

# Isolation of natural universal indicator and preparation of pH paper from *Calotropis gigantea* Linn. floral aqueous and ethanolic extract and study of their applications and benefits.

R. B. More<sup>a</sup>, S. S. Lalsare<sup>b\*</sup>, G. B. Nikam<sup>c</sup>, C. K. Nerkar<sup>d</sup>, S. L. Sangle<sup>e</sup>, S.V.Chavan<sup>f</sup> & B. S. Kale<sup>b</sup>

<sup>a</sup>Department of Chemistry, MVP'S S. S. S. M. Arts, Science and Commerce College, Saikheda, Nashik, Maharastra, India
<sup>b</sup>Department of Botany, MVP'S S. S. S. M. Arts, Science and Commerce College, Saikheda, Nashik, Maharastra, India
<sup>c</sup>Department of Zoology, MVP'S K.R.T. Arts, B.H. Commerce and A.M. Science College, Nashik, Maharastra, India
<sup>d</sup>Department of Chemistry, MGV's L.V. H. Arts, Science and Commerce College, Panchavati, Nashik, Maharastra, India
<sup>e</sup>Department of Chemistry, MVP'S Arts, Commerce and Science College, Nandgaon, Nashik, Maharastra, India
<sup>f</sup>Department of Chemistry, R.L. Mahavidyalaya, Parola, Jalgaon, Maharastra, India

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**Abstract**- A variety of products, including test papers, pH paper strips, acid-base indicators, and medications, were made using floral extract. Many pigments, including minerals, flavonoids, and anthocyanin, are found in floral extracts. These pigments are highly sensitive to pH changes. The ethanolic and aqueous extracts of the petals of the *Calotropis gigantea* flower (Rui) were made in the current study. In various pH ranges of buffer solutions, noticeable color differences of flower extract of *C. gigantea* are observed. Many color pigments found in aqueous and ethanolic floral extract of *C. gigantea* are extremely sensitive to different chemical as per their pH variations. The synthetic indicators which are commonly used in our laboratory and the natural indicators which are prepared from aqueous and ethanolic floral extracts, provide almost identical outcomes. Using the same extracts, the pH paper and indicator have been prepared. The pH paper prepared is environmentally friendly, biodegradable and is helpful for laboratory work. This kind of research is a cheap, efficient, and natural substitute for artificial litmus paper, dye and Chemicals. *C. gigantea* extract indicator is more cost-effective and yield results with the same accuracy as synthetic indicators in acid base type titrations

Key words: *Calotropis gigantea*, Aqueous and ethanolic extracts, Natural Indicator, Universal Indicator, pH paper, Eco friendly, Economic

#### **INTRODUCTION**

There are many ways to identify a compound's acidic, basic, or neutral nature in chemistry practicals and other medical fields. For detection of acidic and basic nature of the given chemicals or solvents, blue and red litmus are preferably used for water soluble compounds. Instead of

\*Corresponding author :

Phone : 9881487858

E-mail : sulabhalalsare@gmail.com

litmus paper and other costly indicators, we used floral petals extracts of *Calotropis gigantea* Linn. It is one of the local plants which is found nearby area of Nashik and easily grow, is commonly known as Ruhi. It has clusters of waxy flowers that are white and faint purple in colour. These flowers have five petals with the stamens held in a tiny, graceful "crown" that rises from the center. This is one of the short shrubs.<sup>1-3</sup>

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### Scientific classification-

Kingdom	-	Plantae
Order	-	Gentianales
Family	-	Apocynaceae
Sub Fami	ly-	Asclepiadaceae
Genus	-	Calotropis
Species	-	C. gigantea

The ethanolic extract of this flower petals were prepared in the laboratory by using simple water bath method with 50-60°C temperature and the aqueous extract are prepared in beaker on heating temperature 70-80°C.<sup>4</sup>

## **MATERIALS & METHODS**

### i. Plant materials:-

The fresh flowers petals of *Calotropis gigantea* were collected in winter season from Saikheda College ground, Niphad, Nashik. Freshly collected flowers are shown in fig.1



## Fig-1 Flowers of *Calotropis gigantea* Linn. ii. Chemicals:-

All chemicals  $H_2SO_4$ , KOH,  $CH_3COOH$ ,  $NH_4OH$ , phenolphthlein, methyl orange, phenol red potassium chloride, disodium hydrogen Phosphate, Phosporic acid, hydrochloric acid, alcohol (ethanol) was used analytical grade and used as it is.

