PHYTOCHEMICAL STUDY OF AERIAL PARTS OF LANTANA CAMARA FOR THE PHARMACOLOGICAL ACTIVE COMPOUNDS

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Abstract

Natural products continue to play an important role in the discovery and development of new pharmaceuticals, as clinically useful drugs, as starting materials to produce synthetic drugs, or as lead compounds from which a totally synthetic drug can be designed. The genus *Lantana* includes 2500 species worldwide and is known for its bioactive secondary metabolites and essential oils. Several Chemical compounds have been extracted and identified from its specie known as *Lantana camara (L .camara)*. The present study is designed for the extraction of compounds using two solvents of different polarities, so that maximum number of compounds can be extracted. TLC has been used for the isolation and identification of the various compounds. Results of the simple TLC showed the isolation of number of UV visible components from dichloromethane and methanolic extract of aerial parts of *L. camara*. Phytochemical analysis of aerial parts showed that alkaloids and Anthraquinones glycosides were absent but Tannins and triterpenes were found. The most important result of phytochemical analysis was the presence of cardioactive glycosides in the aerial parts of *L. camara*, cardioactive glycosides are first time reported in this specie. Therefore *L. camara* can be exploited for further phytochemical studies in the future for isolation and structural determination of cardioactive compounds.

Keywords: Lantana Camara, anthraquinones glycosides, cardioactive glycosides, phytochemical study

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Introduction

For many centuries, it is a known fact that humankind depends on plants as an indirect source of energy, and shelter. It has been found that near about 80% of all established natural products originate from plants (Phillipson, 1990). These natural products have a significant use in the finding and production of new pharmaceuticals which are then clinically useful. They can be used as primary materials to produce some drugs of synthetic origin or they can be used to make products, which then assist in making fully synthetic drugs (Soejarto and Farnsworth, 1989).

L. camara (Figure 1) belongs to the family *Verbenaceae*. This family consists of more than 100 genera and contains nearly 2600 species. Many of these species are of tropical or subtropical region. *L. camara* is cultivated as decorative plant or for making a boundary in a garden, lawn or field. It is found in Pakistan mainly in Muzaffarabad, Rawalpindi, Karachi, Maleer and some parts of Multan and surroundings. Its various parts are used in the conventional and traditional system of medicine for the cure of several

problems which include itching, ulcer, inflammation, eczema, malaria, tumors and rheumatism (kirtikar and Basu, 1981).

A variety of new compounds like 3,24-dioxo-urs-12-en-28-oic acid (Yadav and Ttripathi, 2003), camaryolic acid, methylcamaralate, pentacyclic triterpenoids, camangeloyl acid and other known compounds such as octadecanoic acid, camaric acid, beta-sitosterol 3-O-beta-D-glucopyranoside, docosanoic acid, palmitic acid, 3(3,19a-dihydroxycersan-28 oic acid), 21,22-epoxy-3β-hydroxyolen-12-en-28-oic acid, lantanolic acid, Lantanone and Lantanoside have been isolated from various parts of *L. camara* (Begum *et al.,* 2000). Verbascoside and Martynoside were also isolated and recognized from *L. Camara* (Syah *et al.,* 1998). Chemical structures of the compounds were detected and studied by various chemical as well as spectroscopic methods and 2D NMR techniques (wahab *et al.,*2003; Begum *et al.,* 2005).

Oleanolic acid was isolated from the roots of *L. camara*. This compound is found to have a hepatoprotective activity and was then converted into 28 → 13ß lactone by the reaction of facile photo-oxidation (Misra and Laatsch, 2000). On the other hand, compounds that cause the inhibition of testosterone-5a reductase were separated from the roots of *L. camara*. Their primary use is in the industry of skin cosmetics and hair preparation (Kanbara and Kishida, 1998).

Flavonoids, triterpenoids and a mixture of stigma sterol, campsterol and 13-sitosterol were also isolated from the stem of pink flowering taxa of *L. camara*. In 1998, flavonoid hispidulin was isolated from the genus lantana (Lai *et al.,* 1998). Production of a variety of 5, 5-*trans* fused lactones, which are very closely related to compounds obtained from the extracts of *L. camara*, has produced a number of new acylating inhibitors of human thrombin, chymotrypsin, trypson and human leucocyte elastase.

Figure 1: Flower of *L. camara*

In this research, we have done the phytochemical assessment of the aerial parts of *L. camara* for the determination of groups which are pharmacologically active.

