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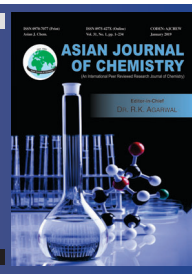

Study of *Brassica oleracea* as Natural Alternative to Synthetic Indicator

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This study was carried out to screen indicator property of *Brassica oleracea* extracts which forms a natural alternative to chemical indicator. Phytochemical analysis of *Brassica oleracea* extracts revealed the presence of 9 phytochemical constituents. Further the extracts were screened for indicator property. Then natural indicator obtained from the extracts was also screened for estimations apart from regular acid-base titrations. In addition, natural indicator was also used to develop pH strips for determining the pH of commercial sample which includes colour change of pH strips shows significant result at different pH and may be used to measure pH of wide range of sample.

Keywords: *Brassica oleracea*, Natural indicator, pH Strip.

INTRODUCTION

Indicators are the dyes or pigments that are isolated from various sources. The natural indicators are very useful, readily available, non-hazardous and economical. The natural indicators have so many advantages over synthetic indicators. The natural indicators which is prepared is affordable, non-toxic and used in acid base titrations. The indicator is very accurate in giving sharp end points [1]. The main importance which is responsible for the developments of natural indicators over chemical indicators is due to the expense and unavailability of chemical indicators which led to lot of attempts to prepare natural indicators. Due to the presence of different pigments, which are widely distributed in leaves, fruits and vegetables and flowers, used in different types of titrations. Plant source used as natural indicators are water melon fruit, beetroot, carrot, raktchandan bark (*Pterocarpus santalinus*), marigold, sweet almond, dalimb seeds, brinjal and ghaneri mixed [1]. Brassica contains bioavailable compounds phenolic compounds,vitamins and minerals [2]. The distinguished characteristic of indicator is that the acid and conjugate base forms are different colours. A good acid-base indicator will change its colour as the concentration of H^+ ions in a solution changes. Often an indicator will have one or more atoms that can gain or lose protons (H^+) [3]. The pH's at which the indicators change colour will be investigated [4].

Cabbage is called a millenniums functional food, often related to health promotion and disease prevention. Cabbage was cultivated from wild cabbage to coastal regions of the Mediterranean. The cabbage has the ability to withstand cold climates and also valued for its medicinal properties. *Brassica oleracea* (red cabbage) which has vitamins, minerals, photochemicals, *etc*. It is unique among cruciferous vegetables in providing a big quantity of anthocyanins, antioxidants and anti-inflammatory nutrients, polyphenols [5]. Red cabbage is just like green cabbage in taste and texture but differentiates by anthocyanins which gives distinctive colour.

The search for natural compounds as acid base indicator and colouring agent in food industries depends on the availability and are free from toxins. Literature survey also revealed that similar works carried out on the flowers of *Rosa indica* and *Hibiscus rosasinensis* [6], *Ipomoea biloba* [7] and *Dahlia pinnata* [8] also suggested that flower extract could be an alternative.

EXPERIMENTAL

Fresh red cabbage (*Brassica oleracea*) (5 kg) was purchased from the local market, Bangalore, India. The sample was washed, cleaned, cut into small pieces and shade dried for 2 days and then finally dried at 50 ºC in hot air oven. The sample was then crushed into powder using mechanical blender.

Crude extraction: This powder sample was dissolved in different solvents such as water, ethanol, methanol, acetone, petroleum ether and chloroform in ratio 1:10 and kept in orbital shaker for three days. After 3 days, the solvents were separated from the powder using Whatman filter paper No. 1. These solvent extracts were concentrated to1/4th using water bath [9].

Phytochemical constituents of *Brassica oleracea* extract: Phytochemicals screening was performed for those extract solvents which showed indicator property. Phytochemicals screening were performed using standard method [10].

