



## Original Research Article

# Application of red mangrove plant (*Rhizophora racemosa*) extracts as pH indicator

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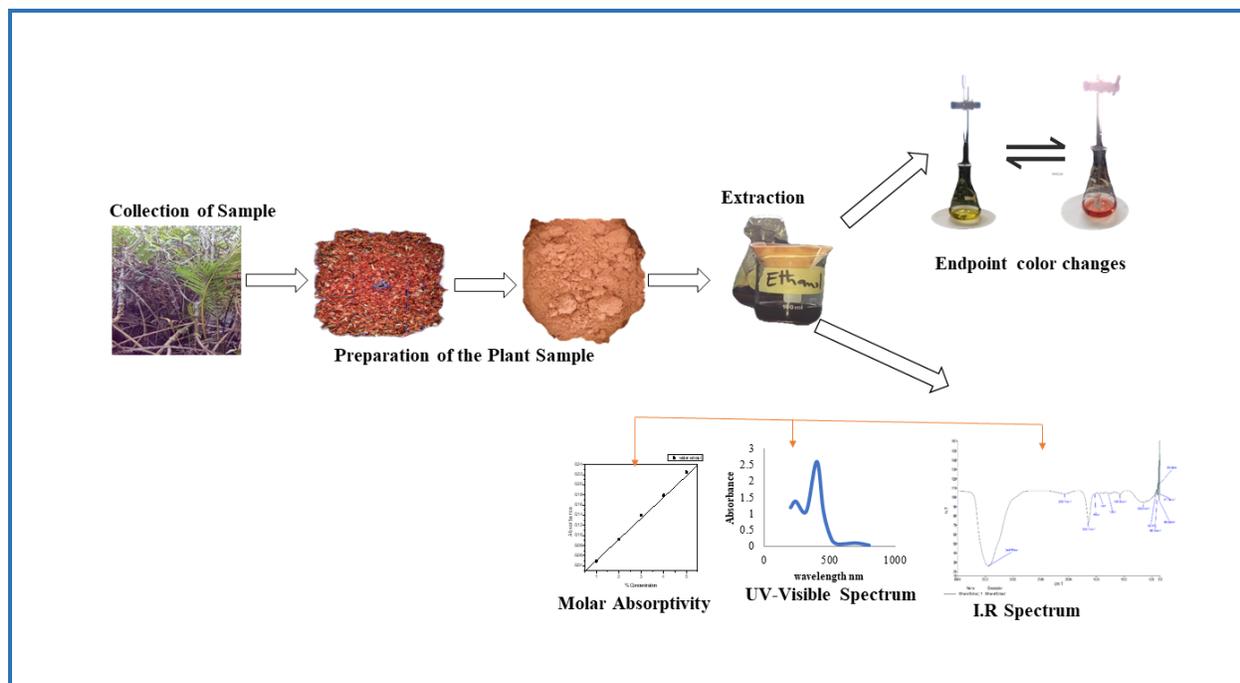
Red mangrove plant

### ABSTRACT

Despite the importance of dyes within the industrial and research settings, the literature lacks information on the utilization of red mangrove forest resources and its applications. In this research study, red mangrove plant (*Rhizophora racemosa*) extracts were characterized and evaluated for its potential as an indicator. The extraction was done using the traditional method of extraction with water and ethanol as solvents. The extracts were characterized using ultraviolet-visible (UV-vis) spectroscopy and Fourier transform infra-red (FT-IR) spectroscopy. Extracts from the plant were applied in different types of acid-base titrations. Following extraction, the UV-vis analyses of the water extract of *Rhizophora racemosa* showed a  $\lambda_{\max}$  at 450 nm while that of the ethanol extract revealed a  $\lambda_{\max}$  at 400 nm. However, after a 72 hour timepoint, the water extract of the *Rhizophora racemosa* showed a  $\lambda_{\max}$  at 559 nm while that of the ethanol extract demonstrated a  $\lambda_{\max}$  at 572 nm. The results of the FT-IR analysis revealed the presence of the O–H for alcohol and phenol, C=O for carboxylic acid, C–H for methyl group, and N–O for the nitro group. Moreover, the endpoints for all titration conducted using red mangrove plant (*Rhizophora racemosa*) extracts were similar to the endpoints obtained using standard synthetic acid-base indicators. This was confirmed by a change in colour of the extracts from yellow in an acidic solution to red wine in the alkaline solution. The results provided established the suitability of *Rhizophora racemosa* as a pH indicator.

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## Graphical Abstract



## Introduction

Environmental pollution issues have awakened the need for green chemicals and processes. Synthetic compounds are likely to pollute the environment [1] and are much more costly [2].