## iii. Method of extraction:-

The *Calotropis gigantea* flower petals were separated and cleaned with distilled water. Petals were kept in sunlight until they get completely withered. The dried petals were grinded into fine powder with a mortal and pestal.<sup>4-9</sup> 5gms of dried powder was dissolved in 50 ml of water and ethyl alcohol separately.<sup>10-18</sup> After 10 minutes ethyl alcohol containing beaker was kept in water bath at constant temperature 60°C for next 10 minutes, while aqueous extract was prepared by continuous heating at temperature 80°C for 10 minutes. After cooling the resulting solution filtered and filtrate were directly used an indicator.<sup>15-18</sup>

## iv. Preparation of buffer solution:-

Different buffer solutions from 2 to 12 pH were prepared from Potassium chloride, disodium hydrogen, Phosphate, Phosphoric acid and hydrochloric acid etc. As per standard procedure and checked for color change properties of both aqueous and ethanolic extract in different pH region. pH meter used to measure the pH of buffer solutions.<sup>19,20</sup>

## v. Preparation of pH paper :-

In this part of work, we select simple filter paper which was used in our day to day work of laboratory. Those papers were cut in simple rectangle strips (1cm X 6cm) and, are dipped in both the ethanolic and aqueous flower extract separately for 1 hour and dried under the shed. Dried strips of papers used for further testing like acidic, basic and pH range testing.<sup>21-28</sup>



**Fig-2-** Strips in extracts

## **RESULTS & DISCUSSIONS**

Acid base titration- Firstly, we observed color of solvents, selected flower petals, and their extract and the observations are recorded in table no.1. The flower was tested for its potential as an indication in acid-base titration, and the outcomes have been compared with those of methyl orange, phenol red, and phenolphthalein, three common indicators. We employed both the aqueous and the ethanolic extract for these titrations. The results for different type of acid base titrations are listed in table no. 2. Chemicals having of 0.01 N concentration acids  $H_2SO_4$ ,  $CH_3COOH$ , and bases KOH,  $NH_4OH$  were utilized for these titrations.<sup>7,20,29,30</sup>

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Name of the solvent and aqueous and ethanolic extract	Color
Flower petals	Purple
Petals powder	Faint pink
H <sub>2</sub> O	colorless
C <sub>2</sub> H <sub>5</sub> OH	colorless
Aqueous extract of C. gigantea	Pink
Ethanolic extract of C. gigantea	Dark Pink

Titrant 10 ml (0.01N)	Titrand (0.01N)	Indicator colour changes	Standard ethanolic floral extract of CG	Aqueous floral extract of CG
H <sub>2</sub> SO <sub>4</sub>	КОН	Colourless to pink (PH)	Pink to green	Pink to greenish yellow
H <sub>2</sub> SO <sub>4</sub>	NH4OH	Pink to yellow (MO)	Pink to dark green	Pink to dark green
CH <sub>3</sub> COOH	КОН	Colourless to pink (PH)	Pink to yellow	Pink to dark green
CH <sub>3</sub> COOH	NH₄OH	Yellow to red (PR)	Pink to green	Pink to fluorescent green

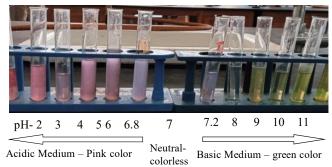
Tab	le 2-	Re	sults	for	differen	t ty	pe	of	acid	base	titration	IS
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PH: Phenolphthlein, MO: Methyl orange, PR: Phenol red, CG- *Calotropis gigantea* 

The color changes of ethanolic and aqueous extracts are tested for all four types of titrations and observed the color change, which are noticeable in comparison with our traditional chemical indicators. When we performed the titrations and note the observations, we found that Phenolphthalein indicator is applicable for strong acid v/s strong base and weak acid v/s strong base and for strong acid v/s weak base methyl orange indicator required and for forth titration weak acid v/s weak base phenol red indicator shows color change. Instead of using 3 different indicators, we may use only single extract so we called it as universal indicator. The end points of this titrations using these extracts are stable.<sup>3,6,15-29,21</sup>