Materials and method:

Materials:

Rotary Evaporator, Extraction bottle, Dichloromethane (DCM), Methanol, Ultrasonic bath, Dragendorff's reagent, dilute ammonia solution, separating funnel, chloroform, acetic acid, Mayer's reagent, carbontetrachloride (CCl4), Five silica gel 60 F_{254} TLC plates (20x20cm) (Merck),

Collection of plant material

Plant material was collected from the surroundings of Multan. The plant was identified as *L. camara* in the Department of Biological Sciences of Bahauddin Zakariya University, Multan.

Extraction

Aerial parts of plant were taken and kept under shade till drying. The plant material was ground in blender and weighed. Extraction from the aerial parts was carried out by simple maceration process.

Two hundred grams of the grounded aerial parts was taken in extraction bottle and 600 ml of. DCM was added. The mixture was occasionally shaken and homogenized using ultrasonic bath. Then it was left for

24 hours. After 24 hours mixture was filtered and the marc was again macerated by the solvent using the same procedure. After the collection of third DCM extract marc was extracted thrice with methanol by following the above mentioned procedure. Extracts of DCM and methanol were concentrated using the rotary evaporator. DCM and methanol extracts were collected separately in sample bottles and weighed.

Phytochemical analysis

Test for the alkaloids

One to three grams of the grounded plant material was extracted with 10ml of acidified water in test tube and boiled for one minute then filtered. One ml of the filtrate was taken and 3 drops of Dragendorff's reagent were added. The remainder of filtrate was made alkaline by addition of dilute ammonia solution. It was transferred to separating funnel and 5ml of chloroform solution was added to the solution, two layers were observed. The lower chloroform layer was pipetted out into another test tube.

Chloroform layer was extracted with 10ml of acetic acid and then was discarded. Extracts were divided into three portions and to one portion few drops of Dragendorff's reagent, to second few drops of Mayer's reagent were added. Turbidity or precipitate was compared with the third untreated control portion.

Test for anthraquinones glycosides

One gram of powdered plant material was taken and extracted with 10ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl4 then CCl4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red color.

One gram of second sample of the same plant material was extracted with 10ml of ferric chloride solution and 5ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for Cardioactive glycosides

One gram of powdered plant material was taken in a test tube and 10mL of 70% alcohol was added. It was then boiled for 2 minutes and filtered. Filtrate was diluted twice of its volume with water and then 1 ml of strong lead sub-acetate solution was added. This treatment leads to the precipitation of chlorophyll and other pigments, which were then filtered off. Filtrate was extracted with an equal volume of chloroform. Chloroform layer was pipetted out and evaporated to dryness in a dish over a water bath.

Residue was dissolved in 3ml of 3.5% ferric chloride in glacial acetic acid and was transferred to test tube after leaving for 1 minute. 1.5 ml of sulphuric acid was then added, which formed a separate layer at the bottom. Cardioactive glycosides were revealed as no appearance of brown color at interface (due to deoxy sugar) on standing, but appearance of pale green color in the upper layer (due to the steroidal nucleus).

Test for tannins

One to two ml of the plant extract solution was treated with 2-3 drops of ferric chloride solution. Presence of hydrolysable tannins like pyrogallola was indicated on the basis of appearance of bluish black color.

A match stick was dipped in plant extract solution and moistened with hydrochloric acid solution and was brought near a flame. Appearance of pink color is indication of presence of condensed tannins.

Thin layer chromatography

Requirements

Test samples, organic solvents (chloroform, methanol, ethyl acetate, n-hexane and isopropyl alcohol), Spotting capillary, coated TLC plates, TLC tank, oven and UV illuminator.

Spotting and developing the TLC plates

Ten mg of each methanolic and dichloromethane extracts of aerial parts were dissolved in 1ml of methanol and dichloromethane (HPLC grade), respectively. Five silica gel 60 F_{254} TLC plates (20x20cm) were marked at 1cm from each side and cut into smaller plates. 5-10ul of sample was applied by capillary on the line marked. Samples were applied at equal distance for simple TLC and spot was no more than 6mm in diameter.

Visualization of TLC Plates

TLC plates were first visualized in the UV light (254nm and 366nm) and visualized spots were marked for the determination of R_f value. Subsequently sprayed with the Godin's reagent.

Godin reagent (Godin 1954)

This reagent was prepared by mixing equivalent volumes of 1% vanillin in ethanol and 3% perchloric acid in water. TLC plates were sprayed with this mixture and then with 10% sulphuric acid in ethanol. Sprayed TLC plates were then heated at 100C°. Different spots were observed.

Measurement of R_f

Both distances, covered by mobile phase and substances were measured and R_f value was calculated as:

R_f - Distance traveled by the component / distance traveled by the solvent front

Results:

Extraction

Extraction of aerial parts of *L. camara* was done with dichloromethane and methanol; results are shown in the Table 1.