Dyes are coloured soluble compounds and are widely used as materials in most industries. Preparation and application of dyestuffs is one of the oldest forms of human activities. Many garden flowers make beautiful dyes ranging from yellow to orange and brown [3, 4]. Dyes can be made using chemicals such as acids or extracted from plant sources [5]. Natural dyes can be obtained from various parts of plants including, flowers, roots, barks, and nuts which can be processed to obtain many colours [5]. Natural dyes produce vibrant colours, creating a palette that is compatible and blending [5]. Plant research has shown that plants with beautiful colours have anthocyanins which are pH-sensitive [6–8]. Examples of these plant species that have been used as acid-base indicators in titration include *Bougainvillea spectabilis*, *Ipomea nil*, *Opuntia ficus indica*, and *Ixora coccinea* [9]. Furthermore, schools and research laboratories use a wide variety of synthetic dyes as indicators. Although synthetic dyes continue to find applications in both research and academic environments, natural dyes have not been sufficiently explored and used as indicators. It is, therefore, expedient in this regard to research basic ways of extracting and applying natural dyes from local plants as a substitute for synthetic indicators currently used in schools and research laboratories. Synthetic indicators have been linked to several complications including, diarrhoea, pulmonary oedema,

hypoglycemia, and pancreatitis [10–12]. In addition, synthetic indicators have been shown to be associated with abdominal cramps, skin rash, eruptions, erythema, and epidermal necrosis [10–12]. These are indications that the continuous use of synthetic indicators would increase human health complications in the future.

Mangroves are notably special plants that have developed and are surviving in the region between land and ocean in many humid climates of the tropics and subtropics [13]. The major family commonly found in Nigeria is *Rhizophoraceae*, and they are collectively known as the red mangrove. Its species are *Rhizophora racemosa*, *R. harrisonii* and *R. mangle*. Among these, *Rhizophora racemosa* is the most abundant, occupying about 90% of the mangrove forest [14, 15]. Red mangroves are easily distinguishable through their unique prop root system and viviparous seeds. Nigeria has extensive mangrove forests in the coastal region of the Niger Delta. Nigeria's mangrove, in terms of area covered, is the largest in Africa and the fourth largest in the world; the largest being Indonesia followed by Brazil and Australia [16]. The mangrove forest extends from Badagry in the West to Calabar in the East covering a total area of 10,000 km<sup>2</sup> along the coast [14, 17]. *R. racemosa* is largely wind-pollinated [18]. Edu et al. [19] in their study on qualitative analyses and profiling of the plant tissues (leaves, barks and roots of *N. fruticans*, *R. racemosa* and *A. africana*) reported that *Rhizophora racemosa* had the highest mean concentrations of flavonoids and tannins [19]. Generally, mangrove plants are rich sources of saponins, alkaloids, flavonoids, and glycosides [20–25]. They are also rich in sterols, terpenes [25–27], and polyphenols [28].

In Nigeria, several researchers have extracted some dyes from a variety of local plants that are used for various purposes. However, there is no published data on dye extracts from red mangrove plants (*Rhizophora racemosa*) and their application as indicators. It has also been observed that one of the biggest challenges of Africa as a continent is under exploitation and utilization of our mangrove forest resources [14]. This utilization gap has called attention to the utilization of mangrove species, creating an enormous need for a database on the properties and usefulness of mangrove plants. It is, therefore, imperative that we shift our attention from the frequent use of synthetic chemicals to extracts from a more natural source.

## Experimental

### *Materials and methods*

Concentrated hydrochloric acid (HCl, 35-37%), absolute ethanol, and sodium hydroxide were purchased from Simmyfranks West Africa Ltd chemical distributors. Acetic acid, ammonium hydroxide, methyl red and methyl orange were purchased from the JHD, China. Phenolphthalein was purchased from the loba chemie, India through joechem venture Nigeria chemical distributors.

### *Preparation and extraction of dyes from plant*

The plant sample was collected from the Buan Mangrove forest in khana local government area, rivers state, Nigeria and was identified. The plant was identified in the department of plant science and biotechnology, faculty of sciences rivers state university. The plant sample was washed with distilled water to remove sand and dirt and was air-dried for 7 days. The dried sample was pulverized and stored in airtight cellophane. 10 gr of the red mangrove plant was soaked in 100 mL of different solvents-ethanol and distilled water and was kept for 48 h to achieve an appreciable exhaustive extraction of the active constituents in the plant samples. The extracts of the samples were filtered, and the filtrates were pre-concentrated [29, 31].

### *UV-vis spectroscopy analysis*

1 mL of each of the plant extracts was diluted in 100 mL of pure distilled water. Each aliquot was transferred into a quartz cell (1 cm pathway) and analyzed in a jenway UV/vis spectrophotometer. The extracts were scanned from 300 nm to 600 nm to generate the characteristic absorption spectra of the sample [29, 31].

