American Journal of Engineering Research (AJER) 2016
American Journal of Engineering Research (AJER)
e-ISSN: 2320-0847 p-ISSN : 2320-0936
Volume-5, Issue-10, pp-10-18
www.ajer.org
Research Paper Open Access

Yellow Dye Extraction from Eucalyptus Grandis Bark.

Chengeto Zvavamwe[1], Cathrine Khetiwe Mkandhla[2], Clever Mpofu[3], Vernon Phiri[4], Felicity Bgwoni[5], Bettina Khonzokuhle Ncube[6], Mafika Sibutha[7], Joel Tshuma[8*]

[1,2, 4,5, 6, 8]*Department of Chemical Engineering, National University Of Science and Technology,, P O Box AC 939, Ascot, Bulawayo, Zimbabwe*

[3]*Department of Applied Chemistry, National University of Science and Technology, P O Box AC 939, Ascot, Bulawayo, Zimbabwe*

[7]*Quality Assurance Department, Datlabs Pvt Ltd,, 45 Falcon Street Belmont, Bulawayo Zimbabwe*

ABSTRACT: *In this article, yellow dye was extracted from Eucalyptus Grandis bark using methanol solvent at a temperature of 25^{0}C and pressure of 1 atmosphere. The extraction process was optimized by varying extraction material-to-liquor ratio, in the ratios of 1:100, 1:50, 3:100, 1:25, 1:20, 3:50, 7:100, 2:25, 9:100 and 1:10. The extraction pH was varied from 1 to 13 at an interval of pH 1. The mass of the dye extract was found to be directly proportional to the mass of the bark at each pH. The optimum quantity of dye was obtained at a pH of 10 and a mass–to-liquor ratio of 1:10. The dye extract was characterized using UV spectroscopy and the UV spectra obtained showed four peaks at wavelengths of 216nm, 228nm, 280nm and 368nm which corresponded to the primary peak of the aromatic ring, benzoyl part of the conjugated aromatic ring of flavonoids and cinnamyl part of the conjugated aromatic rings of flavonoids functional groups respectively. The UV spectroscopy results were confirmed by analysing the same samples using the IR spectroscopy analysis. The IR spectra showed peaks at 3 300cm^{-1}, 2 900cm^{-1}, 1 665cm^{-1}, 1 450cm^{-1} and 1 050cm^{-1} which corresponded to phenol, alcohol, alkane, ketone and alkene functional groups respectively. The confirmed functional groups are the same functional groups which make up the chemical structure of quercetin which is the yellow dye.*

Keywords: *natural dye, extraction, Eucalyptus, bathochromic shift, polyphenol oxidase.*

I. INTRODUCTION

The natural dyes have been traditionally extracted from animal and plant sources for use in colouring food substrate, leather, wood and natural fibres such as silk, cotton and flax from time immemorial. The invention of synthetic dyes towards the end of the nineteenth century brought about a diminish in use of natural dyes owing to the former's comparative cheapness, reliability of supply, consistency of quality, greater colour fastness with textiles and superior stability in food media [1]. The demand in the use of synthetic dyes has brought about an increase in the research and development in the manufacturing techniques of more dyes suited for a wide array of applications. Currently, the annual productivity of synthetic dyes in the world is estimated at 700 000 tons. The textile industry alone produces up to 200 000 tons of dyes lost to effluents every year, due to the inefficiency of the dyeing process [2]. However, environmental threats posed in the production and application of synthetic dyes has recently steered the revival of consumer interest in natural dyes [3, 4].

The main advantage of using natural dyes is the fact that their source is renewable, biodegradable and reduces environmental impact. They produce very uncommon, soothing and soft shades which are refreshingly different from the strong bright colours produced by synthetic dyes [2].

There are many natural dye sources that have been exploited by researchers. For instance, natural yellow dye has been obtained from Eucalyptus Camaldulensis leaves and was used to dye wool fibre [2].Not much work has, however, been done on the Eucalyptus species which is an important source of natural dye that gives yellow to brown colorants. The Eucalyptus is native of Australia. Over 900 species of the Eucalyptus are in existence [2].

Over 31 000 hectares of Zimbabwean land is covered by Eucalyptus plantations, mostly E. Grandis species [5]. On maturation, E. Grandis trees are de-barked before commercial use. The most common way of disposing the stripped barks is to incinerate them [6]. The incineration of plant material in open air produces carbon dioxide, a greenhouse gas which contributes to global warming. Incineration, destroys habitats for fauna in the surrounding environment, produces smoke which obscures vision causing accidents, and if mismanaged, can result in veld fires which damage property. Eucalyptus Grandis tree bark, however, contains quercetin, a polyphenol, which is its major colouring component [2]. Quercetin is yellow in colour which makes it attractive as a dye compound as primary colours are in demand in the dyeing industry. It has been used in the dyeing of food media in several applications. Quercetin has other pharmaceutical applications. Some studies have shown it to combat human diseases such as cardiovascular illnesses, allergies and possibly immunosuppressant conditions including HIV/Aids [7].

The aim of this study is to extract yellow dye from E. Grandis bark using methanol solvent. The method to be used is the conventional method of extraction which is cheap and environmentally friendly. To the best of our knowledge and literature survey carried out, no work was found on the extraction of yellow dye from Eucalyptus Grandis bark.

