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Physicochemical and phytochemical screening in *Lantana camara* leaves

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Abstract
Phytochemicals are a field of increasing attention, both in science and in commerce. As is now generally recognized, many plant compounds and pigments have effects on animals and human beings. There is a great effort now to study and understand at a fundamental level and significant health effects of these compounds. In the present study to investigate the physicochemical analysis and phytochemical screening in *Lantana camara* leaves. The results of the present study concluded that *Lantana camara* leaves may be a good source of phytochemicals, vitamins and minerals. Supplementation of this *Lantana camara* leaves may be useful for human health associated emerging diseases such as cardiovascular diseases, diabetes, hypertension and cancer.

Keywords: Phytochemicals, *Lantana camara*, physicochemical analysis and phytochemical screening

Introduction
Phytochemicals are a field of increasing attention, both in science and in commerce. As is now generally recognized, many plant compounds and pigments have effects on animals and human beings. There is a great effort now to study and understand at a fundamental level and significant health effects of these compounds. This field is maturing and the health effects of these compounds are now getting the careful study they warrant at both a chemical and a molecular biological level. Identifying bioactive compounds and establishing their health effects are active areas of scientific inquiry. There are exciting prospects that select bioactive compounds will reduce the risk of many diseases, including chronic diseases such as cardiovascular disease. Recent findings have established that cardiovascular disease is a disease of inflammation, and consequently is amenable to intervention via molecules that have anti-inflammatory effects Prabhu *et al.* (2011) [21]. In the present study to investigate the physicochemical analysis and phytochemical screening in *Lantana camara* leaves.

Material and methods
Plant materials
The *Lantana camara* leaves were collected in January 2015 from Alangottai, Mannargudi taluka, Tamil Nadu from a single herb. The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (EB001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of extracts
The collected *Lantana camara* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted with methanol for 24 hours. A semi-solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

Determination of Physicochemical parameters
Physicochemical parameters of plant powdered such as ash value, extractive value, loss on drying and crude fiber content were performed according to the method described in WHO guidelines WHO (1998) [28].

Preliminary phytochemicals screening
Chemical tests were carried out on the alcoholic and aqueous extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Harborne (1973) [7], Trease and Evans (1989) [26] and Sofowora (1993) [24].

Quantitative analysis of phytochemicals

Determination of total Phenols Edeoga *et al.* (2005) [4]. Tannin was determined by the method of Van-Burden and Robinson (1981) [27]. Saponin was determined by the method of Obdoni and Ochuko (2001) [17]. Alkaloid determination using Harborne (1973) [7] method. Flavonoid was determined by the method of Bohm and Kocipai-Abyazan (1994) [3]. Qualitative analysis of Vitamins carried out by Pearson (1976) [19].

Qualitative analysis of inorganic elements

Ash of drug material (500 mg) was prepared and treated with HNO3 and HCl (3:1 v/v) for 1hour. After the filtration, the filtrate was used to perform the following tests Khandelwal (2006) [10].

Histochemical and Fluorescence analysis

Histochemical analysis carried out by the method of John Peter Paul (2014) [10]. and Gersbach *et al*. (2001) [5]. Fluorescence analysis carried out by the method of Gupta *et al.* (2006) [6], Kokashi *et al.* (1958) [11].

Results and discussion

Physicochemical analysis of *Lantana camara*

In the physicochemical analysis, the parameters studied are moisture content, loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractive values value, etc. Ash value content can be used to be determining the quality and purity of crude drug. It indicates the presence of various impurities such as carbonate, oxalate, and silicate. Water soluble ash is used to estimate the amount of inorganic compound present in drugs while the acid insoluble ash includes mainly silica and indicate contamination with the earthy material. Minimal moisture content of drugs is crucial to discourage the growth of bacteria, yeast, or fungi during storage. The amount of the active constituents in a given amount of plant material when extracted with a particular solvent is determined by the estimation of extractive values. A solution containing different phytoconstituents is obtained by the extractions of any crude drug with a particular solvent. Whether the crude drug is exhausted or not is indicated by the compositions of these phytoconstituents which depend on the nature of the drug and the solvent used Tatiya *et al*. (2012) [25]. The results of physicochemical parameters such as description, loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol soluble extractive and water soluble extractive are shown in Table 1.