### *FT-IR spectroscopy analysis*

5 mg of lyophilized dye extracts were mixed thoroughly with 195 mg of potassium bromide (KBr) until homogenized in an agate mortar. The mixture was placed into the sample compartment of a smart collector diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). This was then analyzed using a thermo nicolet nexus 670 FT-IR spectrophotometer equipped with a DTGS KBr detector and a purge gas generator at a spectral resolution and wavenumber precision of 0.09 and 0.01  $\text{cm}^{-1}$ , respectively. For each spectrum, 32 scans were used [31].

### *Titration using plant extracts and standard indicators*

Standard solution (0.1 M) of HCl,  $\text{CH}_3\text{COOH}$ , NaOH and  $\text{NH}_4\text{OH}$  was prepared in a 50 mL flask. Then 0.1 M HCl or 0.1 M  $\text{CH}_3\text{COOH}$  was titrated against 25 mL of 0.1 M NaOH or 0.1 M  $\text{NH}_4\text{OH}$  using three drops of the water and ethanol extracts each as indicators: in the order of strong acid/strong base (0.1 M HCl/0.1 M NaOH); strong acid/weak base (0.1 M HCl /0.1 M  $\text{NH}_4\text{OH}$ ); weak acid/strong base (0.1 M  $\text{CH}_3\text{COOH}$ /0.1 M NaOH) and weak acid/weak base (0.1M  $\text{CH}_3\text{COOH}$ /0.1 M  $\text{NH}_4\text{OH}$ ). The same procedure was adopted for similar titration using three drops of methyl orange, methyl red and phenolphthalein as standard indicators [1, 2, 4, 11, 12, 29].

### *Molar absorptivity of red mangrove (*Rhizophora racemosa*) extracts*

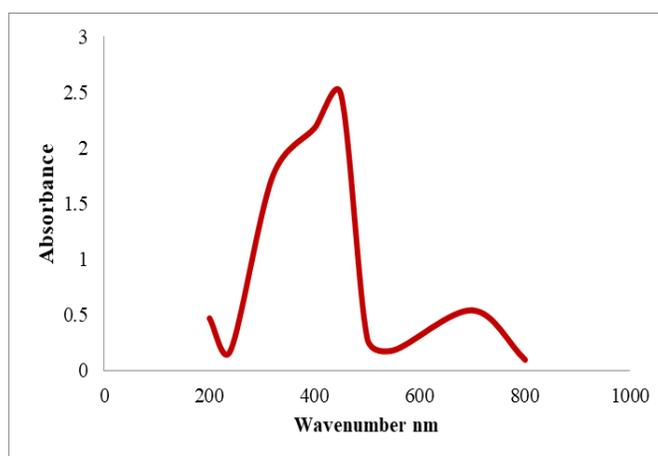
1% solution was prepared by measuring 1 mL of the plant extracts and diluted in 99 mL of pure distilled water for each of the extracts. Same was repeated for 2%, 3%, 4%, and 5% respectively [29]. The absorbances of the prepared solutions were measured at 450 nm for water extract and 400 nm for ethanol extract using the CECIL CE 1021 1000 series spectrophotometer. The concentration versus absorbance was plotted. The slope of the line-of-best-fit was determined through the data points. The slope of the line was divided by the path length (depth of the cuvette) to calculate molar absorptivity [29].

## Results and discussion

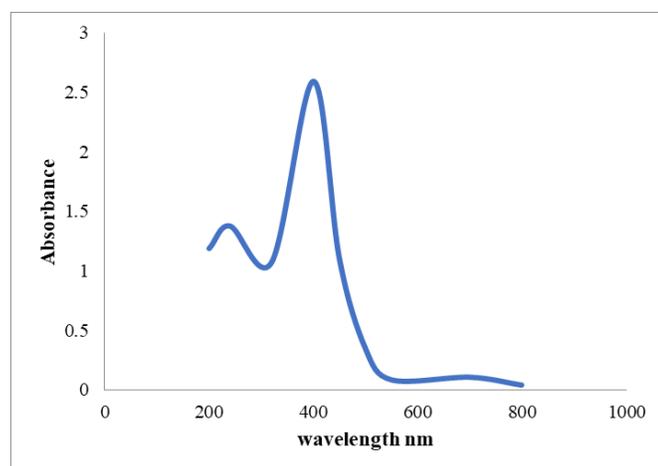
### UV-vis spectroscopy results of red mangrove plant (*Rhizophora racemosa*) extracts

