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Studies of Eco- friendly natural acid-base pH indicators properties of two flowering plants from Adi-Nifas and Mai-Nefhi Eritrea

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Abstract
Today, synthetic indicators are the choice of acid base titrations. But due to environmental pollution, availability and cost, the search for natural compounds as an acid base indicator was started. The present vocation highlights the exploit of the aqueous extract of the flowers of *Catharanthus rosea* and *Nerium oleander* as an acid base indicator in acid base titrations. Natural indicators are easy to prepare as well as they are easily available. Promising results were obtained when they were tested against standard synthetic indicators. Titration shows sharp color change with respect to change in pH at the equivalence point. The equivalence points obtained from the flower extracts coincide with the equivalence point points obtained by the standard indicators. Thus natural indicators are found to be a very useful, economical, simple and accurate for the said titration.

Keywords: Synthetic Indicators, Natural pigment, flowers extract of Catharanthus rosea and Nerium oleander, equivalence point Acid-Base titration

1. Introduction
Eritrea is the country of colorful flowers. People use the colorful flowers for their ornamental use and also to some extent for their medicinal uses since ancient time. Such flowers, like *Catharanthus rosea* and *Nerium oleander* are easily available in Eritrea and also throughout the world in high quantity and present in almost every garden for enhancing the beauty of gardens. The method of wet chemistry such as, titrimetric analysis and gravimetry still has an important role in modern analytical chemistry. The term titrimetric analysis refers to quantitative chemical analysis carried out by determining the volume of solution of accurately known concentration which is required to react quantitatively with a measured volume of a solution of the substance to be determined [1].

Indicators are dyes or pigments that can be isolated from a variety of sources, including plants, fungi, and algae. Almost any flower, for example, that is red, blue, or violet in color, contains a class of organic pigments called anthocyanins (flavonoids) that change color with pH. Anthocyanidins (anthocyanins) are notable plant pigments, which are responsible for the red-violet-blue color seen in plant flowers [2]. They are water-soluble and generally occur in the aqueous cell sap. Structurally, anthocyinidins are polyhydroxy derivations of flavylium chloride [2]. Anthocynidins are classified as flavonoids because of their structural similarities to flavone – the base structure of certain plant pigments (flavones, flavonols, etc) known as flavonoids. The anthocyanidin pigments are amphoteric, their acid salts are usually red, base salts green, metal salts blue and in neutral solution anthocynidins are violet [3]. The use of natural dyes as acid-base indicators was first reported in 1664 by Sir Robert Boyle in his collection of essays "Experimental History of Colors." Indeed, Boyle made an important contribution to the early theory of acids and bases by using indicators for the experimental classification of these substances [1].

Almost any highly colored fruit or vegetable or flower petal has the potential for use as an acid base indicator. Acid-base indicators are commonly employed to mark the end of an acid-base titration or to measure the existing pH of a solution. These are substances that reveal, through characteristic color changes, the degree of acidity or basicity of solutions. Indicators are weak organic acids or bases that exist in more than one structural form (tautomers) of which at least one form is colored. Intense color is desirable so that very little indicator is needed; the indicator itself will thus not affect the acidity of the solution. Care must be used to compare colors only within the indicator range. The indicator range is the pH interval of color change of the indicator. Some add the most common indicators used for beginning chemistry, because their color change is very obvious which makes them easy to use.

A pH indicator is a halochromic chemical compound that is added in small amounts to a solution so that the pH (acidity or alkalinity) of the solution can be determined easily[4]. Hence a pH indicator is a chemical detector for hydronium ions (H_3O+) or Hydrogen ions (H+) in the Arrhenius model. Normally, the indicator causes the color of the solution to change depending on the pH [5].