Table 1: Proximate analysis of *Lantana camara*

S. No	Tests	As per analysis
1	Description	Pale green coloured fine powder
2	Loss on drying at 105 ºC	1.50%
3	Total ash	6.30%
4	Acid insoluble ash	1.84%
5	Water soluble ash	0.84%
6	Sulphated ash	3.12%
7	Alcohol soluble extractive	9.24%
8	Water soluble extractive	13.25%

Phytochemical analysis of leaves extract of *Lantana camara*

Qualitative analysis

Phytochemicals are responsible for medicinal activity of plants Kumar *et al.* (2009) [12] these are non-nutritive chemicals that have protected human from various diseases. The major constituent consists of alkaloids, flavonoids, saponins, phenolic compounds, phytosterols, proteins and amino acids, gums and mucilage and lignin. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries the constituents are playing a significant role in the identification of crude drugs. The medicinal value of these plants lies in some chemical substances that produces a definite physiological action on the human body. The most important property of these bioactive constituents of plants is that they are more effective with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The anti-inflammatory, antispasmodic, anti-analgesic and can be attributed to their high steroids, tanning, terpenoids and saponins Mallikaharajuna *et al.* (2007) [14]. The present study indicates that the qualitative phytochemical analysis of methanolic extract of *Lantana camara leaves* contains tannin, saponin, flavonoids, terpenoids, alkaloids, carbohydrate, anthroquinone and polyphenols (Table 2).

Table 2: Qualitative phytochemical analysis in *Lantana camara* leaves extract

S. No	Phytochemicals	Methanol Extract	Aqueous Extract
1	Tannin	+	+
2	Phlobatannins	-	+
3	Saponnin	+	+
4	Flavonoids	+	+
5	Steroids	+	+
6	Terpenoids	+	+
7	Triterpenoids	+	+
8	Alkaloids	+	+
9	Carbohydrate	+	+
10	Protein	-	-
11	Anthroquinone	+	+
12	Polyphenol	+	+
13	Glycoside	+	+

Note: (-) Absence (+) Presence (++) present with high intensity of the colour

Quantitative analysis

Quantitative analysis revealed that the plant has phenols, alkaloids, tannin and saponin. Significant amount of total phenol (324 mg/gm), alkaloids (35 mg/gm), tannin (98.4 mg/gm), saponin (121 mg/gm) and flavonoids (165 mg/gm) ware presented (Table 3). The above phytoconstituents were tested as per the standard methods. This is because of the pharmacological activity of this plant is used to trace the particular compound.

Table 3: Quantitative phytochemical analysis of *Lantana camara* leaves powder

S. No	Phytochemicals	Concentration (mg/gm)
1	Total Phenols	324.00 ± 22.68
2	Alkaloids	35.00 ± 2.45
3	Tannin	98.40 ± 6.88
4	Saponin	121.00 ± 8.47
5	Flavonoids	165.00 ± 11.55

Values are expressed as mean ± SD for triplicates

Reported by Yanishlieva (2001) [29], flavonoids are found to be better antioxidants and have multiple biological activities including vasodilatory, anti-carcinogenic, antiinflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral and Radioprotective effects. Polyphenolic compounds is a highly inclusive term that covers a wide group of phytochemicals, including well known subgroups of phenolic acids,

flavonoids, natural dye, lignins etc., it is produced by plant as a secondary metabolites is represent a potential source with significant amount of antioxidants to prevent oxidative stress caused by free radicals. In the present study, methanol extract of Indigofera trita was reported to possess polyphenoilc compounds exhibits its antioxidant activity by chelating redox- active metal ions, in activating lipid free radical chains and preventing hydroperoxide conversion in to reactive oxyradicals and other biological properties includes diffusion of toxic free radicals, altering signal transduction, activation of transcription factors and genes expression by Raju Senthilkumar *et al.* (2013) [22].

Qualitative analysis of vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams.

Vitamin C, or ascorbic acid, is one vitamin humans cannot make; they have to get it from food. Vitamin C helps hold the cells together, heal wounds, and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons, and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times (Okwu, 2003) [18]. The vitamins of the *Lantana camara* leaves investigated and summarized in Table 4. The vitamin analysis of *Lantana camara* leaves showed that the presence of Vitamin A, C, E and A was absent.

Table 4: Qualitative analysis of vitamins in *Lantana camara* L

Vitamins	Result
A	+
C	+
D	++
E	++

(+) indicates presence; (+++) indicates high concentrations

Qualitative analysis of inorganic elements in *Lantana camara* leaves

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities.

The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water Indrayan *et al.* (2005) [28]. Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects New Wall *et al.* (1996) [16]. The elements of the *Lantana camara* leaves investigated and summarized in Table-5. The following elements were found in *Lantana camara* leaves. They are calcium, magnesium, sodium, potassium, sulphate, phosphate, chloride while iron was present.