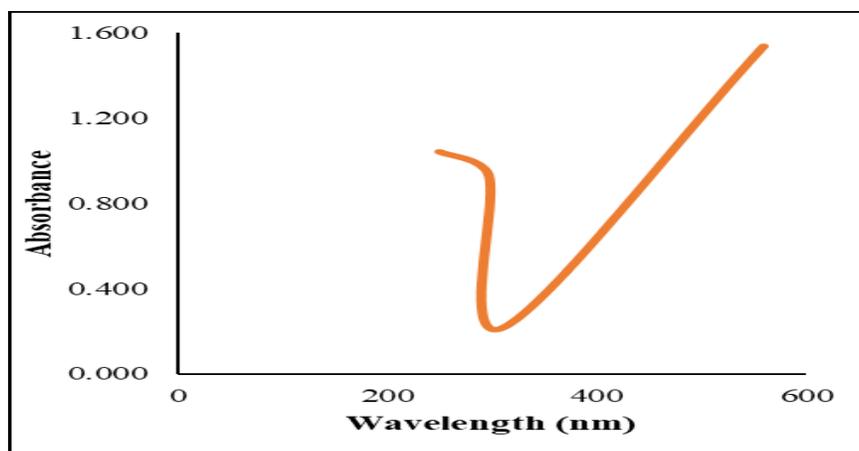
Following extraction, the UV-vis analysis of the water extract of *Rhizophora racemosa* revealed a  $\lambda_{\max}$  at 450 nm (Figure 1) while that of the ethanol extract showed a  $\lambda_{\max}$  at 400 nm (Figure 2). However, after a 72 h timepoint, the water extract of the *Rhizophora racemosa* demonstrated a  $\lambda_{\max}$  at 559 nm (Figure 3) while that of the ethanol extract showed a  $\lambda_{\max}$  at 572 nm (Figure 4).