Catharanthus rosea is commonly known as Madagascar periwinkle or rose periwinkle related to family *Apocynaceae* (dogbane Family). Madagascar periwinkle is a semiwoody evergreen perennial, usually grown as an annual in the flower bed. In frostfree climates, it develops a woody stem near the base and can get 2-3 ft (0.6-0.9 m) tall and spread out just as wide. As annuals, they are usually smaller and more prostrate. Madagascar periwinkle has opposite glossy leaves about 2-3 in (5.1-7.6 cm) long, borne on fairly rigid stems. The five petaled flowers are typically rose pink, but among the many cultivars are those with pink, red, purple and white flowers. The flowers are tubular, with a slender corolla tube about 1 in (2.5 cm) long that expands to about 1.5 in (3.8 cm) across. They are borne singly throughout most of the summer. Like other members of the dogbane family, the broken stem of Madagascar periwinkle exudes a milky latex sap [5, 6]. This periwinkle is native to Madagascar. It has escaped cultivation and naturalized in most of the tropical world where it often becomes a rampant weed. It is established in several areas in the world. Madagascar periwinkle is grown commercially for its medicinal uses in Australia, Africa, India and southern Europe.

Madagascar periwinkle contains a virtual cornucopia of toxic and useful alkaloids. The leaves were sometimes smoked for their narcotic (but dangerous) effects. The plant has been used for centuries to treat diabetes, high blood pressure, asthma, constipation and menstrual problems. More recently, extracts from Madagascar periwinkle have been shown to be effective in the treatment of various kinds of leukemia, skin cancer, lymph cancer, breast cancer and Hodgkin's disease. Indeed, Madagascar periwinkle is a modern day success story in the search for naturally occurring anticancer drugs. However, Madagascar periwinkle is poisonous if ingested or smoked. It has caused poisoning in grazing animals. Even under a doctor's supervision for cancer treatment, products from Madagascar periwinkle produce undesirable side effects [6].

Nerium oleander is commonly known as *oleander*, is an evergreen shrub belongs to Apocynaceae family which can reach up to 240 in (6.1 m) height but is usually seen trimmed at 72-120 in (1.8-3.1 m) [7]. It forms a rounded mound to about 120 in (3.1 m) wide. It is a tough, versatile plant with showy summertime flowers in white, red, pink, salmon and light yellow. Leathery, lance shaped leaves range from about 4-10 in (10.2-25.4 cm) long, depending on variety and are bright green. Oleanders have a tendency to become leggy - overgrown individuals should be pruned as needed to maintain a nice shape. A popular variety is 'Petit Salmon' which is a dwarf that grows to only 48 in (1.2 m). It is widely distributed in Mediterranean and subtropical Asians regions [8, 9]. This plant is also cultivated in tropical and subtropical parts of the world. It is an urbanite plant widely used for ornamental purposes in streets, gardens, and hospitals.

All parts of the plant, including the sap, either fresh, dried or boiled, are poisonous to humans, animals, certain insects, fish and birds, but now a day's number of pharmacological activities are determined by different scientists[10-17].The leaves contain cardiac glycosides like oleandrin, oleandrigenin, digoxin, digitonin, digitoxigenin, nerizoside, neritaloside, odoroside [18, 19].The flowers and leaves are used in folk medicine for the treatment of a wide variety of diseases including infection, malaria, autoimmunity, abscesses, asthma, allergy, eczema, dysmenorrheal, epilepsy, anti-bacterial activity, antnociceptive activity, HIV and cancer [20, 21]. The root is bitter, 6aphrodisiac; tonic is good for chronic pain in the abdomen and pain in the joints, very poisonous, but an antidote to snake-venom and reveals antibacterial activity [22].

There have been numerous reports of poisoning and death from ingestion of oleander, oleander leaf tea, and its extract. It has killed adults, children, pets, and livestock. Even a small amount of oleander can cause death due to its effects on the heart [23-25]. Inhaling the smoke from burning oleander or eating honey made from its nectar can produce poisonous effects.

Extracts from any part of the oleander plant should not be used except under the careful observation and controlled conditions of a clinical trial according to the American Cancer Society [26]. As flavonoids are present in flowers of *Catharanthus rosea* and *Nerium oleander* and are pH sensitive [27] it was hypothesized that the flower extract could be utilized as an indicator for different types of acid base titrations.