Screening of indicator property of *Brassica oleracea* extracts: Acid base titrations were performed using *Brassica oleracea* extracts as an indicator. Titre value was confirmed by using standard indicator (phenolphthalein). The titration was carried by using same glassware, so that titration by using standard indicator (phenolphthalein) and *Brassica oleracea* extract solvents, the reagents were not calibrated. The equimolar titrations were performed using 10 mL of titrant in conical flask against titrand taken in burette with 2-3 drops of indicator. Four different sets of titration performed were strong acid/strong base, strong acid/weak base, weak acid/strong base, and weak acid/weak base. The strength of acid and bases (HCl, CH_3COOH, NaOH and NH_4OH) taken were 1 N, 0.1 N and 0.5 N. This is done to show whether *Brassica oleracea* extract indicator exhibits indicator property at all strength. In each set of titration, the base is first taken as titrant in conical flask and acid is taken as titrand in burette. Titration is repeated for concordant values. Then the acid is taken as titrant in conical flask and base is taken as titrand in burette. Titration is repeated for concordant values. This is done to show that whether *Brassica oleracea* extracts indicator exhibits indicator property irrespective of which titrant is present in conical flask or titrand present in burette [3].

Application of *Brassica oleracea* extract: *Brassica oleracea* extract indicator was also used for other titrametric estimations. The experiment tests performed by using *Brassica oleracea* extract solvents indicator are (a) estimation of acid value of fats; (b) determination of saponification of oils; (c) estimation of acetyl number of fats; (d) estimation of lactic acid in curd; (e) estimation of citric acid produced by *A. niger*; (f) estimation of acidity and volatile acid in graph juice, and (g) estimation of potassium hydroxide using standard oxalic acid.

Preparation of pH strip using *Brassica oleracea* extract indicators: *Brassica oleracea* extract was utilized in the preparation of pH strips using Whatman filter paper No. 1. The Whatman filter paper strips was soaked in solvent extract for 15 min. After soaking, Whatman filter paper was dried in hot air oven at 50 ° C for 10 min. These dried Whatman filter paper are ready for measuring pH of different solvents. These developed pH paper is now tested for pH range from pH 1 to pH 13 using litmus paper as control. The change in colour of developed pH strip at different pH indicates that these strips of *Brassica oleracea* extract can be used to measure pH of any solution [11].

RESULTS AND DISCUSSION

Yield of *Brassica oleracea* extracts: Percentage yield was evaluated to *Brassica oleracea* extract with various solvents including water, ethanol, methanol, acetone, petroleum ether and chloroform are shown in Fig. 1. It is found that the aqueous extract of *Brassica oleracea* was 27 %, whereas methanol extract yield was 23 % and ethanolic extract yield was 21 %.

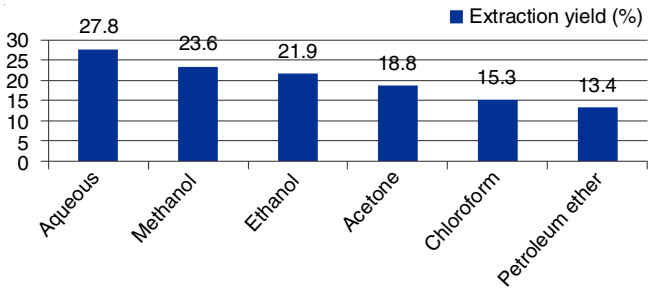

Fig. 1. Percentage yield of *Brassica oleracea* extracts

Phytochemical screening of *Brassica oleracea*: The phytochemical screening showed the presence of 9 different constituents for aqueous extract of *Brassica oleracea* (Table-1).

Screening of indicator property of *Brassica oleracea* extract

Acid-base titration: Titrations results obtained using *B. oleracea* extract are shown in Tables 2 and 3. Four different sets of titration performed were strong acid/strong base, strong acid/weak base, weak acid/strong base and weak acid/weak base.

Since, the pH of indicator of *Brassica oleracea* of aqueous, ethanolic and methanolic extract shows almost neutral. Hence, it can be concluded that on addition of these indicator, it allow to identify the pH of the solution without making major change in the pH solution as pH of all these indicators are almost neutral and also all natural components are ecofriendly. Moreover,

TABLE-1
PHYTOCHEMICAL CONSTITUENTS OF *Brassica oleracea*

Phytochemical test	Aqueous	Methanol	Ethanol	Acetone	Chloroform	Petroleum ether
Terpenoids	+	+	+	+	+	+
Flavonoids	+	+	+	+	-	-
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Alkaloids	+	+	+	+	–	–
Reducing sugar	+	+	+	+	+	–
Anthraquiones	–	–	–	–	–	–
Steroids	+	–	+	+	+	+
Phenols	+	+	+	–	–	–
Oil and Resins	+	+	+	+	+	+

