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Antimicrobial Activity of *Clitoria ternatea* L. flower extract and use as a natural indicator in acid base titration

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ABSTRACT

In present scenario natural indicators are more efficiently prepared as it has several advantages over synthetic indicator. Clitoria ternatea Linn is a species of the genus clitoria , belonging to family Fabaceae. In the present research work natural indicator of Clitoria ternatea flower extract were prepared and it is use as an indicator in various acid base titration. The equivalence points obtained by the flower extract compared with the equivalence point obtained by standard indicators. In case of weak acid and weak base titration, the results obtained by the flower extract matched with the results obtained by mixed indicator and also the prepared methanolic flower extract were tested for antimicrobial activity against Staphylococcus aureus and showed significant anti-microbial activity. This natural indicator was found to be very effective, useful , cost effective, simple and accurate for the said acid base titration.

Key words: *Clitoria ternatea*, Acid base indicator, Natural Indicator, Antimicrobial

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INTRODUCTION

The plant species used for this study is *Clitoria ternatea* (common name including butterfly-pea, blue-pea and cordofan-pea) which belongs to family fabaceae. The native of this plant is tropical equatorial Asia, but has been introduced to Africa, Australia and America. It is a perennial herbaceous plant and its leaves are ethiptic and obtuse. It grows as a vine or creeper, growing well in moist soil. One of the important features about this plant is its vivid deep blue flowers. They are solitary, with light yellow marking having dimension about 4 cm long and 3cm wide. Some varieties of this plant yield white flowers. The fruits are 5-7 cm long, flat pods with 6 to 10 seeds in each pod. They are edible when tender[1].

It is grown as an ornamental plant and as a revegetation species requiring little care when cultivated. Its roots fix nitrogen and therefore this plant is also used to improve soil quality. This plant is very useful as it has several therapeutic activities like antistress, anxiolytic, antidepressant, anticonvulsants, tranquilizing and sedative agent [2]. In southeast asia the flowers are used to colour foods. In animal tests the methanolic extract of clitoria trenatea roots found to possess anxiolytic, antidepressant, anticonvulsant and antistress activity[3].

MATERIALS AND METHODS

All the apparatus and instruments required for the present research work were calibrated[4,5]. Analytical grade reagents were made available by Dadasaheb Balpande college of Pharmacy, Besa Nagpur. Reagents and volumetric solutions were prepared as per standard books[6,7]. The flowers and leaves of *Clitoria ternatea* Linn were collected from the Nagpur region in the month of september 2012. The plant was identified and authenticated at Department of Botany, R.T.M. Nagpur University, Nagpur and authentication number was **9788**.

1. Solvent Extraction:
The fresh petals were cut into small pieces and kept at room temperature. The petals were dried, ground into fine powder with a mechanical blender. The resulting powder was extracted with methanolic hydrochloric acid and the anthocyanins were converted into their corresponding soluble chlorides. From this solutions , anthocyanins were isolated by using ether[8]. Finally extract was filtered and used as indicator in several acid base titration. The experimental work was carried out by using the same set of glasswares for all type of titrations. As the same aliquots were used for both titrations i.e. titration by using standard indicator and flower extract, the reagent were not calibrated. The equimolar titrations were performed using 25 ml of titrant with three drops of indicator. All the parameters for experiment are given in Table **1**. A set of five experiments was carried out and mean and standard deviation were carried out. The mean and standard deviation were calculated from results and also the solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use for anti microbial activity.

2. Stock Solution:
Prepared flower extract (50 mg) were dissolved in DMF (100ml) and volume was made up to 10 ml to produce a concentration of 500 µg/ml. Further dilutions were made with DMF to produce 50, 100, 200 µg/ml.

3. Procedure for antimicrobial activity[9]:
All the operations were carried out under aseptic conditions. Sterile medium was melted on water bath and kept at 45^0c in constant temperature water bath. In each sterile petri dish molten medium was added so that thickness was approximately 4-5 mm and sub cultured organism under study was inoculated. The inoculated dishes were allowed to set for 30 min. at room temperature. Cups of 6 mm diameter were then made with the help of sterile stainless still bore ; 1 ml of test solution of flower extract was added to each cup . Petri dishesh were kept in refrigerator for 30 minutes so as to allow diffusion of the of the solution in the medium, and then incubated at 37^oc for 24 hrs. for antibacterial activity. Zone of inhibition produced by test compounds were measured in mm and the compounds were selected on the basis of their MIC. The results are shown in Table **3.**