II. THEORETICAL BACKGROUND

The major colouring component of E.Grandis bark is quercetin. Quercetin is a brilliant citron yellow coloured flavonoid falling under the flavonol subclass. By definition, it is an aglycone, lacking an attached sugar. The International Union of Pure and Applied Chemistry (IUPAC)) nomenclature for quercetin is 3, 3', 4', 5, 7 – pentahydroxyflavanone [8]. The melting point of quercetin is 316^0C and its density is 1.8g/cm^3. Its chemical structure is shown in Fig. 1:

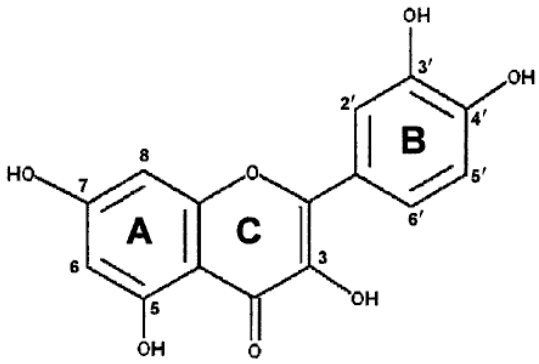


Fig. 1 Quercetin Chemical Structure.

The quercetin chemical structure illustrated in Fig 1 has a benzene ring (A) condensed with a six membered ring (C), which in the 2-position carries a phenyl ring (B) as a substituent. Its major functional groups are diphenyl groups, that is, the A and B rings, alkene group in position 2, 3 of the C ring, an alcohol group on position 3 of the C ring, and a ketone group in position 4 of the C ring [9, 10]. These functional groups can be easily detected using spectroscopy techniques. Quercetin undergoes many chemical reactions but of importance to the extraction process are its solubility and oxidation reactions.

Solubility

Quercetin is generally soluble in glacial acetic acid and aqueous alkaline solutions; slightly soluble in alcohol, and insoluble in water. The solvents that have been successfully used in extraction of quercetin from plant material are methanol and its mixture with water, ethanol and its mixture with water, acetone, dimethylformamide in extraction from onion peels and tetrahydrofuran. Methanol has proven to be a superior solvent for extraction of quercetin for several types of plant material [17].

A quercetin molecule dissolves in methanol solvent by formation of hydrogen bonds between quercetin's alcohol and phenol groups and the alcohol group on the methanol molecules [12]. The reaction is shown in Fig 2.

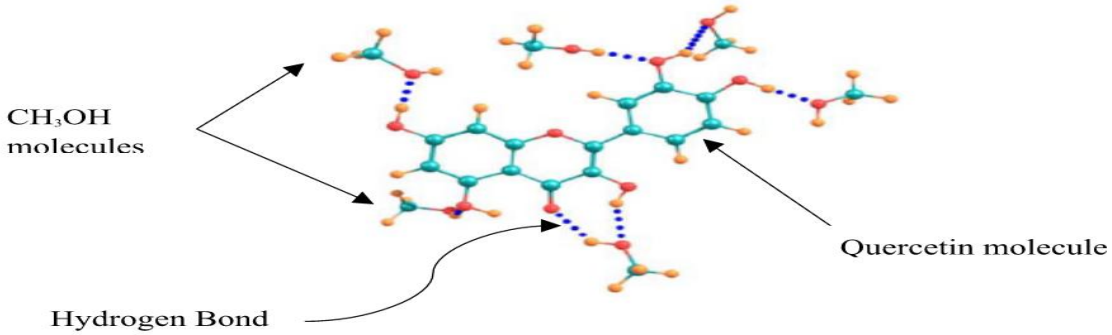


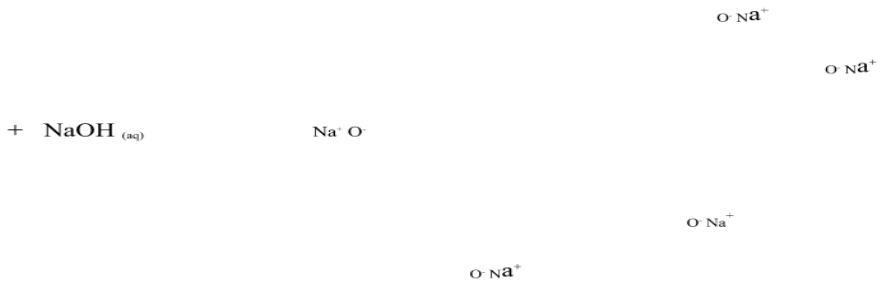
Fig 2 Hydrogen Bond Interaction between Quercetin and Methanol Solvent [14].

The dissolution of quercetin in methanol is limited by the fact that both quercetin, a diphenyl compound and methanol, are weakly acidic, hence the environment created by methanol does not favour high quercetin dissolution rates. The hydrogen bond formation between the quercetin molecule and methanol molecules causes a wavelength shift of the peaks observed when analysing the solution using UV spectroscopy. The degree and direction of shift is dependent upon the number of hydrogen bonded hydroxyl groups in the quercetin structure. A shift towards longer wavelengths is observed as the number of hydrogen bonds formed within a quercetin molecule increases [12].

Alkaline Extraction

Adjusting the pH of the methanol extraction bath to alkaline conditions has a positive effect on the dissolution rate of quercetin. In ionic environments, the phenol groups in quercetin, which are responsible for its weakly acidic nature, can lose the H^+ ion to form a phenoxide ion which is stabilised to some extent. In the presence of Na^+ ions from sodium hydroxide solution, the phenoxide ion reacts with Na^+ ion in a neutralization reaction to form a salt, that is, sodium phenoxide, thus aiding dissolution, according to the reaction below [13, 14]:

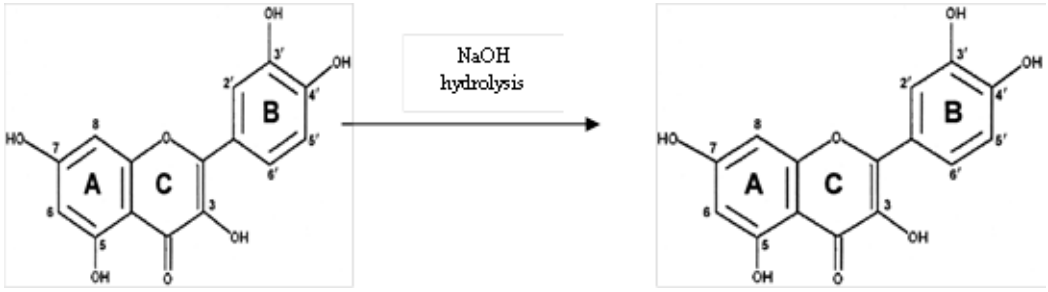
Equation 1:



The formation of sodium phenoxide, an organic compound different from the quercetin aglycone molecule, will result in a wavelength shift of the peaks in the UV-vis spectrum of quercetin. Alkaline environments generally cause a shift to shorter wavelengths, that is, hypsochromatic or blue shift [11].

The quercetin molecule is typically found in plants in its free form as an aglycone, but also as a carbohydrate conjugate. Quercetin glycone conjugates including rutin also known as Quercetin-3-rutinoside, thujin also known as quercitrin, Quercetin-3-L-rhamnoside and 3-rhannosyl quercetin, are hydrolysed in alkaline environments to give the quercetin aglycone molecule [18, 19]. A typical reaction of a rutin molecule is given below:

Equation 2:



rutin quercetin

The extraction in alkaline environments therefore increases quercetin yield through hydrolysis.

Inasmuch as alkaline hydrolysis is beneficial in increasing quercetin yield by breaking down its glycosides, it also results in the breaking down of cell wall structures of the plant material which results in release of normally insoluble Non- Extractable Polyphenols (NEPP) which are found in the matrix of the cell wall, as well as dissolution of the holocellulose and suberin of the plant material into the extraction solvent [20, 21]. The dissolved holocellulose and suberin increase the concentration of non-dye impurities in the liquid dye extract. One major NEPP that is released into the solvent is tannin [20]. Tannin is brown in colour therefore it affects the colour of the extracted dye. Tannin also contains chromophores which means that it will absorb radiation in the UV-vis region of the electromagnetic spectrum. This has the effect of distorting the UV spectrum of quercetin. Alkaline hydrolysis is dependent on concentration of NaOH, implying that its effects increase with increase in NaOH concentration [20].

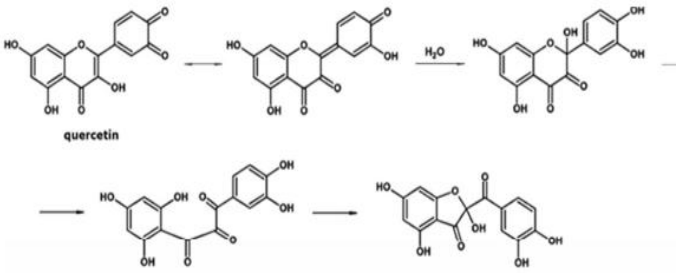
Acid Extraction

Acidic environments have no positive effect on extent of recovery of quercetin. This is because quercetin is a weak acid and since the extraction conditions are acidic, there is a high concentration of hydrogen ions in the bath, and therefore quercetin will retain the H^+ ions on its phenol groups. However, in acidic conditions, acid hydrolysis takes place in much the same way as in alkaline hydrolysis [17]. Quercetin glycosides are broken down to quercetin aglycone and cell walls of plant material are broken down releasing NEPP and dissolving holocellulose and suberin into the solvent [20, 21]. Acid hydrolysis is temperature dependent and its effects are felt at temperatures above room temperature [20].

Oxidation

Quercetin is highly unstable and will readily decompose when exposed to UV-irradiation, electrochemical oxidation, enzyme catalyzed oxidation but also to atmospheric oxygen. Reports on the oxidative degradation of quercetin have been made by several researchers. The oxidation pathway of hydroxyl compounds is given by the distribution of various dissociation forms in solution. Quercetin is decomposed to 2-(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one and subsequently to 2-(3,4-dihydroxyphenyl)-2-oxoacetic acid, 2,4,6-trihydroxybenzoic acid and 3,4- dihydroxybenzoic acid [22].

Equation 3:



At a pH interval between 5 and 7, polyphenol oxidase (PPO), the main enzyme involved in the oxidation of phenolic compounds, oxidises quercetin in a process known as enzymatic browning. The activity of this enzyme is pH

dependent hence it will readily oxidise polyphenols when the pH is between 5 and 7. The activity of PPO is irreversibly inhibited at lower pH values. The enzymatic browning results in deactivation of the dye compound which leads to lower dye yields being obtained. The phenomena also distorts the colour of the dye [23].
The oxidation of quercetin in plant material can be minimized by treating the plant material with tert-butylhydroquinone or ascorbic acid of concentrations not greater than 2g/L before the extraction process [24, 25].

III. METHOD

The following equipment were used:

3.1 Equipment

- UV-Visible Spectrophotometer, Model: UV-1800, Manufacturer: Shimadzu Corporation Analytical & Measuring Instruments Division, 2008.
- FT-IR Spectrophotometer, Model: IR Affinity 1S, Manufacturer: Shimadzu Corporation Analytical & Measuring Instruments Division, 2008.
- Electronic top-loading Sartorius AG Gottingen Electric balance, Model: CP323S, 1997.
- Genlab convection oven, Model: N53c, 1998.
- pH meter, Model: pH 700 pH electrode ECFC7252101B, Manufacturer: Eutech Instruments, 2002.
- Dessicator.
- 25x500ml Borosilicate glass beakers.
- 1 x 500ml measuring cylinder.
- 1 x 1 000ml volumetric conical flask.
- 25x50ml volumetric conical flasks.
- 25 x petri dishes.
- 50-500µl micro pipette.
- Spatulas.
- Stirring rod.
- Droppers.
- Filter funnels.
- Ø 3cm Whatman filter papers.