*(+) indicates presence of phytochemical constituents and (–) indicates absence of phytochemical constituents

TABLE-2
COLOUR CHANGE OF *Brassica oleracea* EXTRACT (INDICATOR) IN ACID-BASE TITRATION (START POINT TO END POINT)

Titrand	Titrant	Colour change of indicator in titration			
		Standard phenolphthalein	*Brassica oleracea* extracts		
			Aqueous extract	Methanolic extract	Ethanolic extract
HCl	NaOH	Pink to colourless	Yellow to colourless	Yellow to colourless	Yellow to colourless
NaOH	HCl	Colourless to pink	Red to colourless	Red to colourless	Red to colourless
HCl	NH_4OH	Pink to colourless	Green to colourless	Skyblue to colourless	Green to colourless
NH_4OH	HCl	Colourless to pink	Red to colourless	Red to colourless	Red to colourless
CH_3COOH	NaOH	Pink to colourless	Yellow to colourless	Yellow to colourless	Yellow to colourless
NaOH	CH_3COOH	Colourless to pink	Pink to colourless	Pink to colourless	Pink to colourless
CH_3COOH	NH_4OH	Pink to colourless	Green to colourless	Skyblue to colourless	Green to colourless
NH_4OH	CH_3COOH	Colourless to pink	Pink to colourless	Pink to colourless	Pink to colourless

TABLE-3
ACID-BASE TITRATION WITH DIFFERENT EXTRACTS OF *Brassica oleracea* AS INDICATOR

Titrand	Titrant	Standard phenolphthalein			Aqueous extract			Methanolic extract			Ethanolic extract		
		0.1 N	0.5 N	1.0 N	0.1 N	0.5 N	1.0 N	0.1 N	0.5 N	1.0 N	0.1 N	0.5 N	1.0 N
HCl	NaOH	10.3	11	9.3	10.3	11.1	9.3	10.3	11	9.3	10.4	11	9.3
NaOH	HCl	11.3	11.2	10.3	11.3	11.2	10.4	11.3	11.4	10.2	11.3	11	10.4
HCl	NH_4OH	9.4	12.3	11.5	9.3	12.3	11.5	9.4	12.3	11.5	9.5	12.3	11.5
NH_4OH	HCl	8.6	10.4	11.5	8.5	10.3	11.5	8.5	10.4	11.5	8.5	10.1	11.5
CH_3COOH	NaOH	10.9	12	10	11	12.2	9.8	10.9	12	10	10.8	12.2	9.8
NaOH	CH_3COOH	9.3	9.8	9.4	9.1	9.7	9.4	9.3	9.6	9.4	9.1	9.8	9.5
CH_3COOH	NH_4OH	8.3	8.9	11.1	8.4	9	11	8.3	8.9	11.1	8.3	8.9	11
NH_4OH	CH_3COOH	11.4	10.7	9.3	11.2	10.7	9.4	11.2	10.7	9.4	11.2	10.7	9.4

colour changes which is observed on addition of *Brassica oleracea* extract of aqueous, ethanolic and methanolic showed that the colour was maintained for a long time. Thus, these indicators are stable. However, aqueous extract of *Brassica oleracea* need to be stored below 4 ºC to avoid microbial contamination. Whereas ethanolic and methanolic extract can be stored at room temperature.

Applications of *Brassica oleracea* extract: *Brassica oleracea* extract indicators were applied in acid base titration to estimate the unknown normality of solution, acid value, *etc*. Titre value was further confirmed by using standard indicator phenolphthalein. The results obtained on performed titration by using *Brassica oleracea* extracts as indicator are shown in Table-4.

The applications of aqueous, ethanolic and methanolic extract of *Brassica oleracea* show the indicator property having distinct colour changes at different pHs. This property is very useful in performing acid-base titration for commercial components for knowing the acid value and saponification number of fat and other natural components. Moreover, by testing these components with *Brassica oleracea* extracts does not cause change in nature of food components. The colour changes which is seen on addition of *Brassica oleracea* extracts showed that the colour was maintained for a long time. There by helping us to know the pH of the solution, without further addition. The results of *Brassica oleracea* extract in titration, it is confirmed that the estimated values of aqueous, ethanolic and methanolic extracts of *Brassica oleracea* showed significant estimated value which are similar to that of phenolphthalein. It is also noted that methanolic and aqueous extract gave more significant values as compared to ethanolic extract.