Table 1: Analyzed Parameters and the Comparison of Color Change

Titrate	Titrant	Indicator Color Change	
		Standard (pH range)	**Flower Extract (pH range)**
Hcl	NaOH	Red to Yellow (3.2-8.4)	Pink to Colorless (1.2-8.2)
Hcl	NH_4OH	Colorless to Pink (5.3-8.2)	Pink to Colorless (5.2-8.6)
Oxalic acid	NaOH	Colorless to Pink (4.7-9.2)	Green to Colorless (5.3-12.2)
Oxalic acid	NH_4OH	Orange to Blue-green (4.3-7.8)	Yellow to Colorless (4.6-9.8)

HCl:- Hydrochloric acid, NaOH:-Sodium Hydroxide , NH_4OH:-Ammonium Hydroxide

Table 2: Screening Results of Various titrations

Sr. No.	Titration (Titrant v/s Titrate)	Strength in Moles	Indicator	Readings with S.D. (+/-)
1	NaOH V/S HCl	0.1	Methyl red	18.3+/- 0.14
			Flower extract	18.2+/- 0.15
		0.5	Methyl red	18.4+/- 0.12
			Flower extract	18.3+/- 0.22
		1	Methyl red	18.5+/- 0.21
			Flower extract	18.4+/- 0.26
2	NH_4OH V/S HCl	0.1	Phenolpthalein	12.2+/- 0.14
			Flower extract	12.3+/- 0.17
		0.5	Phenolpthalein	12.5+/- 0.10
			Flower extract	12.4+/- 0.16
		1	Phenolpthalein	12.7+/- 0.20
			Flower extract	12.7+/- 0.22
3	NaOH V/S Oxalic Acid	0.1	Methyl red	22.4+/- 0.18
			Flower extract	22.3+/- 0.20
		0.5	Methyl red	22.6+/- 0.18
			Flower extract	22.4+/- 0.20
		1	Methyl red	22.5+/- 0.21
			Flower extract	22.4+/- 0.21
4	NH_4OH V/S Oxalic Acid	0.1	Mixed indicator	10.8+/- 0.21
			Flower extract	10.7+/- 0.27
		0.5	Mixed indicator	10.6+/- 0.21
			Flower extract	10.5+/- 0.22
		1	Mixed indicator	10.4+/- 0.21
			Flower extract	10.3+/- 0.23

Hcl:- Hydrochloric acid, NaoH:- Sodium Hydroxide, NH_4OH:- Ammonium Hydroxide, DMF:- Dimethyl Formamide.

Table 3: Antimicrobial activity data against *Staphylococcus aureus* Bacteria along with zone of inhibition (mm)

50 µg/ml	**100** µg/ml	**200** µg/ml	**500** µg/ml
13	17	20	24

RESULTS AND DISCUSSION

The Prepared flower extract was screened for its use as an acid base indicator in various acid base titrations, and result of screening compared with the result obtained by standard indicators methyl red , phenolphthalein and mixed indicator [methyl orange: bromocresol green (0.1-0.2)] for strong acid v/s strong base (Hcl and NaOH), strong acid v/s weak base (Hcl and NH_4OH), weak acid v/s strong base (oxalic acid and NH_4OH) titrations respectively[10,11].

All these parameters are shown in Table **1**. For all titrations the equivalence points obtained by the flower extract matched with the equivalence points obtained by standard indicators. The results of screening were listed in Table **2**. The prepared flower extract were also screened for antimicrobial activity against *S. aureus* using agar diffusion method and the result obtained are shown in Table **3**.

CONCLUSION

It is concluded from the data, the antimicrobial activity is directly proportional to concentration. As increase in concentration of solution results in an increase in zone of inhibition, and also the present research work of the *Clitoria ternatea* flower extract alone can serve the purpose of indicator in weak acid and weak base titration, where generally mixed indicators employed. Another benefit of this titration is that it gives colorless end point at the equivalence point. If we add more amount of titrant (base) it gives Prussian blue colored solution.

The results obtained in all types of acid base titrations lead us to conclude that it was due to the presence of flavonoids and anthocyanins sharp color changes occurred at the end point of the titrations. At last we can say that it is always beneficial to use *Clitoria ternatea* flower extract as an indicator in all types of acid base titrations because of its cost effectiveness, simplicity and availability. This flower extract also have significant antimicrobial activity.

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