# Testing of aqueous and ethanolic CG extracts in buffer solution:

Different buffer solutions were prepared as per standard procedure to check out the color change in different pH solutions from 2 to 12, we used ten different test tubes, in which 4 to 5 ml buffer solution was taken. All test tubes kept in test tube stand and 2 drops of aqueous extract added respectively in each test tube. After addition of the aqueous extract in buffer containing test tubes of various pH colour change was observed. In acidic medium extract shows dark pink color and at neutral range extract show colorless solutions and at basic pH green color appears in test tube. We recorded same observations for ethanolic CG petals extract in different buffer solutions. Further observation reveals that the prepared extract used as a universal indicator. It is one of the innovative works in chemistry.



# Fig. 3- Results of aqueous and ethanolic CG extracts in buffer solution

# Use of CG pH Paper in different buffer solutions for testing its color change

We attempted to create CG pH Paper, which is among the greatest substitutes for artificial chemicals and litmus papers. We made this conclusion on the basis of examining its property as a universal indicator in many titrations and buffer solutions. Results of sections of simple filter paper

## Table 4- CGEE and CGAE paper color change in acid, base and buffer solutions

Chemicals and Buffer solutions	CGEE paper color	CGAE paper color
$H_2SO_4$	Dark pink	pink
CH <sub>3</sub> COOH	pink	pink
КОН	Dark green	Dark green
NH4OH	green	green
2 pH	Dark pink	pink
3 pH	Dark pink	pink
4 pH	Dark pink	pink
5 pH	pink	Faint pink
6 pH	Milky pink	Very Light pink
7 pH	Colorless	Colorless
8 pH	Faint green	Light green
9 pH	Yellowish green	green
10 pH	Green	Green
11 pH	Dark green	Dark green

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strips soaked in CGEE(*Calotropis gigantea* ethanolic extract) and CGAE (*Calotropis gigantea* aqueous extract) after being dipped in acid, water, base and various buffer solutions are shown in observation table 3. The best outcomes are shown by CGAE paper strips based on various observations. The greatest substitute for traditional pH paper is CGEE and CGAE papers.<sup>3,24,25,31-33</sup>

## CONCLUSION

The work demonstrates both the inventive notion of preparing pH paper and the inventive choice of flower. This work is safe, affordable, and environmentally beneficial. In addition to being useful as an acid-base indicator, the aqueous and ethanolic extracts also exhibit highly stable end points and equivalence points. Ethanolic as well as aqueous extracts are used to prepare pH paper that is stable and exhibits color change over time. We can seal and stored them and may use them after a long time as a pH paper. It is also highly helpful in laboratories of schools and colleges, located in remote areas. Students from such places get the opportunity to prepare the extracts and used it in practical's by collecting the flowers of *C. gigantea*.

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### REFERENCES

- Patil S. S., S. S. Kadam, P. M. Salgar, P. T. Sakate. 2019. Effectiveness of *Calotropis gigantean* Linn Flower Extract as Indicator for Acid Base Titration and Development of Litmus Paper. *Am. J. Pharm Tech Res.*, 9(03): 74-79.
- Gupta P., Jain P., & Jain P. K. 2012. Isolation of natural acid base indicator from the flower sap of *Hibiscus rosa sinensis. Journal of Chemical and Pharmaceutical Research*, 4(12): 4957-4960.
- 3. Rekha M., & Ghumare S. S. 2014. Isolation of natural acid base indicator from *Bougainvillea spectabillis* flower bracts. *Journal of Biological and Chemical Research.* 31(2): 1130-1134

