

**USE OF ETHANOLIC EXTRACT OF ACALYPHAHISPIDA FLOWERS AS PH
INDICATOR**

Nitin Bahir, Vaibhav Bangar, Pratik Kadam* and Shriniwas Patil

Alard College of Pharmacy, Hinjewadi-411057, Pune, India.

***Corresponding Author: Pratik Kadam**

Alard College of Pharmacy, Hinjewadi-411057, Pune, India.

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ABSTRACT

Objective: *Acalypha hispida* 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 *A. hispida* was extracted using 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 *Acalypha hispida* flowers can be used as pH indicator.

KEYWORDS: *Acalypha hispida*, anthocyanins, ethanolic extract, pH indicator.

INTRODUCTION

Anthocyanins are the flavonoid derivations; wide in the factory area; conducting utmost of the brilliant colours like blue, pink, red, grandiloquent, orange; observed substantially in leaves, fruits and flowers (Zheng et al., 2011). Some of the naturally being anthocyanins of shops are cyanidin, peonidin, malvidin, delphinidin, pelargonidin and petunidin (Liliana et al., 2012). Chemically, the anthocyanin has a flavylium nexus attached to one or further sugar remainders, which may be D- glucose, D- galactose, L- rhamnose, D- xylose, and D- arabinose, are 3- glycosides or, 5- di- glycosides (Miguel, 2011). piecemeal from being a natural color, anthocyanins have gained considerable exploration interest due to health benefits shown as a result of their antioxidant parcels. Several times, antioxidants exertion of anthocyanins have been demonstrated using several models (Miguel, 2011).

Acalypha hispida (Fig. 1), commonly known as Red hot cat's tail or Chenilleplant belong to family Euphorbiaceae. It bears the red flowers in raceme inflorescence. Its pendulous red flower tassels that hang down from leaf axils and resemble caterpillars or soft yarn. Individual flowers are very small, mostly just feathery pistils, but are tightly packed along the raceme to form the furry catkin. Different region uses *A. hispida* flower for different purposes. In Indonesia, the flowers, fresh or in decoction, are considered a remedy for haemoptysis. In Malaysia a decoction of the leaves and flowers is externally applied as an emollient to wounds and ulcers. When taken internally, it exhibits laxative action and diuretic in treating gonorrhoea. Phytochemical analysis revealed that red colour of flower is because of

presence of cyanidin 3-O- β -galactopyranoside, cyanidin 3-O- (2''-O-galloyl- β -galactopyranoside), and cyaniding 3-O-(2''-O-gal-loyl-6''-O-a-rhamnopyranosyl- β -galactopyranoside) (Fig.2) (Reiersen et al. 2003).

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



Figure 1: *Acalyphahispida* flowers.

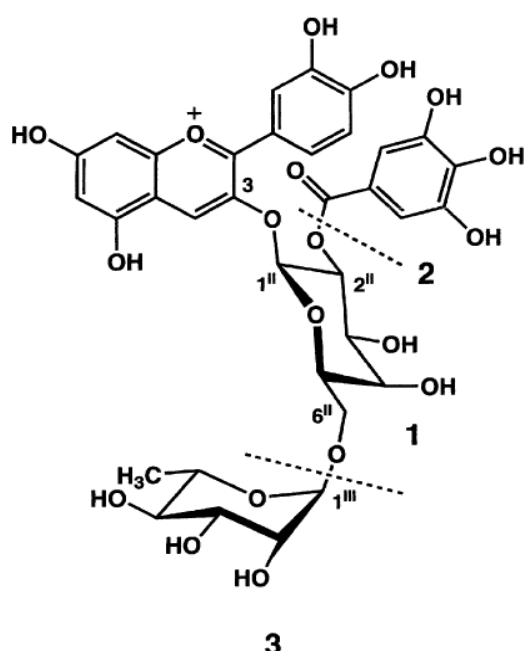


Fig. 2: The structures of the anthocyanins in flower extract of *Acalypha hispida*. 1=cyanidin 3-O-beta-galactopyranoside, 2=cyanidin 3-O-(200-O-galloyl-beta-galactopyranoside), and 3=cyanidin 3-O-(200-O-galloyl-600-O-alpha-rhamnopyranosyl-beta-galactopyranoside) (Reiersen *et al.* 2003).