3.2 Reagents

- 99.5% pure ascorbic acid powder, Glassworld Limited, South Africa.
- Distilled water.
- 99.99 wt% pure methanol, SASOL Chemical Industries Limited, South Africa.
- 32 wt% HCl solution, Minema Chemicals Private Limited, South Africa.
- 50wt% NaOH, Minema Chemicals Private Limited, South Africa.

3.3 Reagents Preparation

The reagents stated in section 3.2 were prepared as described below:

3.3.1 Ascorbic Acid Solution Preparation.

2g/L ascorbic acid solution was prepared as below:

- 10g of ascorbic acid powder was measured and poured into a 5 litre container.
- 5 litres of distilled water was poured into i and mixed thoroughly by stirring to obtain a homogeneous solution.

3.3.2 0.1M HCl Solution Preparation.

- 650ml of distilled water was measured into a 1 000ml conical flask.
- 11.4ml of 32wt% HCl solution was pipetted into the 1000ml conical flask in i. above
- The solution was stirred and allowed to cool down,
- Distilled water was added to the solution to the 1 000ml mark, and the solution was mixed thoroughly.

3.3.3 0.1M NaOH Solution Preparation.

- 4g of NaOH pellets were measured into a 500ml beaker.
- 200ml water was added and mixed thoroughly to dissolve the pellets.
- The solution was poured into a volumetric flask.
- The beaker was rinsed with distilled water and poured into the flask.
- More distilled water was added to the volumetric flask to top it up to the mark.

3.4 Bark sampling and preparation.

- Bark was obtained from 100 Eucalyptus Grandis trees occupying 500m^2, whereby 0.5 kilograms of bark was collected from each tree.
- The collected bark was shredded using a percolator into smaller pieces approximately 25cm^2 in size.
- The bark pieces were soaked in 2g/L ascorbic acid solution for 15 minutes to prevent oxidation of dye compounds [17].
- The treated bark pieces were naturally sun dried for a week and continuously weighed until constant weight was obtained [14].
- The dried bark was ground using a knife mill and sieved to a particulate size of less than 0.18mm [21].
- The ground bark was heaped up on a clean surface to form a cone.
- A sample of one kilogram was obtained from the heap in vi using a cone and quota method of sampling (this was the sample used to carry out the experiments).

IV. Experimental Procedure

3.5.1 Dye Extraction

- 200ml of methanol solvent was poured into a Beaker.
- The pH of solution in 1 was adjusted using 0.1M HCl solution to pH 1.
- The pH of the extraction bath was confirmed to be 1 using a pH meter.
- 4g of treated E.Grandis bark obtained from Section 3.4 vii was measured and poured to the beaker.
- The beaker was left for 24 hours at room temperature whilst stirring occasionally.
- The extract was filtered and collected.
- A dry petri dish was weighed (m_1).
- The filtered dye extract was poured into the weighed petri dish.
- The petri dish was put in an oven maintained at 65^{0}C until all the solvent was evaporated.
- The petri dish was placed in a desiccator for 12 hours to cool.
- After cooling, the petri dish was weighed (m_2).
- The mass of the dried extract (m_{de}) was calculated using the formula:

$$m_{de} = m_1 - m_2 \quad (4)$$

Where, m_1 is weight of empty petri dish,
m_2 is weight of petri dish + dried dye extract, and
m_d is mass of the dried extract.

- The steps 1-12 were repeated three times and the masses obtained averaged using the formula:
- (5)
- The experiments were carried out following the same procedure from 1-13 changing the pH at pH intervals from 1 to 13 and Material to Liquor ratio (Weight of bark in gram; amount of solvent in millilitres) in the ratios of 1:100, 1:50, 3:100, 1:25, 1:20, 3:50, 7:100, 2:25, 9:100 and 1:10.
- The average mass of dried extract obtained from each experiment was re-dissolved in 200ml of methanol solvent.
- The obtained solution was used in the UV and IR Spectroscopy analysis.

3.5.2 Analysis Using the UV Spectroscopy

The SHIMADZU UV-Vis Spectrophotometer was used in the analysis.

- 0.5ml of the re-dissolved liquid dye extract was measured into a 50ml conical flask and diluted with pure methanol to the mark.
- The solution was vigorously shaken to ensure uniform dispersion of the dye in methanol solvent.
- Absorbance was selected as the measurement mode for the run and its recording range was set at 1 to 4.

- The range of the wavelength scans was set from 200nm to 400nm and a sampling interval of 1nm.
- 1ml of the liquid dye was pipetted in the cuvette and the sample run.
- The results were recorded and saved.

3.5.3 Analysis Using the IR Spectroscopy

The SHIMADZU FT-IR Spectrophotometer was used in the analysis.

- 0.5ml of the liquid dye extract was measured into a 50ml conical flask and diluted with pure methanol to the mark.
- The solution was vigorously shaken to ensure uniform dispersion of the dye in methanol solvent.
- The spectrophotometer was switched on and left to warm up for about 15 minutes.
- % Transmittance was selected as the measurement mode.
- The range of the wavelength scans was set from 500cm^{-1} to 4 500cm^{-1} at a sampling interval of 1cm^{-1}.
- The cuvette was rinsed with distilled water several times and then with methanol.
- A micropipette was used to measure 1 ml of the methanol solvent used for extraction into the cuvette. The methanol was used as a blank sample.
- The cuvette from 7 was emptied.
- 1ml of diluted dye sample from 1 was poured into the cuvette and the cuvette was inserted into the spectrophotometer.
- The sample was run.
- The results were recorded and saved.