The intention behind this study is to explore the potentials of using some of these flowers: *Catharanthus rosea* and *Nerium oleander* with water extract as a source of natural indicator for simple acid-base titration. The outcome of the study could promote the use of the plant materials as a raw material for industrial production of chemicals such as indicators, pH papers, dyes and other potential products for foreign exchange. Unlike some commercial indicators that are known to have detrimental effects, this study anticipates that indicators from natural sources could reduce both environmental pollution and the toxic effect on users. This will also encourage farmers regarding cultivation, collection of plants as well as to industry regarding preparation of above indicators, which lead financial support to both farmers as well as industry [28].

Materials and Methods

Collection of plant materials

Flowers of *Catharanthus rosea*, from Bietgergish, and of *Nerium oleander*, from Adi-Nifasi collected in the months of April and May, 2014 and were identified and authenticated by Prof. Ghebrehiwet Medhanie, Taxonomist in Eritrea Institute of Technology (EIT), Mai Nefhi, Eritrea (East Africa).

Chemical and Reagents

All chemicals and reagents of analytical or synthetic grade were used. HCl, NaOH, CH_3OOH, NH_3, methyl red indicator, phenolphthalein indicator etc were obtained from the laboratory of Department of Chemistry in Eritrea Institute of Technology (EIT). The reagents and volumetric solutions were prepared as per stander procedures.

Glass wares

Burettes, pipettes, Analytical beam balance, evaporating dishes, pH meter, spectrophotometer, refrigerator and pestle and mortar were among the important apparatus and instruments utilized etc were calibrated as per the procedures.

Preparation of flower extract [27, 29].

Flowers *Catharanthus rosea* and *Nerium oleander* were cleaned by distilled water and the petals of both the flowers were shade air-dried and the dried petals were grinded into fine powder with a mortar and petals. The dried powder of petals were soaked in 100 ml hot water (45 ºC) in separate conical flasks, for about one hour. Then the materials were filtered through a what man filter paper to get the clear filtrates (free from suspended material). The filtrates were preserved in amber bottles and stored away from direct sun-light to prevent photolysis and decomposition.

Test for color change

0.1 ml of the extract was added to 20 ml each of buffer solutions of pH ranging from 1.2 to13.78.
The results of the test are listed in table 7.

Experimental for Titrations [27-29].

10 ml of the extract was added as indicator for each titration type- strong acid against strong base, strong acid against weak base. Weak acid against strong base and weak acid against weak base and the trials were repeated 3times to check the precision. The titrations were again performed using phenolphthalein and methyl orange indicator as standard and the results obtained were compared with the results of titrations using plant extract indicator. The results for titrations are depicted in the tables 1 to 3.

Table 1: Titration of strong acid Vs strong base and weak base Vs weak acid.

Titration	0.1N HCl Vs 0.1N NaOH				0.1N Na_2CO_3 Vs 0.1N CH_3COOH			
Extracts	Vol. of titre in ml	Final Vol. of titrant in ml	Vol. of titrant in ml (mean)	pH	Vol. of titre in ml	Final Vol. of titrant in ml	Vol. of titrant in ml (mean)	pH
Catharanthus rosea	10	10.4	10.3	6.17	10	6.7	6.7	3.85
	10	10.2			10	6.5		
	10	10.2			10	6.5		
Nerium oleander	10	10.3	10.2	6.66	10	6.4	6.3	4.23
	10	10.2			10	6.3		
	10	10.2			10	6.3		

Table 2: Titration of weak base Vs strong acid and strong base Vs weak acid.

