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Extraction and Characterisation of Mucilage from the herb *Dicerocaryum senecioides* and its use a potential hair permanent

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Abstract

The study was undertaken to isolate and evaluate extracts from the leaves of *Dicerocaryum senecioides* and explore on its potential use as a hair permanent. A mucilage containing bioactive compounds was extracted from dried and pulverized leaves of *Dicerocaryum senecioides* using four extracting solvents with varying polarities *viz* methanol, n-hexane, dichloromethane and water. Metal ions in the extract were analysed by Atomic Absorption Spectrometry (AAS) and removed by Amberlite cationic exchange resins. Solvent-solvent extraction, column chromatography and Thin Layer Chromatography (TLC) were used to further isolate and characterize target compounds. Extracts were tested for reducing ability by iodometric titration and Ultraviolet-Visible (UV-Vis) Spectrophotometric reducing power method. Extracts' hair curling ability was tested on African and Asian hair samples at various pH levels. The dichloromethane (D_2) fraction exhibited both the highest reducing power and best hair curling ability supported by a perm set of 86 % compared to 34 % of hexane (D_1) and 41 % for water (D_3) at an ammonium buffered pH of 9.5. The removal of metal ions was found to

improve extract curling capacity from 62 % to 86 %. Phytochemical analysis of D_2 extract by thin layer chromatography suggests the presence of an unsaturated and polar compound which quenched the fluorescence of Ultra-violet (UV) light at wavelength 254 nm and reacted with vanillin-sulphuric acid reagent to give a dark brown colour.

Keywords: *Dicerocaryum senecioides,* hair permanent, phytochemical, extraction, characterization.

INTRODUCTION

The herb *Dicerocaryum senecioides* is distributed throughout Southern and Central Africa where it is found as a prostate plant with trailing annual stems that grows from a perennial rootstock. In the rainy season the plant forms a mat like structure covering an area of up to $10m^2$ [1]. Its sprawling stems, grow vigorously in summer and less so for the rest of the year. The plant bears distinctive hard fruits with two sharp spines on the upper side. The leaves are opposite, petioled, wedge shaped and deeply lobed. Underneath, the leaves are whitish and deeply hairy while the top layer is gray green [2]. Flowers are trumpet shaped, solitary, axiliary and pedicellate with white to pinkish colour [3]. *Dicerocaryum senecioides* grows in grassland, particularly trampled areas and abandoned fields; usually in sandy soils and shown in Figure 1.

Figure 1: Picture of *Dicerocaryum senecioides*

Fabian and Germishuizen described the genus name *Dicerocaryum* as derived from the Greek words *dikera,* meaning two horns and *karyon*, meaning a nut; referring to

the fruit with its two erect, straight, conical spines [3]. The herb is called by different names in different clans.

Significant work have been done on the use of extracts from *Dicerocaryum senecioides* as an anti-oxidant, anti-inflammatory and anti-proliferate. The findings by Mampuru *et al*.,[4] Madiga *et al*., [5] and Maphahlele [6] confirmed that *Dicerocaryum* species are useful anti-oxidants in the body with strong anti-inflammatory and anti-proliferation activity against cancerous cells. Very little information is available on the nature, composition and structure of the mucilaginous material that occurs in *Dicerocaryum senecioides*. Studies done by Benhura *et al.*, [1] on *Dicerocaryum zanguebaricum*, conspecific to *Dicerocaryum senecioides* revealed that structural elucidation of the extracted mucilage is complicated by the presence of many different sugar monomers found in its backbone and the presence of a complex branching. This together with heterogeneity in molecular weight makes the elucidation of polysaccharide structures more difficult than that of other biological polymers.

Benhura *et al*.'s work on mucilage isolated from *Dicerocaryum zanguebaricum* revealed the presence of sugars: galactose, xylose, arabinose and mannose in the ratio 21:19:12:1. In another study, Benhura and Marume discovered that the mucilage from *Dicerocaryum zanguebaricum* can make an excellent emulsifying agent [7]. No work has been done so far to investigate the use of the herb's extracts as a shampoo or hair permanent despite its use in the rural communities of Southern Africa.

The need to improve personal image has led hair beauty to be the most targeted form of investment in the beauty industry [8]. However, severe consequences for human health due to toxicity of current hair actives have been so rampant and prompted the need to search for safer alternatives [9]. Where applicable, plant products serve as better alternatives to synthetic products because of local accessibility, an eco-friendly nature and affordability. Making use of the plant *Dicerocaryum senecioides* to make hair permanents is worth exploring as the plant lies idle as it has lost its significance in herbal medicine to more sophisticated forms of medication [5].