IV.ANALYSIS OF RESULTS

The results obtained from section 3.5 of the relationship between the mass of the dried extract and the mass of bark at constant temperature of 25^{0}C, extraction time of 24 hours, volume of solvent of 200ml, and varying pH values are shown in Fig 3:

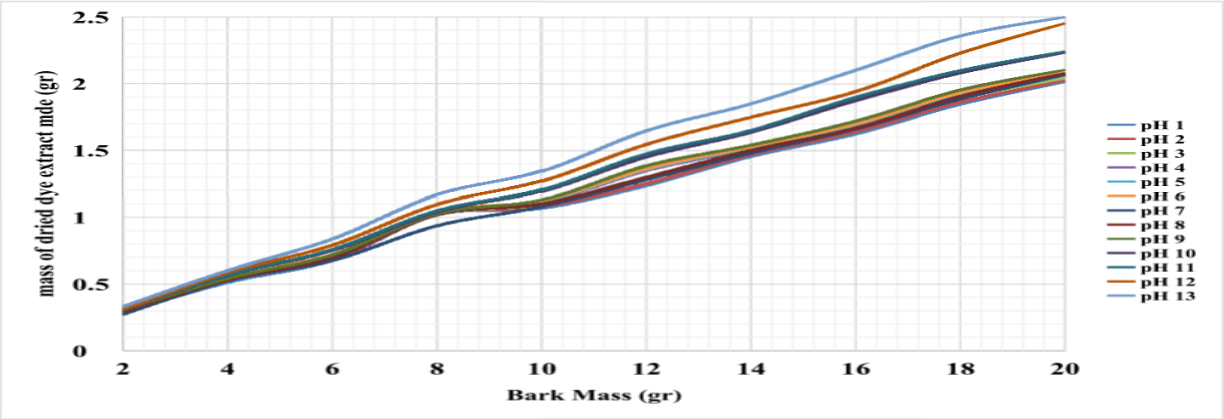


Fig 3 Graph of Mass of Dried Extract against Bark Mass.

The graphs in Fig 3 clearly illustrate a direct proportionality between the mass of dried extract and mass of bark used. The higher yields are obtained at higher bark masses and the lower yields at lower bark masses. The dye yield increases proportionally as the pH increases, that is, higher yields are obtained at higher pH values and lower yields at lower pH values. The visual inspection of the liquid dye extracts showed that the colours obtained at the same pH were lighter at lower bark masses becoming darker as the bark mass increased. The colour intensity increased with increase in pH, from pale yellow in very acidic conditions to brown in very alkaline conditions. A high yield of dried extract is obtained on increase in pH due to the fact that;

- The quercetin molecule is weakly acidic owing to its phenol functional groups, hence will readily dissolve in alkaline environments by the acid-base neutralization reaction therefore increasing the dye yield obtained. The dissolution of quercetin will increase with increase in NaOH solution concentration, hence alkalinity [13, 14].
- In alkaline conditions, alkaline hydrolysis takes place. During alkaline hydrolysis;

- The quercetin glycosides dissolved in the solvent, for example rutin, are hydrolysed into quercetin aglycone increasing the quercetin yield [18].
- The holocellulose structure of the bark is broken down from polysaccharides to monosaccharides which are soluble in methanol therefore increasing the dried extract yield [21].
- Non-extractable polyphenols (NEPP) trapped in the cell walls making up the holocellulose structure are released hence dissolved into the solvent due to the breaking down of the holocellulose structure as described in b [20].

Alkaline hydrolysis is linearly dependent on concentration of NaOH, and will therefore increase with increase in alkalinity of the solution [20].

At lower pH values, the prevailing acidic conditions do not favour dissolution of the weakly acidic quercetin into methanol solvent. This explains why dye yield increases with decrease in acidity of the extraction environment. Acid hydrolysis of the holocellulose structure, as in alkaline hydrolysis, possibly takes place. Acid hydrolysis is only slightly dependent on HCl solution concentration, but highly dependent on temperature, increasing with increase in temperature. Since extraction is carried out at room temperature, the effects of acid hydrolysis on dried extract yield are insignificant compared to those of alkaline hydrolysis [20].

The yield of the dried extract using bark masses less than 6g is approximately the same in the whole pH range. The effect of pH on dried extract yield is not pronounced for these lower bark masses. This suggests that at lower bark masses, the dominating mechanism of the dissolution of dye compounds into the solvent is hydrogen bond formation between the dye compounds and methanol solvent. The other dissolution mechanisms which are acid-base neutralization and alkaline/acid hydrolysis, only significantly increase the dried extract yield at higher bark masses.

Contrary to the general pattern of graphs observed in Fig 3, whereby the dye yield increases with increase in pH, The graphs for pH 5-7 indicate an anomaly in that the yields at this pH values are lower than those obtained at pH 4, which is more acidic. This anomalous behaviour is analysed in the relationship between yield and pH as shown in Fig 4:

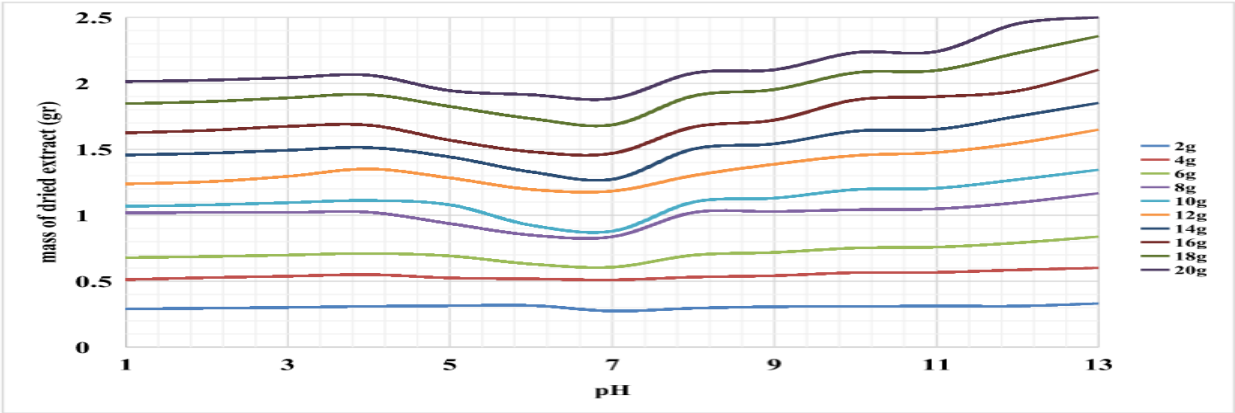


Fig 4 Graphs of Mass of Dried Extract against pH.

The graphs in Fig 4 clearly illustrate that the dye yield increases with the increase in the mass of the bark. The yield at low pH values increases between 1 and 5, and suddenly decreasing from pH 5-7, then starts increasing again to pH 13. The decrease in the yield in the pH range of 5 to 7 is mainly due to the fact that this pH range is the neutralization region. Little to no ions are contained in the solvent, implying that neither dissolution of quercetin by the acid-base neutralisation reaction as in alkaline conditions, nor acid or alkaline hydrolysis for the hydrolysis of quercetin glycosides, release of NEPP from cell wall structures of the bark material into solvent and dissolution of holocellulose and suberin in the solvent occur, both mechanisms which increase dye yield [14, 17, 20, 21] . The dissolution of the dye compounds is by hydrogen bond formation and this results in decrease in dye yield [12]. The significant drop in dye yield in the pH interval between 5 and 7 indicates the importance of the additional dissolution mechanisms introduced by extraction pH adjustment. Also, in this pH interval, that is, between pH 5 and 7, polyphenol oxidase (PPO), the main enzyme involved in the oxidation of phenolic compounds, oxidises quercetin in a process known as enzymatic browning. The activity of this enzyme is pH dependent hence it will readily oxidise polyphenols when the pH is between 5 and 7 [23]. This results in decomposition of the quercetin dissolved in the solvent thereby reducing the dye yield obtained.

UV Spectroscopy Analysis

The UV spectra of some of the dye obtained from section 3.5 are shown in Fig 5:

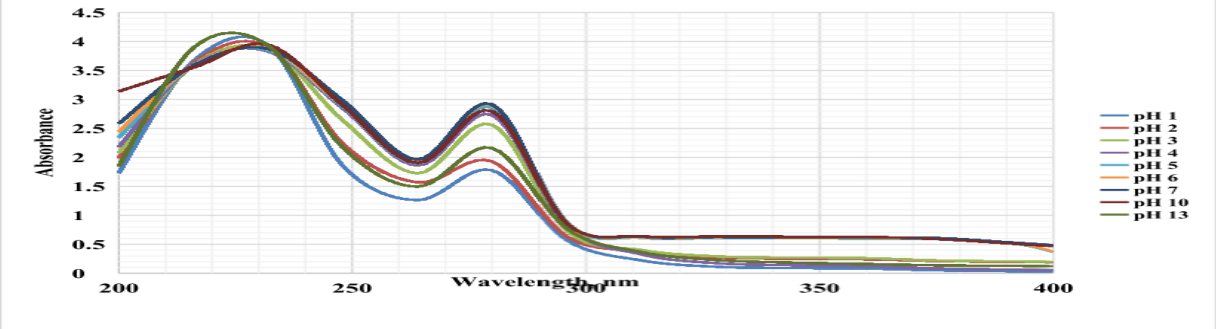


Fig 5 UV Spectra for Liquid Dye Extracts.

The graphs in Fig 5 illustrate a peak at 216nm which disappears in alkaline conditions. In the alkaline media there is a peak which appears at 228nm. This peak observed at 216nm in acidic conditions and 228nm in alkaline conditions can correspond to the primary peak of the aromatic ring functional group. The aromatic ring functional group is the only functional group which produces a primary peak with a tendency to shift to longer wavelengths (bathochromatic shift) in acidic conditions, and to shorter wavelengths (hypsochromatic shift) in alkaline conditions. In the spectra, there is a peak at 280nm in both acidic and alkaline conditions. A peak in this region may be attributed to the electronic transitions of benzene and its derivatives, which may include various aromatic compounds such as phenolics. All flavonoids including quercetin, give a characteristic peak in the 240-285nm range which is attributed to the benzoyl part of their conjugated aromatic rings [11].

The radiation absorption was also evidenced at 368nm for all extracts except those at pH 1, 2, 12 and 13 where the spectrum appeared more or less flat in this region. All flavonoids, including quercetin, give a characteristic peak in the 350-390nm region which is attributed to the cinnamyl part of their conjugated aromatic rings [11]. In highly acidic conditions (pH 1 and 2) and highly alkaline conditions (pH 12 and 13), the cinnamyl structure is oxidized thus destroyed, forming decomposition products which give a different spectrum to that of non-oxidized quercetin [22]. This explains why the graphs of pH 1, 2, 12 and 13 flatten out in this region.

The UV spectrum peaks of quercetin have been shown to shift depending on extraction conditions. The yellow dye extract UV spectra peaks possibly shift due to effects of methanol-quercetin hydrogen bond formation during dissolution, changes in pH conditions and decomposition of quercetin structure caused by oxidation, thus forming new products peaking at different wavelengths [12, 13, 22]. Since the obtained spectra are in close correspondence to that of quercetin, the results therefore suggest presence of quercetin in the liquid extract.

