

**Research Article****Use of ethanolic extract of *Lantana camara* L. flowers as pH indicator**

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**Abstract**

**Objective:** *Lantana camara* L. is omnipresent notorious weed. Present study was aimed towards evaluation of ethanolic extract of its petals as pH indicator. Material and methods: Powder of dried flower petals of *L. camara* L. was extracted with 0.5% HCl in ethanol. Ethanolic extract was then studied preliminary for phytochemicals present and added in aqueous solutions of different pH to observe change in colour. Results and conclusion: On phytochemical screening, pH sensitive anthocyanins were found to be present. It can be concluded that ethanolic extract of *Lantana camara* L. flowers can be used as pH indicator.

**Keywords:** *Lantana camara* L. , anthocyanins, ethanolic extract, pH indicator

**Introduction**

Anthocyanins are the flavonoid derivatives; widespread in the plant kingdom; imparting most of the brilliant colours like orange, red, pink, purple and blue; observed mostly in fruits, flowers and leaves (Zheng et al., 2011). Some of the naturally occurring anthocyanins of plants are cyanidin, peonidin, malvidin, delphinidin, pelargonidin and petunidin (Liliana et al., 2012). Chemically, the anthocyanin has a flavylum nucleus attached to one or more sugar residues, which may be D-glucose, D-galactose, L-rhamnose, D-xylose, and D-arabinose, are 3-glycosides or 3,5-di-glycosides (Miguel, 2011). Apart from being a natural colorant, anthocyanins have gained considerable research interest due to health benefits shown as a results of their antioxidant properties. Several times, antioxidants activity of anthocyanins have been demonstrated using several models (Miguel, 2011).

*Lantana camara* L. (Figure 1) is an ornamental but notorious weed belong to family Verbenaceae (Patil and Kumbhar, 2017). It bears the flowers of colour ranging from yellow-white or pink to deep red or purple. (Huang et al., 2009) proved the presence of pigments, identified as 3-glucoside, 3,5-diglucoside and 3-malonylglucoside of cyanidin, and 3,5-diglucoside and 3-

malonylglucoside of peonidin in flowers (Huang et al., 2009) (Figure 2).

An acid-base indicator is a compound which changes color according to change in pH. For synthetic acid-base indicators, it has been reported that they may have carcinogenic effect (Dunnick and Hailey, 1996). In addition, some of these synthetic indicators exhibited toxic effects such as pulmonary edema, diarrhea, hypoglycemia and pancreatitis and they can result in abdominal cramps, skin rash, eruptions, erythema, and epidermal necrosis and cause environmental pollution (Pathade et al., 2009; Abugri et al.,



**Figure 1:** *Lantana camara* L. flowers

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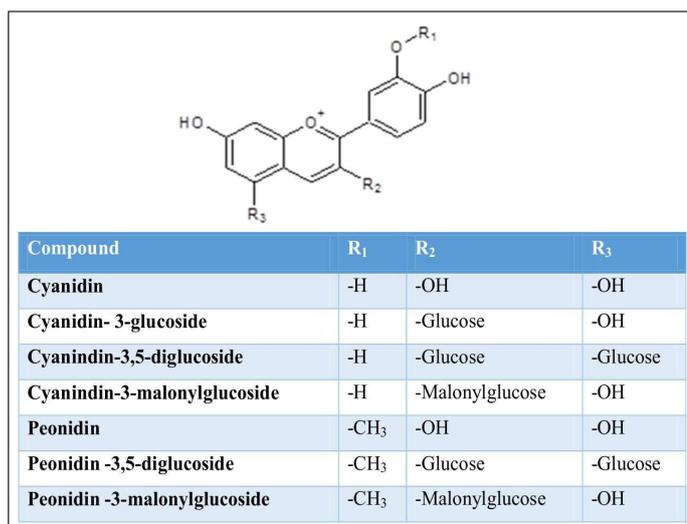


Figure 2. Anthocyanidins present in *L. camara* L. flowers

2012). Considering the wide availability of *Lantana camara* L plant and adverse effects of synthetic pH indicators, present study was aimed towards checking the feasibility of anthocyanin- rich extract of *L. camara* L. flowers to be used as pH indicator in acid –base titrations.