Preparation of pH paper using *Brassica oleracea* extracts: The *Brassica oleracea* extracts screened for indicator property were also used to generate pH indicator strip. The developed pH paper tested for pH range from pH 1 to pH 13 by using known pH solution and litmus paper is used as the control. The change in colour of developed pH paper at different pH indicates that pH papers consisted of *Brassica oleracea* extract solvents can be used to measure pH of unknown solution.

The developed pH paper tested for pH range from pH 1-13 by using know pH solution and litmus paper is used as to show significant colour at different pHs. The change in colour of

TABLE-4
RESULTS OBTAINED ON PERFORMED TITRATION BY USING *Brassica oleracea* EXTRACTS

Estimations	Estimated value			
	Standard phenolphthalein	*Brassica oleracea* extracts		
		Aqueous	Methanolic	Ethanolic
Acid value of fats	1.60	1.60	1.60	1.12
Saponification of oil	2.14	2.14	2.28	2.00
Acetyl number of fats	2.68	2.24	2.68	1.79
Lactic acid in curd (%)	0.36	0.37	0.36	0.48
Citric acid produce by *A. niger* (%)	0.07	0.09	0.09	0.11
Acidity in grapes juice (%)	0.33	0.36	0.37	0.40
Volatile acid in grapes juic (%)e	0.27	0.28	0.30	0.32
KOH using standard oxalic acid (N)	0.094	0.095	0.095	0.096

developed pH paper at different pH indicates that these pH paper are sensitive to the developed pH range pH 1-13 (Table-5). The colour change in different extract was observed when compared to aqueous and ethanol extracts methanolic extract showed significant colour change. *Brassica oleracea* extract can be used as a natural alternative to synthetic pH paper.

TABLE-5
Brassica oleraceaextract pH STRIPS COLOUR CHANGE WHEN DIPPED IN DIFFERENT pH

pH	*Brassica oleracea* extracts pH strips		
	Aqueous	Methanolic	Ethanolic
1	Red	Red	Red
2	Pale red	Pale red	Pale red
3	Pink	Pink	Pink
4	Pale pink	Pale pink	Pale pink
5	Colourless	Colourless	Colourless
6	Colourless	Colourless	Colourless
7	Colourless	Colourless	Colourless
8	Colourless	Colourless	Colourless
9	Colourless	Sky blue	Colourless
10	Dark green	Blue	Light green
11	Light green	Green	Green
12	Yellowish green	Yellowish green	Yellowish green
13	Yellow	Yellow	Yellow

Application of pH paper: The developed pH strip from *Brassica oleracea* extract was used for determining the pH of unknown pH solution and the results were confirmed with litmus paper. The results of pH of components tested by using developed pH strip of *Brassica oleracea* extract indicator are shown in Table-6.

TABLE-6
DETERMINATION OF pH OF DIFFERENT SAMPLES USING pH STRIPS OF *Brassica oleracea* EXTRACTS

Hard water	pH 9	Soft drinks	pH 3
Curd	pH 4	Detergent	pH 10
Grapes juice	pH 4	Soil	pH 8

The results revealed that the *Brassica oleracea* extract pH paper gives a significant colour for samples tested, based on their pH. It was noted that *Brassica oleracea* methanolic extract pH paper is more effective than compared to ethanolic and aqueous extract pH paper.

Conclusion

Phytochemical analysis of *Brassica oleracea* extracts revealed the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, reducing sugar, steroids, phenols, oil and resins. Further, the extracts were screened for indicator property based on the colour change using acid-base titration using standard reference indicator. The natural indicator obtained from the extract was also used for other estimations apart from regular acid-base titrations. The natural indicator was also used to develop pH strips for determining the pH of the commercial sample which includes the colour change of pH strips shows significant result at different pH and may be used to measure pH of wide range of sample. The presence of bioactive compounds in *Brassica oleracea* extract can be substituted as natural indicator with wide range of samples instead of use of existing chemical indicators.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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