**Figure 1.** UV-visible spectrum for water extract

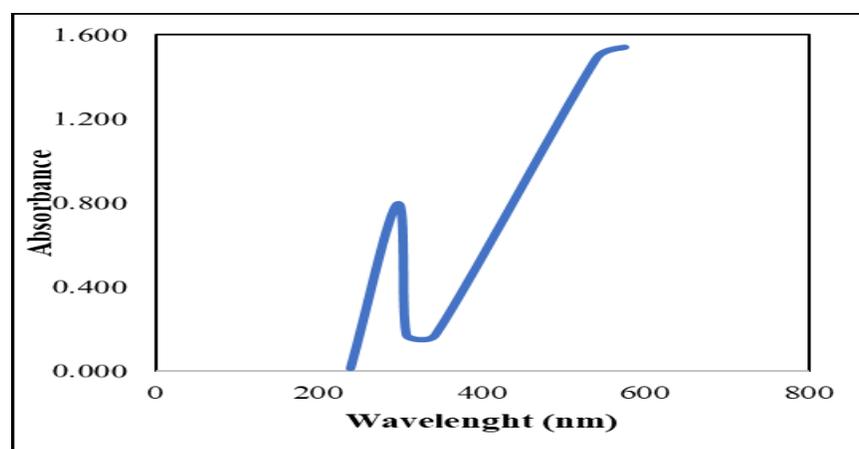


**Figure 2.** UV-visible spectrum for ethanol extract





**Figure 3.** UV-visible spectrum for water extract

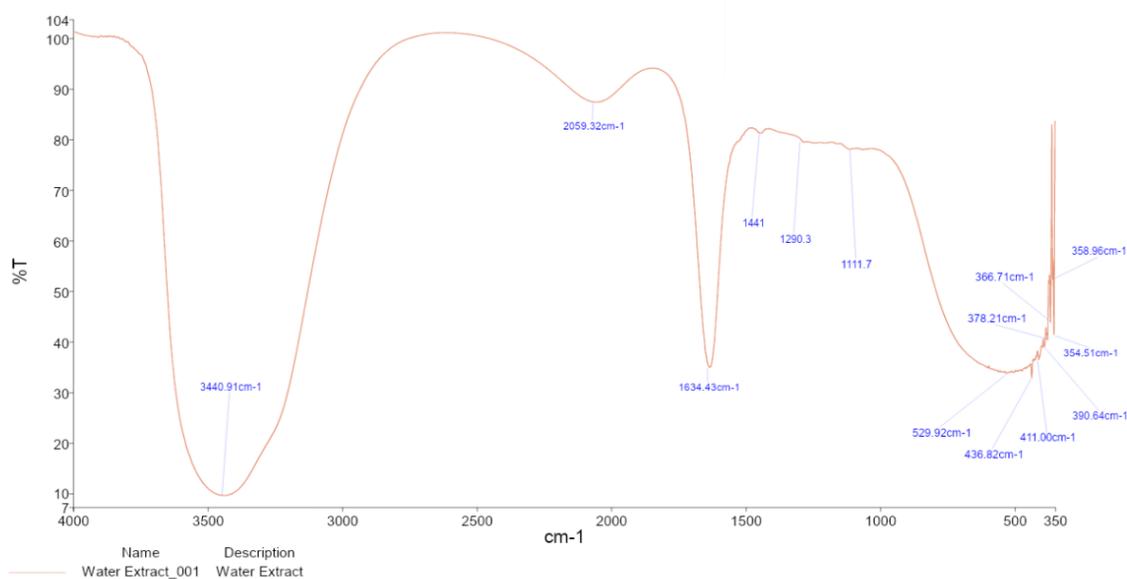


**Figure 4.** UV-visible spectrum for ethanol extract

Izonfuo *et al.* [29] also obtained a  $\lambda_{\max}$  of 520 nm for the ethanol extracts of *Hibiscus rosasinensis* [29]. Findings from the present study agree with the reports by Sudarshan *et al.* [30] and Espinosa-Morales *et al.* [31] that extracts from plants could absorb at 596 nm, 555 nm and 537 nm, respectively. Okoduwa *et al.* [10] also reported the maximum absorption of the plants *Rosa setigera*, *Allamanda cathartica* and *Hibiscus rosa-sinensis* to be 620 nm and 640 nm, 580 nm and 640 nm, 640 nm and 670 nm. These findings agree with the findings from the present study which showed that plant extracts absorbed within the visible region of the electromagnetic spectrum. Phenolphthalein absorbs at 557 nm, methyl red absorbs at 410 nm and methyl orange absorbs at 505 nm. Findings from the present study showed that the plant extracts absorb similar to those of standard indicators.

*FT-IR results of red mangrove plant (Rhizophora racemosa) extracts*

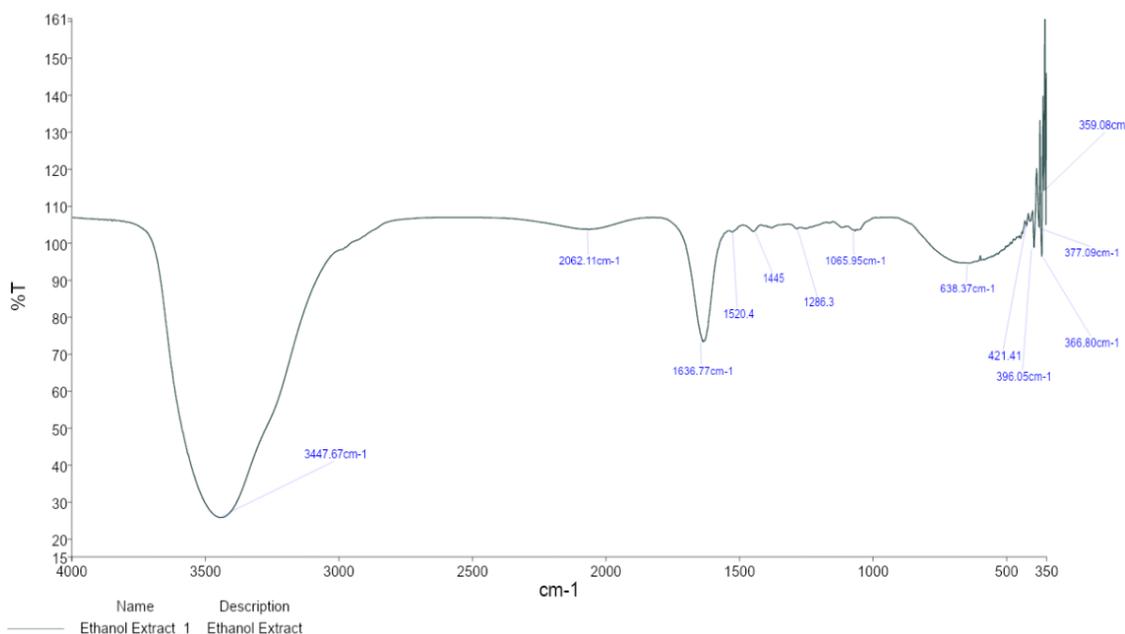
Results of the FT-IR characterization of the water and ethanol extracts of red mangrove plant to determine the functional groups are presented in Figures 5 and 6, respectively. For the water extract (Figure 5), the vibrational frequency at  $3440.91\text{ cm}^{-1}$  can be assigned to O-H vibrational stretching. The vibrational band at  $3440.91\text{ cm}^{-1}$  can be due to the presence of the C-H and O-H groups related to the stretching vibration, alcohol and phenol O-H groups [32]. The vibrational band at  $1634.43\text{ cm}^{-1}$  can be assigned to C=O group since it falls within the range of C=O stretching,  $1849\text{-}1634\text{ cm}^{-1}$ . The peak at  $1441\text{ cm}^{-1}$  can be due to the C-H in a ring group,  $1290.03\text{ cm}^{-1}$  can be assigned to C-O stretching. The peak at  $1111.07\text{ cm}^{-1}$  can be assigned to the C-O stretching.



