natural dye extraction

A bright yellowish-green dye was obtained from the leaves of *Ampelocissus latifolia* with the above method of extraction. Different shades of dyed fabrics using dye obtained from leaves of plant were obtained. Effects of mordant and dye colour and effect of dye without mordant is presented in Table 2. Cotton fabric showed slight greenish-brown colouration, and silk fabric showed light brown colouration, while wool yarn and cotton yarn showed honey mustard and bright gold colouration respectively (figure 2). The application of natural dyes in textile industry is for various purposes, viz. dyeing of fabrics, ropes, food stuffs, block printing, where the textile materials are printed with the help of printing blocks; Kalamkari where the "Kalam" or pen is used to draw beautiful designs on the cloth [24]. Natural dyes are in demand not only in textile industry but also in pharmaceuticals, leather, food and cosmetics industries. We can get different shades of colour using different mordants and the colour fastness, wash fastness properties also can be improved by different treatment procedures [25]. The literature reveals the chemical composition of the different parts of *Ampelocissus latifolia*, but no report exists so far on the extraction of natural dyes from this plant species and their applications. Natural dyes have poor to moderate wash and light fastness as

compared to synthetic dyes having moderate to excellent colour fastness properties. So, an extensive work has to be carried out to improve the light fastness properties of different natural dyed textiles [26].

Table 2: Different shades of dyed fabrics using dye obtained from leaves of plant.

S. No	Fabric	Shade without mordant	Shade with mordant
1.	Cotton pure	Straw	Tinsel
2.	Silk	New wheat	Cocoon
3.	Wool yarn	Yellowish brown	Honey mustard
4.	Cotton yarn	Light brown	Bright gold

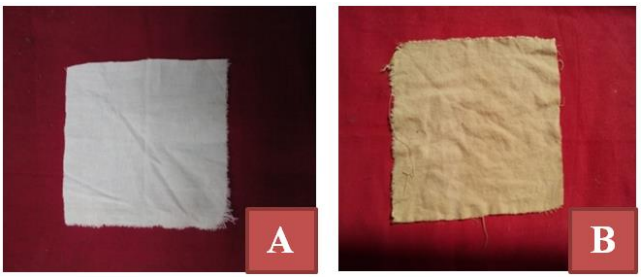

Fig A and B: Untreated and dyed cotton cloth. (Tinsel color)

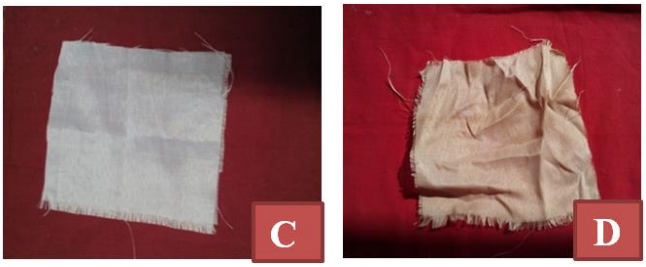

Fig C and D: Untreated and dyed silk cloth. (Cocoon color)

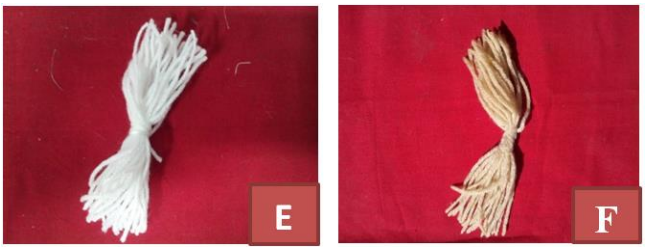

Fig E and F: Untreated and dyed wool yarn. (Honey mustard color)

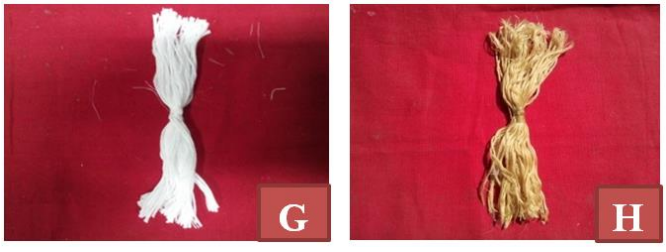

Fig G and H: Untreated and dyed cotton yarn. (Bright gold color)

Fig 2: Application of dye obtained from leaves on different textile fabrics

Conclusion

Quantitative evaluation of different phytometabolites confirmed the medicinal value of Ampelocissus latifolia in ethnomedicine. Presence of high amount of secondary metabolites like phenols and flavonoids reflects the therapeutic efficacy of this plant species to be used in phyto-pharmaceutical industries. It is the first report on Dye extraction from leaves of *Ampelocissus latifolia* and its application on different textile fabrics. This study was aimed in search of better and natural alternative to satisfy the consumer's growing demand of eco-friendly and biodegradable products, and progress has been made with this study in the use of *Ampelocissus latifolia* leaves extract. It was observed that different fashion hues were obtained on cloth fabrics from the same dye extract with and without using mordant.

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