#### Materials and Methods

##### Collection of *L. camara* L. flowers and preparation of anthocyanin-rich extract (ARE)

Flowers of *L. camara* L. were collected from Medicinal Plant Garden of Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune in month of July. Plant was already identified for our previously reported study on same plant (Patil et al., 2017). Flowers were washed with purified water; dried in shade and pulverised to powder. About 50gm of powder was extracted in 200 ml of 0.5% hydrochloric acid (v/v) in 80% ethanol using Soxhlet apparatus for 6 hrs. Extract so collected was the filtered and concentrated on rotary evaporator at temperature less than 35°C. On further drying, around 2gm of dried extract was obtained..

##### Phytochemical evaluation of extract

Physical evaluation of extract included the checking of appearance, colour and odour, while phytochemical analysis included testing for the presence of different secondary metabolites (alkaloids, terpene, tannins and flavonoids). Then UV-Visible spectrum of extract (0.001%) was noted on Shimadzu UV-1700 Pharma Spec UV-visible spectrophotometer. Further, extract was analysed by forestal paper chromatographic technique, where about 10% solution of extract was spotted on aluminium foil pre-coated with silica gel (0.2 mm thick); run through acetic acid based mobile phase, BAW (butanol: acetic acid: water; 4:1:5) and R<sub>f</sub> values of different coloured bands were noted (Horbone, 1998).

##### Evaluation of extract as pH indicator in acidic and basic solutions

A solution of extract (10%) was prepared by dissolving 5 gm in 50 ml of absolute ethanol. Then, performance of extract was tested in solutions with different pH (1, 4, 7, 9 and 14). For solutions of pH 1 and 14; 0.1 N solutions of hydrochloric acid and sodium hydroxide were prepared respectively. For solutions of pH 4 and 9, ready-to-use solutions were used; while for solution of neutral pH, double distilled water was employed. To each of these solutions, about 0.5 ml of 10% solution was added, stirred well and change in colour was noticed. Further, UV spectrum of extract, exhibiting different colours in extreme pH values (pH 1 and 14) were noted for detection of possible change in structure of anthocyanins in the extract.

##### Results and discussion

Extract so prepared from *L. camara* L. flowers, was found to be dark maroon coloured and viscous. Preliminary phytochemical analysis of extract showed the presence of only flavonoids and polyphenols. UV-Visible spectrum (Figure 3) of extract showed two absorption maxima at  $\lambda$  290.5 and 319 nm. This shifting of wavelength towards shorter wavelength may be because of presence of traces of hydrochloric acid placed while making extract of *L. camara* L. flowers. Further, forestal paper chromatographic analysis revealed considerable separation of different colouring pigments. Observations were two different coloured bands at different R<sub>f</sub> values (Brown, 1.0, Pink, 0.33 and Dark pink, 0.20) (Figure. 4). With this phytochemical analysis, it could be concluded that acidified ethanolic extract of *L. camara* L. flowers was rich in anthocyanins. However Horbone quoted malonylated anthocyanidins exhibit higher R<sub>f</sub> values. But in present attempt no spot was observed at higher R<sub>f</sub> value. Hence, reported malonylated anthocyanidins may be present in only in traces (Horbone, 1998).