**Figure 5.** FT-IR spectrum of the water extract

For the ethanol extract (Figure 6), the vibrational frequency at  $3447.67\text{ cm}^{-1}$  can be assigned to the O-H vibrational stretching. The vibrational band at  $3447.67\text{ cm}^{-1}$  can be due to the presence of the C-H and O-H groups related to the sugar vibration, alcohol and phenol O-H groups. The vibrational band at  $1636.77\text{ cm}^{-1}$  can be assigned to C=O group since it falls within the range of C=O stretching,  $1849\text{-}1634\text{ cm}^{-1}$ . Peaks at  $1445.03\text{ cm}^{-1}$  can be assigned to  $\alpha\text{-CH}_2$  stretching. Vibrational frequencies at  $638$  and  $421.41\text{ cm}^{-1}$  can be C-H deformation of the compounds present in the sample. The frequencies observed in this study agree with those reported by Al-Alwani [32] and Nhapi [33] for the characterization of *Strelitzia reginae* flowers dye and *Eichhornia crassipes* dyes. The present findings also agree with the reports by Espinosa-Morales *et al.* [31] in their research on the characterization of a natural dye by spectroscopic and chromatographic techniques on *Justicia Spicigera Schltidl* (Chak Lool plant). From the vibrational bands obtained from the extracts, it could

be concluded that the functional groups associated with the carbohydrate molecules, phenols, flavylum cation and cyanidin-derived anthocyanins might be present in the red mangrove plant extracts.



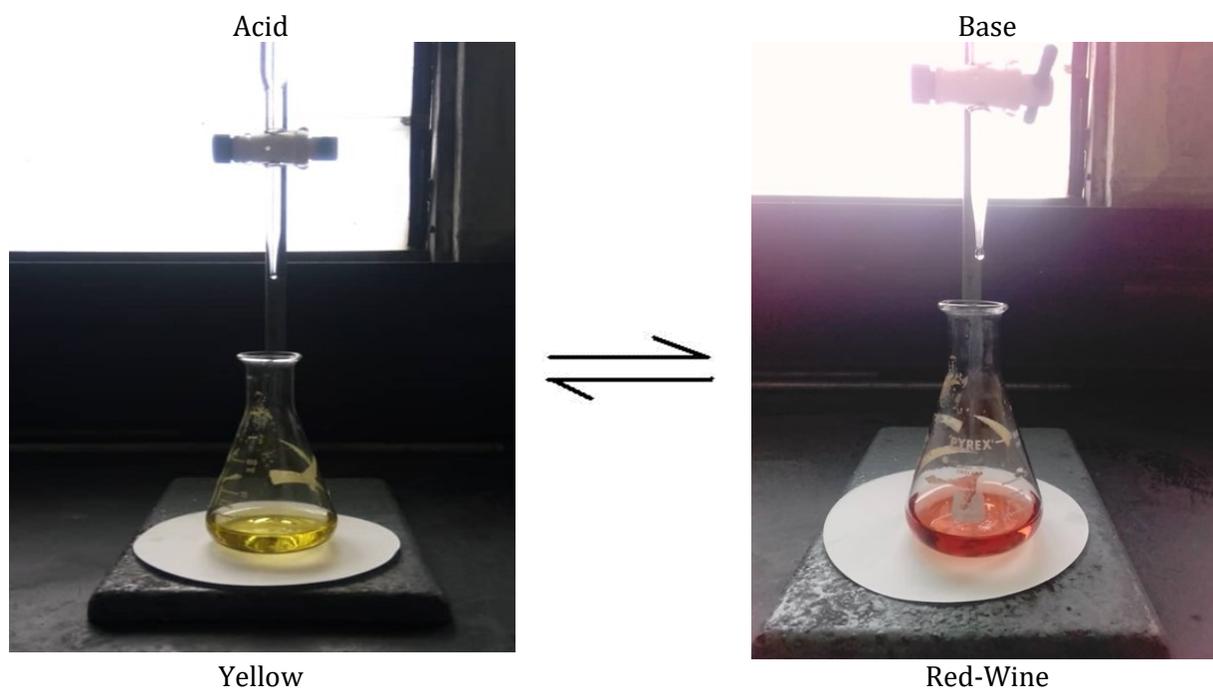
**Figure 6.** FT-IR spectrum of the ethanol extract

### *Titration*

The endpoints for all titration conducted using red mangrove plant extracts as an indicator were very close to the endpoints obtained using the standard synthetic acid-base indicators-methyl orange, methyl red and phenolphthalein (Figure 7). The results of the titre values obtained using the red mangrove plant extracts and the standard indicators are presented in Table 1.