Table 1: Extraction of plant material with different solvents

Plant	Part	Solvent	Extract (Wt)
Lantana camara	Aerial parts (200g	Dichloromethane	10.80 gm
		Methanol	12.70 gm

Phytochemical Analysis

Phytochemical studies were done for the detection of alkaloids, glycosides, tannins in the aerial parts of the plant *L. camara*. The details of the tests employed are given in the following tables.

Table 2: Detection of alkaloids in aerial parts of *L. camara*

Plant Part	Reagent	Observation	Result
Aerial Parts	Dragendorff's reagent	No reddish brown precipitates	__
Aerial Parts	Mayer's Reagent	No Cream Colour precipitates	__

+++ *Strongly positive results*
__ *Negative results*

Chromatographic method.

Dichloromethane Extract

Thin layer chromatographic analysis of the aerial parts of dichloromethane extract of *L. camara* was performed. Stationary phase used was Silica gel 60 F254. The Mobile phase was n-hexane: dichloromethane (1:1) and UV detection of the extracted material was done at 254 nm, 366 nm. Godin's Reagent was used for detection of invisible components of the chromatogram. UV active components 1,

(Rf = 0.123). 2, (Rf = 0.246) and 3, (Rf = 0.323) were observed at 254 nm. and components 4, (Rf = 0.4), 5, (Rf = 0.507) were observed at 366 nm. Some components became visible after spraying the chromatogram with the Godin Reagent i.e, p, bg, g, lp, lg, dg, as shown in the figure 2.

Table 3: Detection of Glycosides in aerial parts of *L. camara*

(Anthraqunone Glycosides)			
Plant Part	**Test Employed**	**Observation**	**Result**
Aerial Parts	Borntrager's Test	No Pink Colour	—
Aerial Parts	Modified Borntrager's Test	No Intense Pink Colour.	—
(Cradioactive Glycoside)			
Aerial Parts	Keller Kelliani Test	Brown color at the interface due to deoxy sugar and pale green color in the upper layer due to steroidal nucleus.	++

+++ *Strongly positive results*
— *Negative results*

Table 4: Detection of Tannins in aerial parts of Lantana camara

Plant Part	Test	Observation	Result
Aerial Parts	Ferric Chloride Test (1 to 2 ml of ferric chloride solution + 1 to 2 ml extract)	Bluish Black colour was produced	+++
Aerial Parts	Catcehin Test (Dip match stick in the plant extract dry and moisten with Hydrogen Chloride solution and warm near flame.	Wood was turned pink	+++

+++ *Strongly positive results*
— *Negative results*

Methanolic extract

Thin layer chromatography analysis of the aerial parts of methanolic extract of *L. camara* was performed. Stationary phase used was Silica gel 60 F254. The Mobile phase was Chloroform, Methanol, Water (1:1:1) and UV detection of the extracted material was done at 254 nm, 366 nm. Godin Reagent was used for detection of invisible components of the chromatogram. UV active components 1, (Rf = 0.178). 2, (Rf = 0.589) and 3, (Rf = 0.798) were observed at 254 nm. and component 4, (Rf = 0.945) were observed at 366 nm. Some components became visible after spraying the chromatogram with the Godin Reagent i.e., b, lg, lg, y, dg, as shown in the figure 3.