The graphs of dye extract obtained at pH values beyond pH 10 show a significant decrease in absorbance implying a lower concentration of chromophores in the liquid extracts, which is contrary to the results shown in Fig 3 that showed a general increase in dye yield with increase in alkalinity up to pH 13. This observation confirms that there are non-dye compounds that are co-dissolved into the solvent therefore increasing the dried extract yield. These compounds consist of soluble sugars and fatty acids which are also soluble in methanol as well as holocellulose and NEPP which are dissolved from the bark cell wall structure through alkaline hydrolysis. Also, the decomposition of quercetin in highly alkaline conditions has the overall effect of reducing its concentration in the dye extract. The graphs in Fig 5 indicate that the optimum peak is obtained at pH 10, and at higher pH values above 10, the peaks are low. It can therefore be concluded that the optimum dried dye yield can be obtained at an optimum pH of 10.

IR Spectroscopy Analysis.

The samples used for UV spectroscopy were analysed using IR spectroscopy and some of the spectra are illustrated in Fig 6:

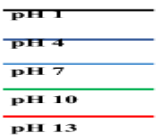


Fig 6 IR Spectra of E.Grandis Bark Dye Extract.

The spectra in Fig 6 clearly show well-defined peaks at 3 300cm^{-1}, 2 900cm^{-1}, 1 665cm^{-1}, 1 450cm^{-1} and 1 050cm^{-1}. The first peak at 3 300cm^{-1} shows presence of either O-H stretch bond, which absorbs between 3500–3200cm^{-1}, or N-H stretch bond which absorbs between 3400-3250cm^{-1}. However, since the band is strong and broad, it is most likely to be caused by presence of O-H bonds which confirms presence of either alcohols or phenols or both in the liquid dye extracts. The presence of the -OH group confirmed by the IR spectroscopy concides with the UV spectroscopy results which gave a peak at 280nm suggesting presence of a phenol. The chemical structure of quercetin shows two phenol rings, that is, A and B ring, and an alcohol group on position 3 of the C ring [10].

The second peak at 2 900cm^{-1} is attributed to C–H stretch bond confirming presence of alkanes. The C ring of quercetin is heterocyclic, composed of an oxygen atom, an alkene group and several alkane groups. Also, on oxidation, quercetin forms decomposition products that have several alkyl groups in their structure as discussed in section 2.0. The alkene group on position 2, 3 of the C-ring is oxidised into a C-C alkane bond. The presence of these alkane groups give rise to the band observed at 2 900cm^{-1}[16].

The third peak at 1 665cm^{-1} confirms presence of either C=O stretch bond which absorbs between 1710–1665cm^{-1} therefore showing presence of α and/or β–unsaturated aldehydes or ketones, or –C=C- stretch bond which absorbs between 1680-1640cm^{-1} therefore showing presence of alkenes. Since the band is strong and not medium, and appears at exactly 1665cm^{-1} which is the cut off range for carbonyls, it confirms presence of a carbonyl [16]. A ketone functional group specifically absorbs in the range of 1680-1700cm^{-1}. However, the position of the band is dependent on hydrogen bonding and conjugation within the molecule. Conjugation with a C=C band results in delocalization of the C=O group, hence causing the absorption to shift to a wavenumber below 1680cm^{-1}, which explains the lower wavelength of 1665cm^{-1} obtained. The band therefore confirms presence of a ketone functional group. Quercetin has a ketone functional group at position 4 of its chemical structure [12].

The fourth peak at 1 450cm^{-1} shows presence of C-C stretch (in-ring) aromatics bond which absorbs from 1500 to 1400cm^{-1}, or C-H bend alkane which absorbs from 1470 to 1450cm^{-1}[12]. Both functional groups are present in quercetin.

The fifth peak at 1 050cm^{-1} may be a result of C-N stretch bond showing presence of aliphatic amines in the dye, or =C-H bend attributed to alkenes, or C-O bond which absorbs between 1320-1000cm^{-1} showing presence of alcohols, carboxylic acids, esters, phenols or ethers. In this region, the band for alkenes is strong, that of amines is medium and that of C-O bond is strong [12]. The observed band in Fig 6 is strong, therefore confirms presence of either alkenes or C-O bond. The quercetin molecule, in its non-oxidized state, has an alkene functional group in position 2, 3 of the C-ring of its chemical structure. The observed peak could be a result of presence of this alkene in the dye extract or the C-O bond in phenol, both functional groups which show presence of quercetin in the dye extract.

The IR confirms that the functional groups present in the liquid dye are phenol, alcohol, alkane, alkene and ketone which are also present in the quercetin chemical structure therefore confirming the presence of quercetin in the dye extract.

V.CONCLUSIONS

In this study, yellow dye (quercetin) was successfully extracted from Eucalyptus Grandis bark using the cheap and environmentally friendly conventional method of extraction. The dye extraction process was found to be dependent on extraction pH, the highest yield of dye being obtained at an optimum pH of 10. The results were not conclusive on optimum material-to-liquor ratio (MLR) of extraction as the highest yield was obtained at the highest MLR of 1:10. Higher yields may possibly be obtained at higher MLRs beyond 1:10 that were not tested for in this research work. The characterisation of the dye obtained using the UV spectroscopy gave four regions of electromagnetic radiation at 216nm, 228nm, 280nm and 368nm which are characteristic of the primary peak of the aromatic ring, the benzoyl part of the conjugated aromatic ring of flavonoids and the cinnamyl part of the conjugated aromatic ring of flavonoids. The presence of these functional groups was confirmed using the IR spectroscopy. The IR spectroscopy gave five peaks at 3 300cm^{-1}, 2 900 cm^{-1} 1 665 cm^{-1}, 1 450 cm^{-1} and 1050 cm^{-1} which confirmed the presence of phenol, alcohol, alkane, ketone and alkene functional groups respectively. These functional groups are the same functional groups which make up quercetin therefore it can be concluded that the dye obtained from the Eucalyptus Grandis bark was the natural yellow dye.