- Pramila Kori, Vijayaishree Nilosey, Prerana Alawa.
   2018. Comparative Evaluation of Phytochemicals, TPC and TFC in Aqueous and Ethanolic Extracts of *Calotropis gigantea* L. Leaves and Roots. *International Journal of Science and Research*, 7(11): 859-861.
- A. Umamaheswari, R. Shreevidya and Aparna Nuni, A. 2008. In vitro antibacterial activity of Bougainvillea spectabillies leaves extract. Advances in Biological Research, 2(1-2): 01-05.
- 6. Karim Nafisi-Movaghar. 2013. Processes for Extracting color from *Hibiscus rosasinensis* plant. patent application no:20130184359.
- Palejkar C. J., Palejkar J. H., Patel M. A., & Patel A. J. 2012. A Comprehensive review on plant Calotropis gigantea. International Journal of Institutional Pharmacy and Life Sciences, 2(2): 463-470.
- Kumar P. S., Suresh E., & Kalavathy S. 2013. Review on a potential herb *Calotropis gigantea* (L.) R. Br. Scholars Academic Journal of Pharmacy, 2(2):135-143.
- 9. Nasrat M. N., Sakimin S. Z., & Hakiman M. 2022. Phytochemicals and antioxidant activities of conventionally propagated nodal segment and *in vitro* induced callus of *Bougainvillea glabra* Choisy using different solvents. *Horticulturae*, **8(8):** 712.
- Lekshmi S. G., Sethi S., Pooja B. K., Nayak S. L., & Menaka M. 2023. Ornamental plant extracts: Application in food colouration and packaging, antioxidant, antimicrobial and pharmacological potential–A concise review. *Food Chemistry Advances*, 3: 100529.
- Singh N., Gupta P., Patel A. V., & Pathak A. K. 2014. Calotropis gigantea: A review on its phytochemical & pharmacological profile. Int. J. of Pharmacognosy, 1: 1-8.
- 12. Kumar G., Karthik L. & Rao K. B. 2010. Antibacterial activity of aqueous extract of *Calotropis* gigantea leaves– an *in vitro* study. *International journal* of pharmaceutical Sciences Review and Research, 4(2): 141-144.
- 13. Pathak A. K., & Argal A. 2007. Analgesic activity of *Calotropis gigantea* flower. *Fitoterapia*, 78(1): 40-42.

More *et al.*- Isolation of natural universal indicator and preparation of pH paper from *Calotropis gigantea* Linn. floral aqueous and ethanolic extract and study of their applications and benefits.

- Ubani C. S., Joshua P. E., & Anieke U. C. 2011. Effects of aqueous extract of *Hibiscus sabdariffa L.* calyces on liver marker enzymes of phenobarbitone induced adult wistar albino rats. *J Chem Pharm Res*, 3(4): 528-537.
- Rajamohan S., Kalaivanan P., Sivangnanam H., & Rajamanickam M. 2014. Antioxidant, Antimicrobial activities and GC-MS analysis of *Calotropis gigantea* white flowers. J. Phytopharmacol, 3: 405-409.
- Dhivya R., & Manimegalai K. 2013. Preliminary phytochemical screening and gc-ms profiling of ethanolic flower extract of *Calotropis gigantea* Linn. (Apocyanaceae). *Journal of Pharmacognosy and Phytochemistry*, 2(3): 28-32.
- Habib M. R., Aziz M. A., & Karim M. R. 2010. Inhibition of Ehrlich's ascites carcinoma by ethyl acetate extract from the flower of *Calotropis gigantea* L. in mice. *Journal of Applied Biomedicine*, 8(1): 47-54.
- Punia G. 2013. A Review on Varieties of Arka Calotropis procera (Aiton) Dryand. and Calotropis gigantea (L.) Dryand. Global Journal of Research on Medicinal Plants & Indigenous Medicine, 2(5): 392.
- Jaworska M., Szulińska Z., & Wilk M. 2005. Application of a capillary electrophoresis method for simultaneous determination of preservatives in pharmaceutical formulations. *Journal of separation science*, 28(2): 137-143.
- Vogel A. I., 1996. Titremetric analysis, Textbook of quantitative chemical analysis, 5<sup>th</sup> edition, Longman group, UK. 262-282.
- Khan P. M. A., & Farooqui M. 2011. Analytical applications of plant extract as natural pH Indicator: a review. *Journal of advanced scientific research*, 2(04): 20-27.
- 22. Patil S. B., Kondawar M. S., Ghodke D. S., Naikwade N. S., & Magdum C. S. 2009. Use of flower extracts as an indicator in acid-base titrations. *Research Journal of Pharmacy and Technology*, 2(2): 421-422.
- Agrawal S., Raj N. R., Chouhan K., Raj C. N., Jain S., & Balasubramaniam A. 2011. Isolation of herbal acid-base indicator from the seeds of *Punica granatum*.