Since the herb has many traditional uses, an understanding of its structure and functional properties would be necessary in order to most effectively exploit it for industrial use. In line with the objective of the Millennium Development Goal number 7 (M.D.G.7) and to ensure environmental sustainability by 2015 (Millennium Development Goals Report, 2014). We hope to further this work and be able to manufacture a green product with ingredients that induce safer hair curl reformations as an alternative to toxic actives currently on the market.

MATERIALS AND METHODS

Instrumentation

UV-Vis Spectrometer (Perkin Elmer UV/VIS lambda 2) was used to determine the reducing potency of the extracts. Atomic Absorption Spectrometer (Varian Spectra AA 20) was used to determine and quantify the various metal ions found in the extracts. The rotary evaporator (Bùch Rotavapor R-134) was used to remove the extracting solvent at reduced pressure at low temperature. Amberlite cationic exchange resins were used to remove the metal ions from the extracts and a Waring Commercial Blender was used to grind the dried leaves into extractable powder. A pH meter (Corning pH meter 220, USA) was used to measure pH of buffer solutions and allow for necessary adjustments using 0.1 M NaOH or HCl.

General experimental procedures

Samples were labelled and spread thinly on stainless steel dishes and allowed to dry at room temperature. The thoroughly dried material of each sample were crushed and shaken vigorously by hand to separate the leaves from the stems. The stems were then discarded and the dried leaves separately ground into a fine powder using a commercial blender.

Extraction process

An extraction process was used to selectively dissolve some components from the dry grounded leaves of *Dicerocaryum senecioides*. Solvents of different polarities as determined by their dielectric constants were used to ensure maximum selectivity of analytes of different polarities from the matrix.

Extraction with method 1

Initially the powdered leaves were extracted with absolute methanol for 72 hours at room temperature (10 g / 100 ml) with periodic shakings. The extracts were then filtered using Whatman number 1 filter paper and passed through Amberlite IRC cationic resins. The extracts were recovered from the solvent by rotary evaporation at 40 °C. The dried crude residue was then resuspended in ethanol/water (3:1 v/v) and fractionated by solvent-solvent extraction into 3 fractions: n-hexane (D_1 fraction), Dichloromethane (D_2 fraction) and water (D_3 fraction). Fractions were concentrated by rotary evaporation at 40 °C. The procedure was repeated for all samples from different places except one sample which could not be passed through cationic resins in order to investigate the effect of metal ions in the extracts.

Extraction with method 2

Ground leaves (70 g) were suspended in distilled water (1 litre) and the mixture homogenized in a blender with continuous stirring in a water bath at 40 °C. The mixture was cooled and centrifuged at 2000 rpm for 1 hour at 4 °C. The supernatant was removed while the pellets were soaked again in water and agitated prior to further centrifugation. The supernatants were combined and two volumes of 96 % ethanol were added with stirring to precipitate the mucilage. Extracted mucilage was washed twice in fresh 96 % ethanol and dried on a rotary evaporator at 40 °C.

Determination of metal ions

Portions of the methanolic extracts (10 ml) were poured into a beaker and digested with concentrated hydrochloric acid (25 ml) overnight. Samples were gently heated to quicken digestion. When the contents were almost dry, HCl (2 ml) was added and the solution further boiled. Distilled water (50 ml) was added with subsequent filtering. The filtrate was diluted with distilled water (100 ml). This was repeated for all the methanolic extracts from different locations. Calibration standards of zinc, copper, calcium, magnesium, iron and lead and potassium were prepared from 1000 ppm stock solutions. Samples were analysed for metal ions using Atomic Absorption Spectrometer.

Preparation of Buffer solutions

Acetate buffer pH 6.2

To make 0.1 M citric acid 21.01 g of citric acid (analytical reagent grade) was diluted in 1 litre of distilled water and 0.1 M solution of sodium citrate was made by diluting 29.41 g of sodium acetate dehydrate in 1 litre of water. 80 ml of the prepared 0.1 M citric acid was mixed with 920 ml of 0.1 M sodium acetate in 1 L volumetric flask to give a pH of 6.2.

Phosphate buffer pH 7.4

39.5 ml of 0.2 M sodium hydroxide was added to 50 ml of 0.2 M potassium dihydrogen phosphate and the solution was diluted to 200 ml with distilled water.