### *Endpoint colour change from titration*

The endpoint colour change from the titration is presented in Figure 7. It was observed that the red mangrove plant extracts showed a sharp yellow colour in acid and at endpoint produced a red wine colour in alkaline solutions. Organic compounds that can be used as an indicator in the titration has characteristics of changing colour when the pH of the solution changes. The red mangrove plant extracts were also sensitive to change in pH and revealed a red wine colour change at alkaline pH and yellow colour at acidic pH. Izonfou *et al.*, obtained endpoints comparable to those obtained using methyl red, methyl and phenolphthalein [29]. The present study reported the suitability of the red mangrove plant extracts as an indicator in weak acid/weak base titrations. Findings from the present



**Figure 7.** Endpoint colour changes

**Table 1.** Endpoint results in titrimetric analysis using synthetic and natural indicators

Titration	Indicator	Titre value	Colour change	pH range
HCl vs NaOH	Methyl Red	25.53±0.7	Red - Yellow	11.7-1.4
	Methyl Orange	26.63±0.2	Red - Yellow	11.8-4.3
	Phenolphthalein	23.53±0.4	Colorless - Pink	
Ethanol Extract of <i>Rhizophora racemosa</i>		26.03±0.1	Yellow - Red-Wine	11.7-0.8
	Water Extract of <i>Rhizophora racemosa</i>	26.70±0.2	Yellow - Red-Wine	11.6-0.8
HCl vs NH <sub>4</sub> OH	Methyl Red	3.00±0.1	Red - Yellow	9.2-1.3
	Methyl Orange	3.02±0.0	Red - Yellow	11.8-4.3
	Phenolphthalein	2.02±0.0	Colorless - Pink	
Ethanol Extract of <i>Rhizophora racemosa</i>		4.03±0.1	Yellow - Red-Wine	11.6-4.3
	Water Extract of <i>Rhizophora racemosa</i>	4.17±0.2	Yellow - Red-Wine	11.6-4.3
CH <sub>3</sub> COOH vs NaOH	Methyl Red	26.37±0.8	Red - Yellow	11.8-4.3

	Methyl Orange	25.67±0.2	Red - Yellow	11.7-4.5
	Phenolphthalein	25.17±0.2	Colorless - Pink	11.6-4.4
	Ethanol Extract of <i>Rhizophora racemosa</i>	25.43±0.1	Yellow – Red-Wine	11.6-4.3
	Water Extract of <i>Rhizophora racemosa</i>	28.1±0.3	Yellow – Red-Wine	11.6-4.3
CH <sub>3</sub> COOH vs NH <sub>4</sub> OH	Methyl Red	9.5±0.1	Red - Yellow	9.2-3.3
	Methyl Orange	5.4±0.1	Red - Yellow	11.6-4.3
	Phenolphthalein	3.02±0.0	Colorless - Pink	9.2-3.2
	Ethanol Extract of <i>Rhizophora racemosa</i>	3.37±0.1	Yellow – Red-Wine	11.6-4.3
	Water Extract of <i>Rhizophora racemosa</i>	3.3±0.1	Yellow – Red-Wine	11.6-4.3

\*All values for titre are mean ± S.D. for n=3

HCl: Hydrochloric acid, CH<sub>3</sub>COOH: Acetic acid, NaOH: Sodium hydroxide, NH<sub>4</sub>OH: Ammonium hydroxide

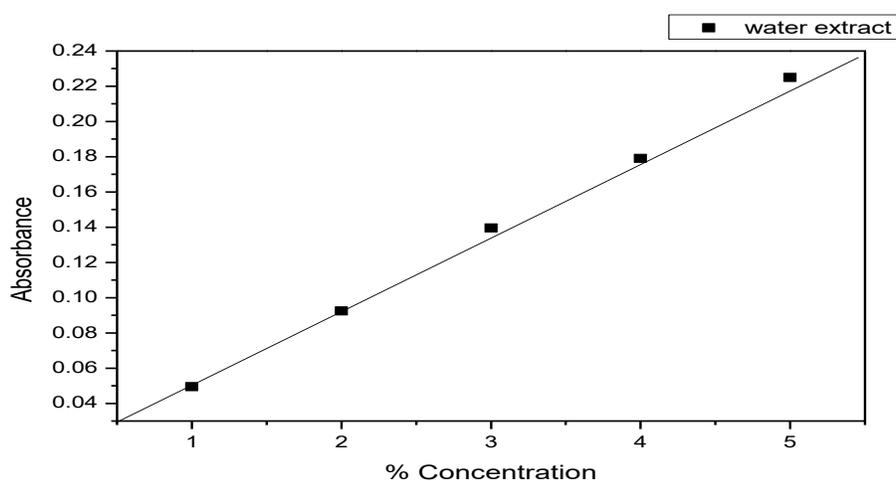
study were found to be in agreement with the study reported by Sudarshan *et al.* [30] which showed that plant extracts changes colour at different pH and can be used successfully as a compound indicator. Onwuachu *et al.* [3] reported that plant extracts can be used as acid-base indicators in the titration of a strong acid with a strong base, as well as weak acid with a strong base. Findings from the present study are consistent with the study conducted by Onwuachu *et al.*