Titration	0.1N Na_2CO_3 Vs 0.1N HCl				0.1N NaOH Vs 0.1N CH_3COOH			
Extracts	Vol. of titre in ml	Final Vol. of titrant in ml	Vol. of titrant in ml (mean)	pH	Vol. of titre in ml	Final Vol. of titrant in ml	Vol. of titrant in ml (mean)	pH
Catharanthus Rosea	10	7.5	7.5	2.66	10	14.4	14.4	8.68
	10	7.6			10	14.4		
	10	7.5			10	14.5		
Nerium oleander	10	6.7	6.6	4.11	10	14.7	14.6	8.67

Table 3: Results of Different Acids and Base Titrations Using Standard Indicators and *Catharanthus rosea* and *Nerium oleander* Flower Extracts as Indicators with pH Change.

Titrant v/s Titrate	Strength in moles	Indicator	Color	pH at color change
NaOH(10ml) v/s HCl	0.1	MR	Yellow to Red	6.96
	0.1	CR	Green to Pink	6.17
	0.1	NO	Green to Red	6.66
Na_2CO_3(10ml) v/s HCl	0.1	MO	Yellow to Red	3.84
	0.1	CR	Green to Pink	2.66
	0.1	NO	Green to Red	4.11
NaOH(10ml) v/s CH_3COOH	0.1	PT	Deep Pink to Pale Pink	9.02
	0.1	CR	Deep Green to Pale Green	8.68
	0.1	NO	Green to Pink	8.67
Na_2CO_3(10ml) v/s CH_3COOH	0.1	MR	Yellow to Red	2.24
	0.1	CR	Green to Pink	3.85
	0.1	NO	Green to Red	4.23

*HCl: Hydrochloric acid, CH_3COOH: Acetic acid, NaOH: Sodium hydroxide, Na_2CO_3: Sodium carbonate,
MO: Methyl orange, PT: Phenolphthalein, CR: Catharanthus rosea extract, NO: Nerium oleander extract.

Chemical Test for flavonoids:

Preliminary chemical tests show the presence of flavonoids in the water extracts of *CR* and *NO*. The results of the chemical tests are shown in Table4.

Table 4: Chemical Tests of Flavonoids on *CR and NO* Water Extract.

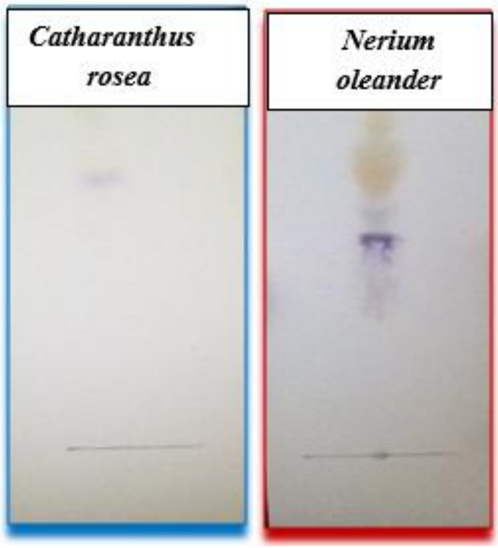
	Chemical tests	Color observed
Catharanthus Rosea	2ml *CR* extract + 2ml 1N NaOH	Green
	2ml *CR* extract + 4ml 1N HCl	Red
	2ml *CR* extract + 2ml saturated $Pb(CH_3COO)_2$	Green-Blue
Nerium Oleander	2ml *NO* extract + 2ml 1N NaOH	Dark Green
	2ml *NO* extract + 4ml 1N HCl	Deep Red
	2ml *NO* extract + 2ml saturated $Pb(CH_3COO)_2$	Dark Green-blue

Preliminary Phytochemical screening

Preliminary photochemical investigation of hot water extracts Catharanthus rosea and Nerium oleander of were presented in table 5. It showed the presence of flavonoids in all the two natural Indicators.

Table 5: Preliminary Phytochemical Screening of Hot Water Extracts of Natural Indicators

Tests	Hot water extract of *Catharanthus rosea*	Hot Water extract of *Nerium oleander*
Alkaloids	-	-
Flavonoids	+	+
Anthocyanidin	+	+
Tannins	+	+
Saponins	-	-



Thin Layer Chromatography of both flower extracts.