Ammonia buffer pH 9.5

Ammonium chloride (32.5 g) was dissolved in distilled water (150 ml). 10 M ammonia solution (42 ml) was then added and the contents were diluted to 250 ml with distilled water.

Testing the curling performance of the extracts

Ring test

Curl tests were performed using the ring test previously reported [10]. 10 hairs, each with a length of 8 cm were wound around a curl rod with a diameter of 3 mm and the ends fixed with fast glue. Care was taken to ensure that the fibre loops were parallel to each other and the fibre axis perpendicular to the rod axis. Each rod was treated in a single prescribed test tube with buffered extract solution for 30 minutes and then rinsed in distilled water before being oxidized in a solution of 1.5 % w/w hydrogen peroxide for 5 minutes. After the treatment the hair loops were cut open with a razor blade and dropped into a petri dish which was filled with distilled water. The loops were soaked for at least 30 minutes after which a 200X enlargement of the petri dish was made using a normal photocopier. The distance between the ends of the hair curls was determined with a ruler. The ratio of the cylindrical rod diameter (d_R) over the mean hair ring diameter ~~d~~ yields the degree of set of permanent wave, S_P (Perm set) as given in equation 1.

$$S_p = d_R \frac{100\%}{d} \qquad 1$$

Where the perm set was less than 50 %, the diameter, d, was not measured directly but determined using equation 2:

$$d = \frac{I_R + S}{\pi} \qquad 2$$

Where S is the measurable distance between the fibre ends and I_R is the length of the hair ring

A plot of d against s was then used to determine the approximate value of ~~d~~.

Comparing the curling ability of extracts

Portions of D_1, D_2, D_3 (0.2 g) and water extracts were dissolved in ammonia buffer pH 9.5 and ring tests performed using 10 hair strands on each fraction. Percentage perm sets were calculated, recorded and compared to determine the fraction responsible for curling hair.

Investigating the effect of pH on hair

Black African hair samples, were identified by professional hair dressers as fine, medium and course. Asian hair was also used for comparison. 8 strands of each of the identified hair samples were subjected to procedural ring tests in 10 ml of the four different buffered solutions at pH 6.2, 7.4, and 9.5) mixed with 0.2 g of D_2 extract.

Effect of extract concentration on curling performance

Concentrations of 40, 80, 120, 160, 200 and 240 mg of D_2 extract were each dissolved in ammonia buffer (10 ml) pH 9.5 and ring tests performed to determine the rate of increase of percentage perm set with concentration. Processing time was fixed at 30 minutes.

Comparison of curling performance of D_2 extracts from the four sampling places

To investigate the effect of geographical location on curling performance of D_2 extract, ring tests were performed on course hair using 0.2 g extract in 10 ml ammonia buffer pH 9.5 for each sample. 8 strands of course hair were allocated to each sample and hair ring tests were performed.

Comparing curl performance on Afro and Asian hair

Ring tests were conducted using D_2 extracts on both Asian and African hair origin and the perm set results compared.

Effect of metal ions on curling ability of D_2 extract

Ring tests were conducted using D_2 fraction of the sample which was not passed through ion exchange resins and the results compared with those passed through ion exchange.

Comparison of performance between D_2 extract and commercial perm lotion

Ring tests were performed on hair using the D_2 fraction and perm lotion separately. Perm sets were calculated for comparison.

Determination of extracts' reducing ability by iodometric titration

Extracts of D_1, D_2 and D_3 (1 g) fractions were each re-suspended in methanol (5 ml). 5 ml of HCl (5 ml) was then added and the flask connected to the second round bottom flask with 10 ml of 0.025 N iodine solution. The flask with the extract was

heated for 2 hours to liberate hydrogen sulphide gas which reacted with iodine in the other flask. The contents of the second flask were gently swirled to achieve a uniform mix and poured into a 500 ml flask followed by titration with 0.025 N standard sodium thiosulphate solution to determine the unreacted iodine by back titration. Towards the end point, a few drops of starch solution were added and the titration continued until the blue colour disappeared. Reducing ability was measured as sulphide concentration and calculated using equation 3:

$$\frac{(mL\ I_2\ x\ N\ I_2) - (mL\ titrant\ x\ N\ titrant) \times \left(\frac{32.06\ g}{2\ eq.}\right)}{sample\ weight\ (kg)\ or\ sample\ volume\ (L)} = sulfide\ (mg/kg\ or\ mg/L)$$

Spectrophotometric determination of extracts' reducing power

The reducing power of extracts were determined according to a previosly described method [11](Alarm *et al*.,). Different amounts of each extracts (200-1000 µg/ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricynade [$K_3Fe(CN)_6$] (2.5 ml, 1 %). The resulting mixture was incubated at 50 °C for 20 minutes in a water bath. After cooling 10 % trichloroacetic acid (2.5 ml) was added and the solution centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power. Ascorbic acid was used as the positive control.