ACKNOWLEDGEMENTS

The authors would like to thank the Datlabs Pvt Ltd Bulawayo Zimbabwe and Border Timbers Company Mutare Zimbabwe for allowing part of this research to be carried out at their premises and providing all the necessary legal information and resources.

REFERENCES

[1.] Welham, R. D. (1963), The Early History of the Synthetic Dye Industry. *Journal of the Society of Dyers and Colourists, 79: 98–105.*

[2.] Mongkholrattanasit R, Kryštůfek J, Wiener J and Studničkova J , (2011), *Natural Dye from Eucalyptus Leaves and Application for Wool Fabric Dyeing by Using Padding Techniques*, Natural Dyes, Dr. Emriye Akcakoca Kumbasar (Ed.), pp 58, 63

[3.] Carmen Z, Daniela S, *Textile Organic Dyes –Characteristics, Polluting Effects and Separation/Elimination Procedures from Industrial Effluents – A Critical Overview*, Romania, Gheorghe Asachi' Technical University of Iasi, pp 56

[4.] Ratna.L, Padhi.B, (2012) Pollution due to synthetic dyes toxicity & carcinogenicity studies and remediation, *International Journal of Environmental Science, 3(3), pp 940, 946*

[5.] Mabugu R and Chitiga M, (2002), Accounting for forest resources in Zimbabwe, *CEEPA Discussion Paper No 7*, CEEPA. University of Pretoria, Pretoria, South Africa, pp 14

[6.] Kenya Forest Services, (2009), *A Guide to On-Farm Eucalyptus Growing in Kenya.*

[7.] Lakhanpal P and Kumar Rai D, (2007), Quercetin: A Versatile Flavonoid, *Internet Journal of Medical Update, 2 (2).*

[8.] Mongkholrattanasit .R, Klaichoi .C, Rungruangkitkrai .N, Punrattanasin .N, Sriharuksa .K, Nakpathom .M, (2013), Dyeing Studies with Eucalyptus, Quercetin, Rutin, and Tannin: A Research on Effect of Ferrous Sulfate Mordant, *Journal of Textiles, pp 1.*

[9.] Pretsch E, Buhlmann P and Badertscher M, (2009), *Structure Determination of Organic Compounds, 4*, Zurich, Switzerland, Springer.

[10.] Pereira D.M et al., (2009), Phenolics: From Chemistry to Biology, *Molecules, 14, pp 2202-2211.*

[11.] Espinosa-Morales Y, (2012), Characterization of a Natural Dye by Spectroscopic and Chromatographic Techniques, *Materials Research Society Symposium Proceedings, 1374.*

[12.] Andrade-Filho T, Ribeiro T. C. S, Del Nero J, (2009), The UV-Vis Absorption Spectrum of the Flavonol Quercetin in Methanolic Solution: A Theoretical Investigation, *The European Physical Journal E, 29 (3), pp 6,*

[13.] Duan Y, (2014), Ultraviolet-Visible spectrum characterizations of Quercetin in aqueous ethanol solution with different pH values, *Journal of Chemical and Pharmaceutical Research, 6 (9), p. 236-240.*

[14.] Mohammad G.U, (2015), Extraction of Eco-friendly Natural Dyes from Mango Leaves and their Application on Silk Fabric, *Textiles and Clothing Sustainability, 1(7).*

[15.] Moffat J, (2000), *Fundamental of UV Visible Spectometry*, Germany, Agilent Technologies.

[16.] Stuart B., (2004), *Infrared Spectroscopy: Fundamentals and Applications*, John Wiley & Sons, Ltd.

[17.] Dmitrienko S.G., Kudrinskaya V.A., and Apyari V.V., (2012), Methods of Extraction, Preconcentration, and Determination of Quercetin, *Journal of Analytical Chemistry, 67(4), pp. 299–311.*

[18.] Orwa et al., (2009), Eucalyptus Grandis, *Agroforestry Database 4.0.*

[19.] Lima M.A et al ,(2013), Effects of Pre-treatment on Morphology, Chemical Composition and Enzymatic Digestibility of Eucalyptus Bark: A Potentially Valuable Source of Fermentable Sugars for Biofuel Production – Part 1, *Biotechnology for Biofuels, 6 (75).*

[20.] Anwei Cheng et al., (2014), Acid and Alkaline Hydrolysis Extraction of Non-Extractable Polyphenols in Blueberries: Optimisation by Response Surface Methodology, *Czech J. Food Sci., 32(3), pp. 218–225.*

[21.] Miranda I, Gominho J, Mirra I and Pereira H, (2013), Fractioning and chemical characterization of barks of Betula pendula and Eucalyptus globulus, *Industrial Crops and Products, 41, p. 299– 305.*

[22.] Bancirova M, (2015), Changes of the Quercetin Absorption Spectra in Dependence on Solvent, *Chemistry Journal 1 (2), p. 31-34.*

[23.] Wissam Z., (2012), Effective Extraction of Polyphenols and Proanthocyanidins from Pomegranate's Peel, *International Journal of Pharmacy and Pharmaceutical Sciences, 4(3).*

[24.] Sultana B and Anwar F, (2008), *Food Chem, 108, p. 879.*

[25.] AlonsoSalces R.M, Barranco A, Corta, E, Berrueta L.A, Gallo B, and Vicente F, (2005), *Talanta, 65 (3), p. 654.*