For TLC of both flower extracts, the solvent system was selected on the basis of the pigments, flavonoids i.e. rutin. The results are tabulated in the Table 6.

Table 6: TLC Results of Both Flower Extracts

Extract	Solvent System	R_f Value	Color OF Spot
Water extract of *Catharanthus rosea*	Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26)	0.93 0.75	Yellow Violet
Water extract of *Nerium oleander*	Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26)	0.87 0.75 0.70	Yellow Blue Violet

The color effect of the *Catharanthus rosea* and *Nerium oleander* stock solution in different buffer solutions are presented in Table 7. The color effects are similar to those of authentic anthocynidines in identical media.

Table 7: Color Effect of *CR* and *NO* in Different pH Buffer Solutions pH Change with Addition of Indicators

TEST	*PH-*CR*	Observed *CR* Color	*PH -*NO*	observed *NO* color	Inference
2 ml of buffer pH 1 + 2 ml of CR/NO stock	1.65	Pink	1.30	Red	Anthocyanidin present
2 ml of buffer pH 2 + 2 ml of CR/NO stock	2.44	Pink	2.06	Red	Anthocyanidin present
2 ml of buffer pH 3 + 2 ml of CR/NO stock	3.38	Pink	2.85	Red	Anthocyanidin present
2 ml of buffer pH 4 + 2 ml of CR/NO stock	3.93	Pink	3.94	Red	Anthocyanidin present
2 ml of buffer pH 5 + 2 ml of CR/NO stock	4.92	Pink	5.00	Red	Anthocyanidin present
2 ml of buffer pH 6 + 2 ml of CR/NO stock	5.78	Pink	5.87	Red	Anthocyanidin present
2 ml of buffer pH 7 + 2 ml of CR/NO stock	6.98	Green	6.92	Pink	Anthocyanidin present
2 ml of buffer pH 8 + 2 ml of CR/NO stock	6.72	Green	7.89	Pink-green	Anthocyanidin present
2 ml of buffer pH 9 + 2 ml of CR/NO stock	8.84	Green	8.96	Red-green	Anthocyanidin present
2 ml of buffer pH 10 + 2 ml of CR/NO stock	9.77	Green	9.76	Red-green	Anthocyanidin present
2 ml of buffer pH 11 + 2 ml of CR/NO stock	10.82	Green-yellow	10.77	Green	Anthocyanidin present
2 ml of buffer pH 12 + 2 ml of CR/NO stock	11.86	Green-yellow	11.82	Green	Anthocyanidin present
2 ml of buffer pH 13 + 2 ml of CR/NO stock	12.69	Green-yellow	12.93	Green-yellow	Anthocyanidin present
2 ml of buffer pH 14 + 2 ml of CR/NO stock	13.87	Green-yellow	13.76	Green-yellow	Anthocyanidin present

* PH-*CR*=PH of mixture of *CR* and buffer solutions, PH -*NO*=PH of mixture of *CR* and buffer solutions Acid Base titration

The titrations of strong acid with strong base (HCl & NaOH), strong acid with weak base (HCl & Na_2CO_3), weak acid with strong base (CH_3COOH & NaOH), and weak acid with weak base (CH_3COOH and Na_2CO_3) were carried out using standard indicators: methyl orange, phenolphthalein and *Catharanthus rosea* and *Nerium oleander* flowers extracts[30-32]. The results of these titrations are given in Table 3. It could be due to these flavonoids, the sharp end point appeared in the above mentioned titrimetric analyses.

Table 8: Vis spectra analysis of natural indicators.