Thin Layer Chromatography

Thin Layer Chromatography was used to determine the chemical components of the plant extracts by UV light visualisation and staining with vanillin-sulphuric acid reagent.

Statistical treatment of data

The experimental results were expressed as mean values ± standard error of the mean (SEM). Levels of statistical significance were calculated using the paired student t-test when comparing two groups, or by analysis of variance (ANOVA) for more than two groups. P values of ≤ 0.05 were considered significant. SPSS software was used for ANOVA data analysis to obtain the $F_{calculated}$ values which were compared with the $F_{critical}$ values at $\alpha = 0.05$ (5% level of significance).

RESULTS AND DISCUSSION

Dichloromethane extract of *Dicerocaryum senecioides* shows the capacity to reduce the disulphide bonds of hair at a pH of 9.5 as evidenced by a perm set of 86% on Afro course hair and 97% perm set for Asian hair. When curling ability of D_2 extract was compared to that of commercial ammonium thioglycolate perm lotion using the *t*-test, there was no evidence at 95% confidence interval to suggest a difference in the curling ability. Commercial ammonium thioglycolate had a hair perm set of 91% compared to 86% for D_2 extract. The capacity of D_2 extract to reduce and soften hair disulphide bonds for curling was found to depend on pH and hair texture as shown in Table 1.

Table 1: Results for Ring test as determined by percentage perm set on different textured hair at different pHs

	MEAN PERCENTAGE PERM SET			
pH of buffer	Fine hair %	Medium hair %	Course hair %	Asian hair %
6.2	71	44	35	67
7.4	83	68	60	71
9.5	99	92	86	97

The D_2 extract, as a reducing agent might have a similar mechanism of cleaving the disulfide bond (CyS-SCy) in hair keratin as that of thio-perms [12].

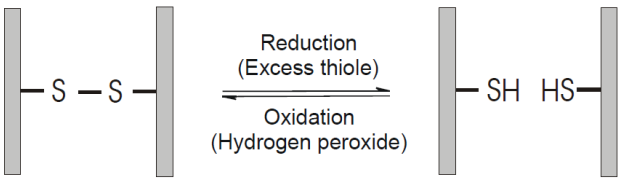

Figure 2: Schematic diagram of reduction and oxidation of two polypeptide chains which are linked by a disulphide bridge [12]

Effect of pH on D_2 curling performance

Hair curling with D_2 extract was shown to be influenced by pH. When tested on virgin course hair, a pH of 9.5 resulted in 86% perm set followed by a 60% perm set at a pH of 7.4 and lastly 35% perm set for a pH of 6.2. Alkaline solutions soften and swell the

hair and open the cuticle. D_2 extract has an acidic pH in distilled water (pH 4.5) and hence had no capacity to swell the hair or penetrate into the cortex. Low pH products and styling treatments reduce the hair's porosity by constricting the cuticle and causing it to tighten, hence the need to add an alkali to the solution. High pH products have the opposite effect and increase the hair's porosity by swelling and lifting the cuticle scales.

Coarse hair with a strong, resistant cuticle layer needs the additional swelling and penetration that is provided by a more alkaline waving solution. By contrast, porous hair, or hair with a damaged cuticle layer, is easily penetrated and could be damaged by a highly alkaline permanent waving solution. Fine hair is more porous than course hair and therefore quickly absorb chemicals into the cortex of hair were disulphide bonds are reduced into thiols for a curl reformation. The alkalinity of the solution should correspond to the resistance, strength, and porosity of the cuticle layer.

Effect of metal ions on hair shampooing and curl reformations

Metal ions have an oxidising effect on the active hair permanent and therefore must be eliminated before effective curl reformations. The extract which was passed through ion exchange had an increased curling ability of 86.2% perm set compared to 66.2% when the extract was not subjected to ion exchange. When present in hair or perm lotion, alkali metal ions compete with hair for thiols. The metallic bond formed between thiols and metal ions is more energetically favoured compared to the temporary thiol-disulphide transition bond formed during disulphide reduction.