MATERIALS AND METHODS

Collection of *Acalypha hispida* flowers and preparation of ethanolic extract

Flowers of *A. hispida* were collected from Medicinal Plant Garden of Alard College of Pharmacy, Marunji,

Pune in month of August. Flowers were washed with purified water; dried in shade and pulverized to powder. About 50gm of powder was extracted in 200 ml of ethanol using Soxhlet apparatus for 6 hrs. Extract so collected was 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 Jasco-V630-UV-visible spectrophotometer. Further, extract was analysed by forestal paper and thin layer chromatographic technique, where about 10% solution of extract was spotted on whatmann filter paper and aluminum foil pre-coated with silica gel (0.2 mm thick) separately; run through acetic acid based mobile phase, BAW (butanol: acetic acid: water; 4:1:5) and Rf values of 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, 10 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 10, 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 *A. hispida* flowers, was found to be colourless and viscous. Preliminary phytochemical analysis of extract showed the presence of only flavonoids and polyphenols. UV-Visible spectrum (Fig. 3) of extract showed two absorption maxima at λ 238 and 323 nm.

Further, forestal paper chromatographic analysis revealed considerable separation of different colouring pigments. Observations were single pink bands at different Rf values (0.18 in paper chromatography and 0.56 in thin layer chromatography) (Fig. 4). With this phytochemical analysis, it could be concluded that ethanolic extract of *A. hispida* flowers was rich in anthocyanins. (Horbone, 1998).

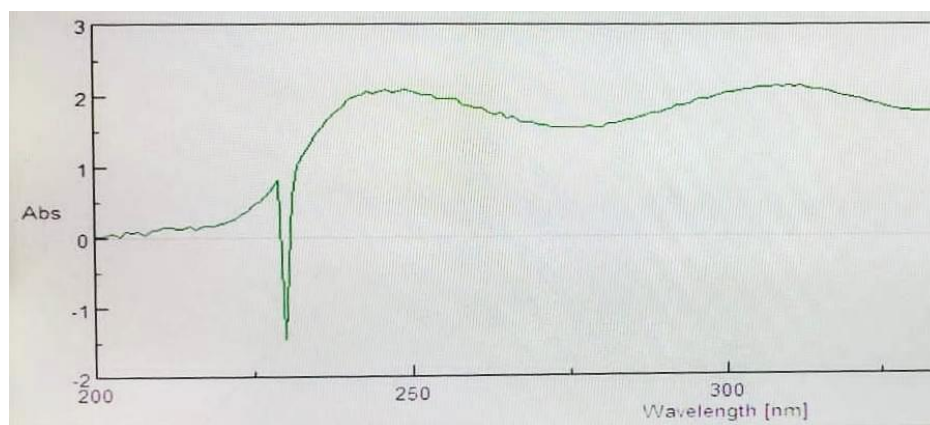


Fig. 3: UV-Visible spectrum of extract.



Fig. 4: Paper chromatographic (A) and thin layer chromatographic (B) profiling of Ethanolic extract of *A. hispida* flowers.

On addition of about 0.5 ml of 10% solution of extract, colour got changed as per change pH of solution and noticed (Fig. 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.

Deconvolution of these spectra proved the parallel factor (PARAFAC) hypothesis about existing of several equilibrium forms of anthocyanin namely the flavylium 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 flaviium ion on enzyme catalysed reactions. It also includes attachment of sugar for formation of glycoside. 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.

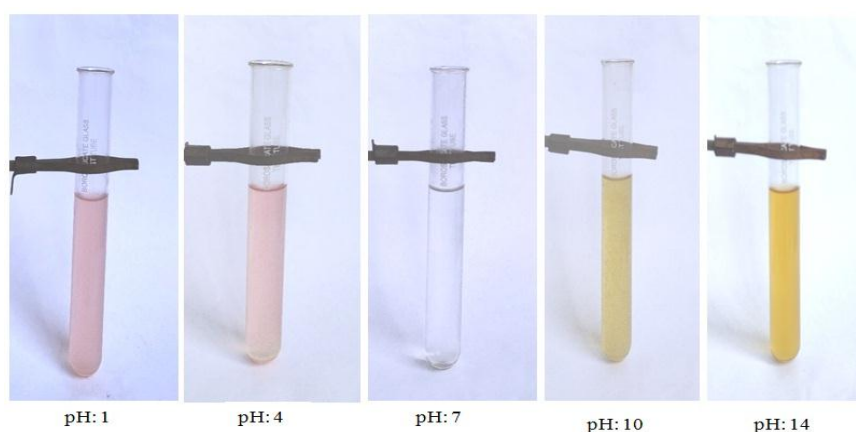


Fig. 5: Colour variations exhibited by extract in solutions of different pH.

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 *A. hispida* flowers contain anthocyanins, and therefore their ethanolic extract exerted different colours in different pH solutions. Hence, though *A. hispida* is a plant, 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.

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