Journal of Chemical and Pharmaceutical Research, **3(2):** 168-171.

- 24. Kadam S., Yadav A., Raje V., & Waghmare K. 2013. Comparative study of natural and synthetic indicators. *Der Pharma Chemica*, 5(1): 296-299.
- 25. R. S. Yadav, A. K. Singh, and R. D. Singh. 2015. Natural pH Indicators from Plant Sources. *Journal of Chemical and Pharmaceutical Research*, 7(5):137-143.
- 26. Bhise S. H., Shinde N. G., Surve B. S., Pimpodkar N. V., & Shikalgar S. S. 2014. Acalypha wilkesiana as natural pH indicator. International Journal of Natural Products Research, 4(1): 33-35.
- 27. Pathade K. S., Patil S. B., Kondawar M. S., Naikwade N. S., & Magdum C. S. 2009. Morus alba fruit-herbal alternative to synthetic acid base indicators. International Journal of Chem Tech Research, 1(3): 549-551.
- 28. A. K. Mishra, R. K. Tiwari, and S. K. Shukla 2017. Preparation of pH Paper Using Natural Indicators. International Journal of Advanced Research in Chemical Science, 4(2): 1-8
- 29. Vaibhav, G. N., Vishal, B. K., Prashant, D. N., Ganpatrao, N. M., Suresh, T. S., & Ashish, S.S. 2014. Study of Nerium odoratum as natural, economical and effective alternative to synthetic indicator and litmus paper. International Journal of Pharmaceutical Chemical Science, 3(2): 440.
- 30. Saloni Desai, Dhruti Ganatra, Anjali Maisuriya, Ishali Patel. 2023. Flower Extract as an Organic Indicator in Acid Base Titration. Int. J. Pharm. Sci. Rev. Res., 82(2): 54-58.
- Kapilraj N., Keerthanan S., & Sithambaresan M. 2019. Natural Plant Extracts as Acid Base Indicator and Determination of Their pKa Value. *Journal of Chemistry*, 2019(1): 2031342.
- Herrera-Ruiz M., García-Beltrán Y., Mora S., Díaz-Véliz G., Viana G. S., Tortoriello J., & Ramírez G. 2006. Antidepressant and anxiolytic effects of hydroalcoholic extract from *Salvia elegans*. *Journal* of *Ethnopharmacology*, 107(1): 53-58.

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**33.** Cermak, R., & Wolffram, S. 2006. The potential of flavonoids to influence drug metabolism and pharmacokinetics by local gastrointestinal mechanisms. *Current drug metabolism*, **7**(7):729-744.

## **ADDITIONAL REFERENCES**

- 34. Kumar G., Karthik L., & Rao K. B. 2010. Antibacterial activity of aqueous extract of *Calotropis* gigantea leaves–an in vitro study. *International journal* of pharmaceutical Sciences Review and Research, 4(2): 141-144.
- 35. Hemalatha M., Arirudran B., Thenmozhi A., & Rao U. S. 2011. Antimicrobial effect of separate extract of acetone, ethyl acetate, methanol and aqueous from leaf of Milkweed (*Calotropis gigantea* L.). *Asian Journal of Pharmaceutical Research*, 1(4): 102-107.

- 36. Manivannan R., & Shopna R. 2017. Antimicrobial and Anti-inflammatory Activity of New 4-methoxy-3-(methoxymethyl) Phenol and (E)-N'- (5-bromo-2methoxybenzylidene)-4-methoxy Benzohydrazide Isolated from *Calotropis gigantean* white. *Natural Product Sciences*, 23(1): 69-74.
- 37. S. K. Singh, R. K. Sharma, P. K. Singh, and S. C. Singh. 2013. Phytochemical Analysis and Antimicrobial Activity of *Calotropis gigantea*. Journal of Pharmaceutical and Scientific Innovation. 2(4): 23-28.

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