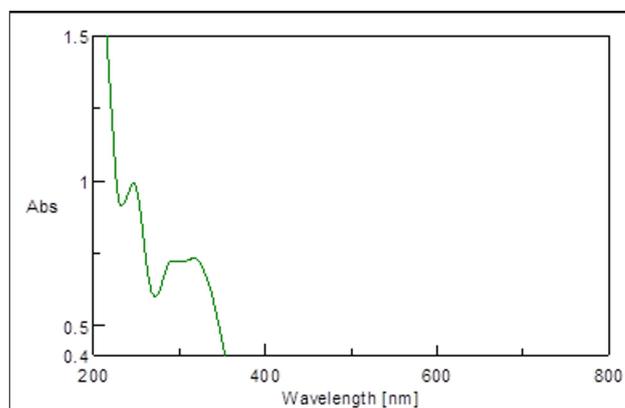


Figure 3. UV-Visible spectrum of extract



**Figure 4.** Paper chromatographic profiling of Ethanolic extract of *L. camara* L. flowers

On addition of about 0.5 ml of 10% solution of extract, colour got changed as per change pH of solution and noticed (Figure 5). In strong acidic solution (pH 1), extract took pinkish colour while in strong basic solution (pH 14) extract showed yellow colouration. At neutral pH, extract was colourless. Previous research carried out on anthocyanins explained the intra-molecular rearrangement of anthocyanin structure that takes place on change in hydrogen ion concentration, pH.

UV-Visible spectrum of extract making solution of pH 1 dark pink and pH 14 yellow coloured were noted and found that, pinkish solution showed absorptions at wavelengths of 511 nm,

328 nm and 288 nm whereas yellowish solution showed at 765 nm and 273 nm;

Deconvolution of these spectra proved the parallel factor (PARAFAC) hypothesis about existing of several equilibrium forms of anthocyanin namely the flavylum cation, carbinol, quinoidal base, and *E*- and *Z*-chalcone and their ionized forms, as well as their relative concentrations as a function of pH (Levi et al., 2004).

Anthocyanins are biosynthesized in flower using aromatic amino acid, phenylalanine as precursor. It is converted to anthocyanins via sequential formation of *trans*-cinnamic acid, coumaryl co-A, chalcone, naringenin, dihydrokaemferol and flavilium ion on enzyme catalysed reactions. It also includes attachment of sugar for formation of glycoside<sup>11</sup>. During life span of plant, flowers arise with anthocyanins reflecting specific colour, which changes with different biochemical processes those take place in flowers. Present research work explains that this change in colour is because of change in pH.

#### Conclusion

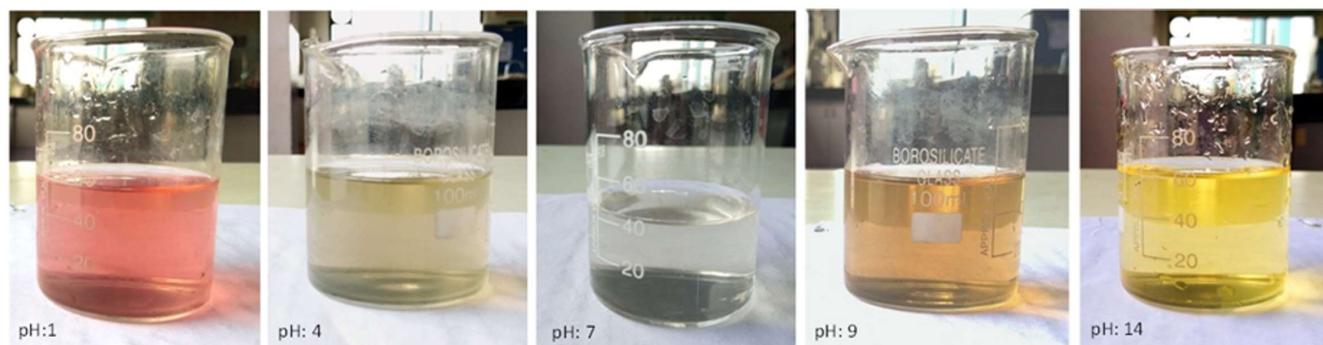
Colouring matter of flower is composed of anthocyanins, generally which are pH sensitive and soluble in ethanol. On phytochemical analysis, it was found that *L. camara* flowers contain anthocyanins, and therefore their ethanolic extract exerted different colours in different pH solutions. Hence, though *L. camara* L. is a notorious weed, ethanolic extract of its flowers can be undertaken for further research for its use as pH indicator in titrations.

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#### Conflicts of interest

We, authors declare no conflict of interest.



**Figure 5.** Colour variations exhibited by extract in solutions of different pH

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## Graphical Abstract