Table 5: Qualitative analysis of elements in *Lantana camara* leaves

S. No	Elements	Result
1.	Calcium	+
2.	Magnesium	+
3.	Sodium	+
4.	Potassium	+
5.	Iron	-
6.	Sulphate	+
7.	Phosphate	+
8.	Chloride	+
9.	Nitrate	+

Note: (-) Absence (+) Presence

Fluorescence behavior of *Lantana camara* leaves powder

Fluorescence is the phenomenon demonstrated by various chemical constituents existing in the plant material. When exposed to ultraviolet (UV) radiation or in the range of visible light, some constituents display fluorescence Kumar *et al*. (2013) [13]. Identification of the powdered drug, extract or fractions of herbs can be carried out by utilizing the property of the organic molecules to absorb light over a specific range of wavelength and re-emit radiations Rashida *et al*. (2012) [23]. Many physiochemical fluorescence are seen when suitably illuminated. The colour of fluorescence is specific for each compound Pimenta *et al*. (2006) [20]. Some crude drugs are often assessed qualitatively because non-fluorescent substances may often be converted into fluorescent derivatives after reacting with different reagents and thus this is an important parameter of pharmacognostical evaluation Ansari (2006) [1]. Fluorescence analysis of entire leaves of *Lantana camara* has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *Lantana camara* was carried out by the treatment of different chemical reagents such as Hexane, Chloroform, Methanol, Acetone H2SO4, HCl, HNO3 and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm) and the results were presented in Table 6.

Table 6: Fluorescence behavior of *Lantana camara* leaves powder

S. No	Fluorescence	Day light	UV light	
			Short wavelength (245nm)	Long wavelength (365nm)
1.	Plant powder (pp)	Green	Green	Black
2.	Plant powder with water	Green	Light yellow	Black
3.	Plant powder with Hexane	Green	Green	Black
4.	Plant powder with Chloroform	Green	Light yellow	Black
5.	Plant powder with Methanol	Black	Black	Black
6.	Plant powder with acetone	Dark green	Light yellow	Black
7.	Plant powder with IN Sodium hydroxide in water	Dark green	Green	Black
8.	Plant powder with IN Hydrochloric acid	Light green	Light green	Black
9.	Plant powder with sulphuric acid with equal amount of water	Light green	Green	Black
10.	Plant powder with Nitric acid diluted with equal amount of water	Light green	Light yellow	Black

Histochemical analysis of leaves powder of *Lantana camara*

Histochemistry deals with localization of chemical compounds within the cells by means of specific colors of the compounds. Staining the cells with different stains or dyes, which render the compounds visible under the microscope, makes the specific color reaction compounds. The importance of histochemistry in solving critical biosystematics problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. The biosystematic importance and implications of histochemical features of ergastics, calcium oxalate crystals, nature of tannins and saponins have been investigated in various plants families such as Dioscoreaceae Mbagwu and Edeoga (2006) [15], Leguminosae-papilionoideae Asokan (2006) [2]. Identification of localization of secondary metabolites in plant parts which are using in the preparation of drug is an immense importance to prevent adulteration and also helpful in taxonomic hierarchy. Hence in the present study an attempt has been made to identification of secondary metabolites in the medicinal plants. The powder of *Lantana camara* leaves were treated with specific chemicals and reagents. The *Lantana camara* leaves powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids and treated with dragant draft reagent gave brown colour indicates alkaloids. The treated plant powder further analysed in light microscope (Table 7 and Fig. 1).

Table 7: Histochemical analysis of leaves powder of *Lantana camara*

S. No.	Secondary metabolites	Observation	Results
1	Flavonoids	Yellow	+
2	Alkaloids	Reddish Brown	+
3	Tannin	Dark Blue to Black	+
4	Starch grain	Blue	+
5	Steroids	Violet to Blue (or) Green	+
6	Poly phenol	Blue green/Red	+

(+) Presence; (-) Absence; (++) Highest concentrations

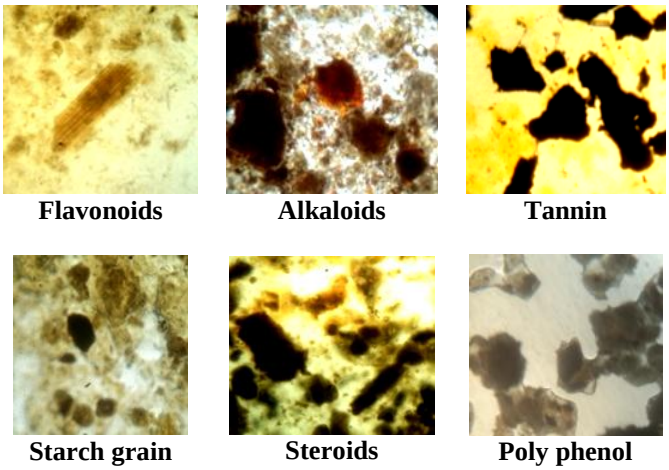

Fig 1: Histochemical analysis in *Lantana camara* leaves powder

Conclusion

The results of the present study concluded that *Lantana camara* leaves may be a good source of phytochemicals, vitamins and minerals. Supplementation of this *Lantana camara* leaves may be useful for human health associated emerging diseases such as cardiovascular diseases, diabetes, hypertension and cancer.

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