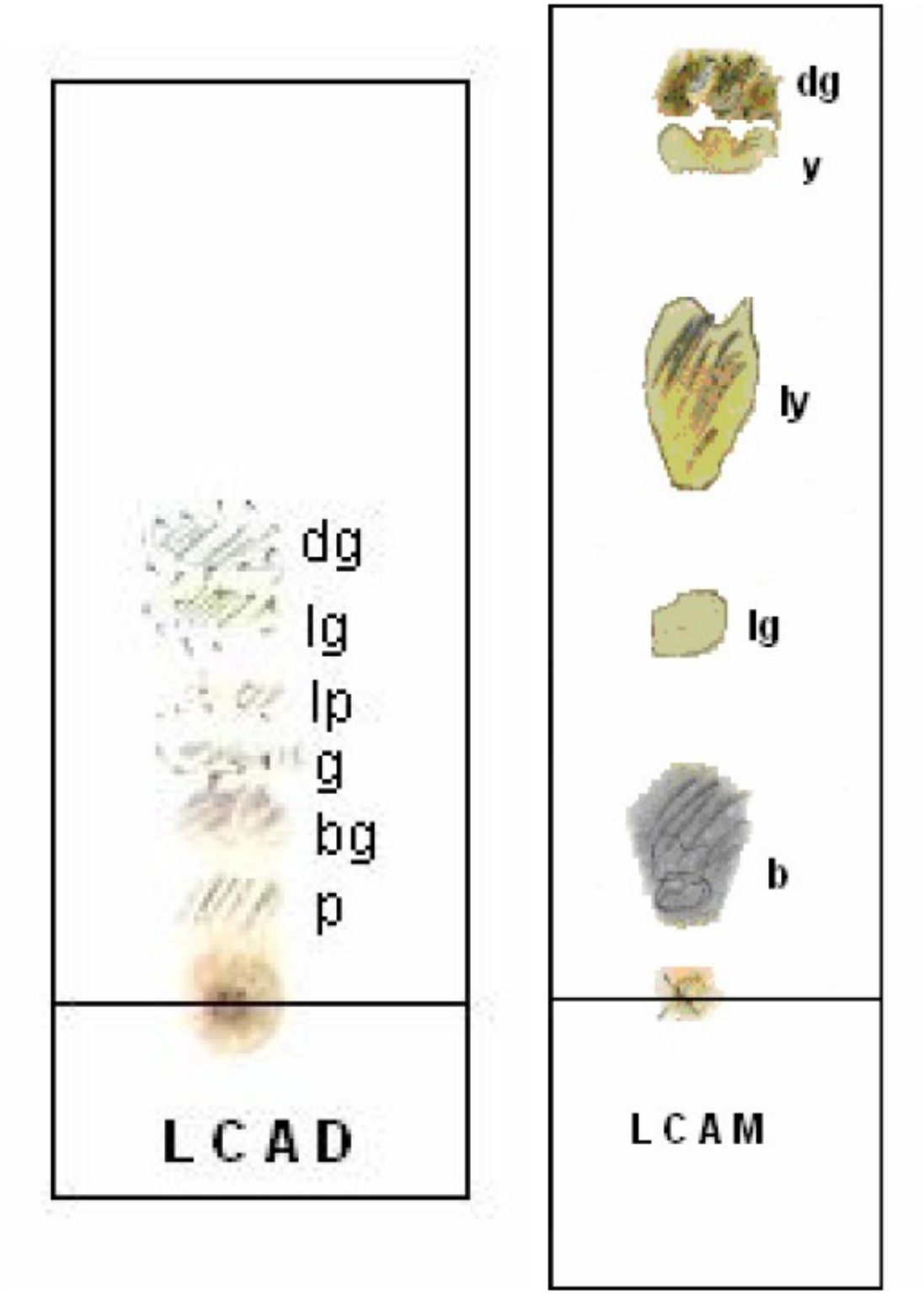

Figure 2: TLC of dichloromethane extract Aerial parts of *L. camara* (LCAD) and TLC of methanolic extract of aerial parts of *Lanatan camara* (LCAM)

Discussion

The bioassay permits convenient and rapid evaluation of various plant parts, ontogenic and seasonal variations within individual plant and highly bioactive genotype within the intra-specific variations. The crude botanical extracts, containing mixture of bioactive compounds are usually effective and these procedures may permit their conventional standardization. The genus *Lantana*, includes 2500 species worldwide reported, is known for its bioactive secondary metabolites and essential oils.

Present study showed that in general methanol is a better solvent for extraction of antibacterial constituents than chloroform from different plant parts. This observation is in agreement with the previous reports. Chandrasekaran and Venkatesalu (2004) proposed that the methanolic extract had higher antibacterial and antifungal activity than that of aqueous extract which may be due to solubility of the different constituents in different solvents having antimicrobial activity. Vlachos *et al.* (1996) also concluded that methanol was the most effective solvent for the extraction of antibacterial compounds from the selected seaweeds.

TLC has been used for the isolation and identification of the various compounds. Results of the simple TLC showed the isolation of number of UV visible components from dichloromethane extract of aerial parts of *L. camara* and methanolic extract of its aerial parts. When the color of spots and their R_f values were compared, it was seen that UV visible compounds present in methanolic extract of aerial parts of *L. camara* were completely different from dichloromethane extract of aerial parts of *L. camara*, because the color in the UV and R_f value are the characteristic of a specific compound..

In present study phytochemical analysis of aeril parts showed that alkaloids were absent. Anthraquinones glycosides were absent. Tannins and triterpenes were found. These compounds are known to have pharmacological activities and therefore are commonly found in medicinal plants. The most important result of phytochemical analysis was the presence of cardioactive glycosides in the aerial parts of *L. camara*, cardioactive glycosides are first time reported in this species. Therefore *L. camara* can be exploited for further phytochemical studies in the future for isolation of cardioactive compounds.

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