It was observed that the natural indicator (red mangrove plant extracts) when added to the acid produced a sharp yellow colour, an observation that is consistent with the work of Abugri *et al.* [11] and Trivedi *et al.* [2]. Nwokonkwo *et al.* [34] also reported the similarity of standard indicators and demonstrated that these plant extracts could be used as acid-base indicators for volumetric analysis [34]. Okoduwa *et al.* revealed that the natural indicators could be an excellent replacement for synthetic indicators since they are cheap, readily available, simple to extract, non-toxic, and are user and environmentally friendly [10]. Trivedi *et al.* [2] observed that natural indicators give sharp and intense colour change at the neutralization point and show promising results when compared to available synthetic acid-base indicators such as methyl orange and phenolphthalein [2]. The trend of the results obtained in this study agree with other findings reported by Kapilraj *et al.* [35], Nair *et al.* [36], Byamukama *et al.* [37], Eze and Ogbuefi [1], Abugri *et al.* [11], Udachan *et al.* [38], Pathade *et al.*

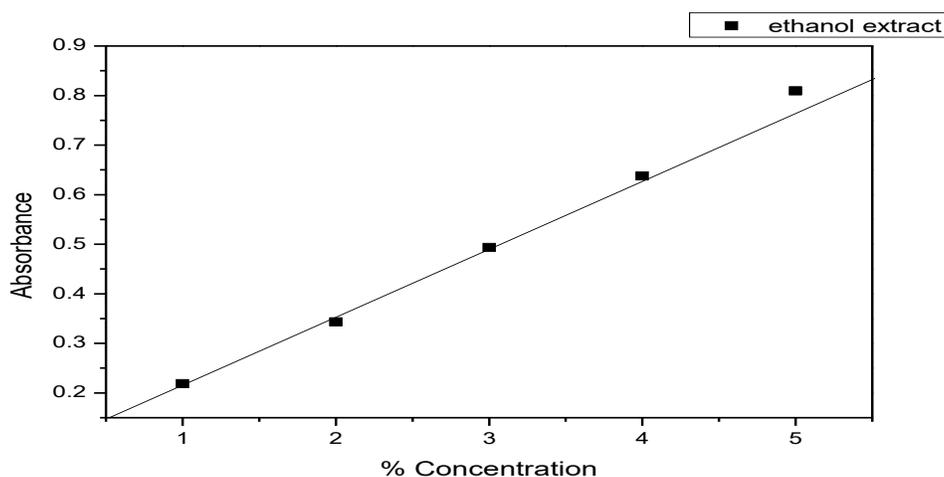
[12], Patil *et al.* [39], Bhagat *et al.* [40], and Nwosu *et al.* [41], all of which support plant extracts as suitable indicators in titration.

#### *Molar absorptivity of red mangrove plant (*Rhizophora racemosa*) extracts*

In this section, how strongly the chemical species in the plant extracts attenuates light at a given wavelength is presented in Figures 8 and 9. From the present findings, it is shown that the molar absorptivity of the extracts from the plant was derived as 0.044 and 0.148 for water and ethanol extracts, respectively. This agrees with the reports of Izonfuo *et al.* [29] in which the molar absorptivity of *H. sabdariffa* and *B. Alba* was determined. The present study agrees with their study showing that as the concentration increases, the beer lamberts' law no longer applies.



**Figure 8.** Molar absorptivity for water extract



**Figure 9.** Molar absorptivity for ethanol extract

## Conclusions

The present study characterized the extracts of the red mangrove plant (*Rhizophora racemosa*) and evaluated their potential as an indicator. The UV-vis analysis of the water extract of *Rhizophora racemosa* showed a  $\lambda_{\max}$  at 450 nm while that of the ethanol extract showed a  $\lambda_{\max}$  at 400 nm for the measurements immediately after extraction. After 72 h the water extract of the *Rhizophora racemosa* demonstrated a  $\lambda_{\max}$  at 559 nm while that of the ethanol extract showed a  $\lambda_{\max}$  at 572 nm. The FT-IR analysis revealed the presence of the functional groups O-H for alcohol and phenol, C=O for carboxylic acid, C-H for methyl group and N-O for the nitro group. The plant extracts showed sharp colour changes at the endpoint and could be used as an indicator in acid-base titration. The colour changed from yellow in an acidic solution to red wine in the alkaline solution at the endpoint. As the extracts produce comparative results with standard synthetic indicators, it can be used with the absolute accuracy and reliability for acid-base titration.

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## Disclosure Statement

No potential conflict of interest was reported by the authors.

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