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Isolation of herbal acid-base indicator from the seeds of *Punica granatum*

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ABSTRACT

In acid- base titrations, indicators are used to show a sharp color changes at interval of pH. Natural pigments in plants are highly colored substances and may show color changes with variation of pH. An attempt has been made to investigate the indicator activity of methanolic fruit extract of Punica granatum and to replace the synthetic indicators as they have certain disadvantages like chemical pollution, availability problems and high cost. Methanolic fruit extract of Punica granatum from family Puniceaceae gives sharp and intense color changes as compared to Phenolphthalein, Methyl Red and Phenol Red. Herbal indicators are evaluated by using strong acid-strong base, strong acid-weak base, weak acid-strong base and weak acid-weak base. In all these titrations the extract was found to be very useful and accurate for indicating the neutralization point.

Keywords: Acid-base titration, *Punica granatum*, Herbal indicator, Methanolic extract.

INTRODUCTION

The fruits of *Punica granatum* L. (Punicaceae), the pomegranate, are commonly eaten [1]. In traditional Cuban medicine pomegranate fruits have been used to treat acidosis, dysentery, microbial infections, diarrhoea, helminthiasis, haemorrhage and respiratory pathologies have also reported popular use of the pomegranate plant in the treatment of respiratory disease [2,3,4]. *Punica granatum* would appear to have interesting anti-viral activity [5]. Extracts have been shown to be effective against the herpes virus and hydroalcoholic extracts of whole fruits have exhibited high activity against the influenza virus [6,7].

Phytochemically the plant has been attributed to contain punicalagin, punicalin, strictinin and granatin [8]. Two new ellagitannins, diellagic acid rhamnosyl glucopyranoside and 5-O-galloylpunicacortein-D were isolated and characterized together with four known tannin

metabolites, punicacortein D, punicalin, punicalagin and 2-O-galloylpunicalin [9].It also contains flavonoids, antioxidants and anthocyanins as well [10]. The intention behind this study is simply to bring in market the use of plant pigments and to increase the wealth of traditional medicinal system of India which is mostly plant based and to help farmers regarding cultivation, collection of plants as well as to industry regarding preparation of above indicators[11-12]. Titrate and Titrant with indicator shows sharp and intense color changes at the equivalence point that is at neutralization [13]. Therefore the objective of this work was to explore the indicator activity of methanolic fruit extract of *Punica granatum.*

MATERIALS AND METHODS

Analytical grade reagents i.e. hydrochloric acid (HCL) , sodium hydroxide (NaOH), acetic acid (CH_3COOH) , ammonia (NH_3) , phenolphthalein and methyl red were procured from the Technocrats Institute of Pharmacy , Bhopal. Reagents and volumetric solutions were prepared as per Indian Pharmacopoeia.

The fresh fruits were purchased from a local market and were authenticated by National Botanical Research Institute , Lucknow. The fruits were cleaned and cut into small pieces. 100 gm of these pieces were macerated with 150 ml of solution containing 9 parts of methanol and 1 part of dilute hydrochloric acid for 3 hrs.The extract was preserved in tightly closed container and stored away from the direct sun light. [11]

The experiment was carried out by using a same set of glass wares for all types of titrations. The reagents were not calibrated: as same aliquots were used for both titrations i.e. titration by using standard indicator and fruit extract.

5 ml of titrant and 3 drops of indicator was titrated. All the parameters for the experiment are given in **Table1**. Each titration was carried five times and results were recorded .Mean and standard deviations were calculated from the results. The Methanolic extract of fresh fruit of *Punica granatum* was screened for its use as indicator for Acid-Base titration and the results of this screening were compared with the results obtained by using standard indicators.

RESULTS AND DISCUSSION

The fruit was screened for its use as an indicator in acid base titration and the results were compared with the results obtained by standard indicators methyl red, phenolphthalein. The results of the screening for strong acid- strong base (HCl & NaOH) , strong acid -weak base (HCl & NH_3) , weak acid-strong base (CH_3COOH & NaOH) and weak acid-weak base(CH_3COOH & NH_3) are listed in **Table 2**.

Table 1 : Parameters for titration

Titrant	Titrand	Indicator colour change and (Ph range)	
		Standard	Fruit Extract
HCl	NaOH	Colorless to pink (PH)	Pink to colorless
HCl	NH_3	Red to yellow (MR)	Pink to colorless
CH_3COOH	NaOH	Colorless to pink (PH)	Pink to colorless
CH_3COOH	NH_3	Yellow to red (PR)	Pink to colorless

Key: PH=Phenolphthalein, MR= Methyl Red PR=Phenol Red

The table represents mean of five titrations ± standard deviation. The screening was carried out using three different molar strength of acids and alkalis viz. 0.1, 0.5, 1.0 M. For all types of titrations equivalence point obtained by the fruit extract either exactly or very closed with the equivalence point obtained by the standard indicators. This represent the usefulness of fruit extract as an indicator in acid base titration .Its use in weak acid and weak base was found to be more significant over standard indicator as it gives sharp colour change in a narrow pH range. The results obtained showed that the routinely used indicator can be replaced successfully by fruit extracts.

Table 2: Mean volume (in ml) at the equivalence point for titrations

Strength (in M)	Hydrochloric acid v/s Sodium Hydroxide		Hydrochloric acid v/s Ammonia		Acetic acid v/s Sodium Hydroxide		Acetic acid v/s Ammonia	
	PH	FE	PH	FE	PH	FE	PR	FE
0.1	7.8±0.20	7.7±0.24	7.4±0.20	7.5±0.62	7.9±0.20	7.8±0.24	9.2±0.30	9.1±0.32
0.5	8.0±0.40	7.9±0.32	7.8±0.24	7.7±0.34	8.1±0.24	8.2±0.42	9.4±0.42	9.3±0.42
1.0	10.2±0.3	10.0±0.34	9.5±0.30	9.4±0.36	9.8±0.30	9.9±0.34	10.0±0.60	10.1±0.32

Mean of five titrations ± S.D. , Key: M= Molar strength, PH=Phenolphthalein, MR=Methyl red, FE=Fruit extract, PR=Phenol Red

CONCLUSION

The results obtained in all the types of acid base titrations lead us to conclude that it was due to the presence of flavonoids sharp colour changes, which occurred at end point of titrations. At the end point of it states that *Punica granatum* fruit extract as an indicator in all types of acid base titrations because of its economic, simple, accurate and precise.

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