Hot water extract	Absorbance			λ_{max} in nm
	Ph 1	Ph 6	Ph 13	541
Catharanthus rosea	2.160	0.593	0.693	541
Nerium oleander	1.940	0.338	1.100	541

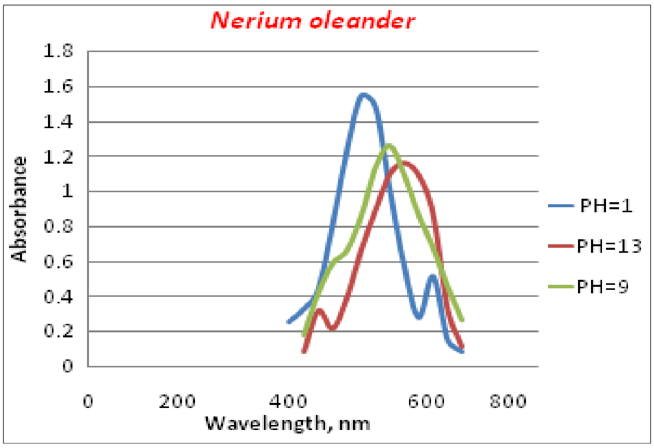


Fig 1: Visible spectra of aqueous *NO* extract in pH 1.0, 13.0 and 9 (where color change occurs) buffers.

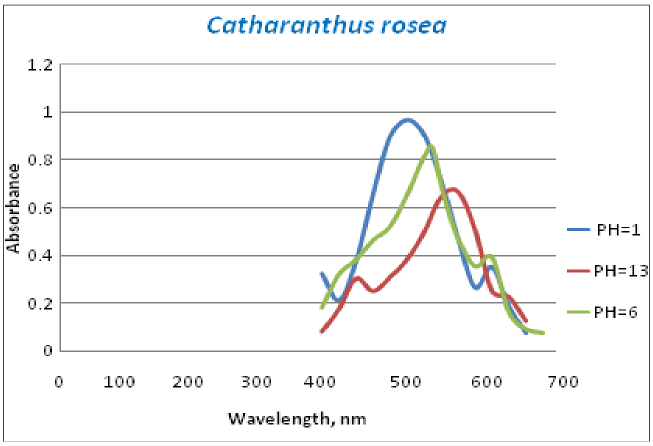


Fig 2: Visible spectra of aqueous *CR extract* in pH 1.0, 13.0 and 6 (where color change occurs) buffers.

Result and Discussion

The solutions of *Catharanthus rosea* and *Nerium oleander* extracts in water are acidic (pH 3.59 and 2.40 respectively) and are red in color. A mixture of an aqueous solution of the extracts and saturated lead (II) acetate gave an intense green-blue coloration; while in concentrated sodium hydroxide solution, the extract became green. These color changes agree with the prescribed colors of anthocynadins in the itemized reagents [31] Thus, indicating the presence of anthocynidins in the petals of *Catharanthus rosea* and *Nerium oleander*. The visible light absorption spectrum of the aqueous extracts of *Catharanthus rosea* and *Nerium oleander* petals with prominent peaks in the 500 – 550 nm wavelength region also confirmed the presence of anthocyanidins Table 8. The typical spectra expressed hypsochromic to bathochromic shift as the medium pH changes from acidic to basic. These spectral changes were in good agreement with the documented spectral behaviour of anthocyanidins in solutions of varying pH.

This, once more, confirms the presence of anthocynidins in *Catharanthus rosea* and *Nerium oleander* petals. Reliable end-points were obtained in titrations, employing the water extracts of *Catharanthus rosea* and *Nerium oleander* petals as end-point indicators. Thus, solutions of *Catharanthus rosea* and *Nerium oleander* can be employed as the endpoint indicators in complexometric and weak acid-weak base titrations. Because, in such titrations the end results were very similar to those reproduced with standard endpoint indicators.

Conclusion

From the results obtained in all the types of acid base titrations, it can be concluded that the sharp color changes which occurred at the end point of titrations is due to the presence of flavonoids (anthocynidins). The standard indicators and aqueous floral extract of *Catharanthus rosea* and *Nerium oleander* show very slight variation in the results. Thus, aqueous floral extract of natural indicators can be used with cent percent reliability and accuracy for acid base titrations. Therefore, the use of natural indicators in acid base titrations is more beneficial because of its economy, easy to prepare, easy availability, pollution free inert and accurate results.

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