Hair is capable for extracting hard ions from water during hygiene practices due to the presence of ligand nitrogen and carboxylic acid functional groups in its amino acids. These ligand compounds contain electron-donating functional groups that form stable, cage-like structures with metal cations [13]. The chelating ability of hair increases with increase in pH as its binding capacity increases due to more carboxylic acid groups of hair protein being deprotonated and thus available for metal interaction. The situation is even worse with damaged and treated hair as it contains more anionic moieties for binding with metal ions.

Permanents which have thiols as their active curling components require hair pre-shampooing in order to remove any metal ions in the hair. However, failure to use compatible shampoos may lead to hair and scalp damage. In the case of hair curl reformations with dichloromethane extract (D_2), the water extract was used as a shampoo since it's known to be an excellent shampoo [1].

Comparison of samples from different geographical places

There was no evidence at 95% confidence interval to suggest a difference in the mean percentage perm set results of D_2 extracts from different geographical places (Table 3).

Table 3: ANOVA table for perm set results as determined by samples from different geographical places

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	**62.075**	**3**	**20.692**	**0.883**	**0.459**
Within Groups	**843.300**	**36**	**23.425**		
Total	**905.375**	**39**			

Decision rule: Reject H_0 if Fcritical < F calculated , F calculated = 2.84

Since $F_{critical} > F_{calculated}$ we fail to reject H_0 and conclude that there is no sufficient evidence at 5 % level of significance to suggest a difference between the curling abilities of D_2 extracts sampled from different geographical places.

Matopo sample had the lowest yield of 11.46% while Ntabazinduna had the highest yield of 12.48%. Thus, the *Dicerocaryum senecioides* species within the four sampled places do not have much difference in curling ability. The small noted differences may be due to different exposure to air and light since the extracts were shown to degrade in the presence of light. Iodometric titration results showed no significant difference in the sulphide concentration of D_2 extracts from different geographical places.

Reducing power for extracts

All the extracts' reducing ability increased with increase in concentration (Figure 3); however D_2 extract exhibited the highest reducing power.

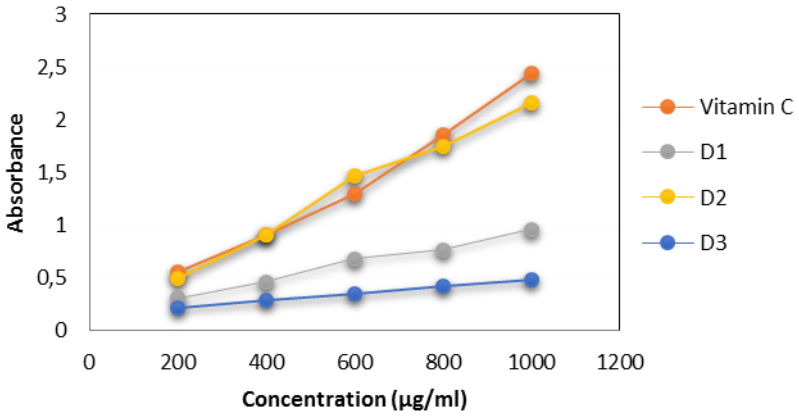

Figure 3: Graphical presentation of reducing power of different extracts as reflected by absorbance of Pearl's Prussian blue at wavelength 700 nm. Vitamin C was used as the positive control

The reducing power of D_2 was comparable to that of ascorbic acid. The high reducing power might be the cause of the extract's curling ability since the curling process involves reduction of disulphide bridges to free thiols that can undergo curl reformation. At a concentration of 1000 ppm, the extracts D_1, D_2 and D_3 showed absorbance of 0.962, 2.157 and 0.485 respectively. At the same concentration, ascorbic acid, the positive control had an absorbance of 2.440. This reducing ability is in agreement with previous work done [4].

Solvent extraction methods

Methanol, a solvent of lower polarity (dielectric constant of 33) had the extract with higher curling ability compared to water extraction alone (dielectric constant of 80). Thus, it is evident that few individuals who use the rural practice of curling their hair only extract a few portion of the active component since water is not the best extracting solvent. This, together with variations in the extracting water's pH and individuals' hair texture contribute to the characteristic associated recurrent inconsistent hair curling results. Solvent-solvent extraction of the methanol extract resulted in the dichloromethane (dielectric constant of 9.1) extract having both the highest reducing ability and curling ability. Thus the compound of interest is polar than hexane but less than water. On developing the proper solvent for TLC analysis, the extract separated well on a polar solvent (EMW) as shown in Figure 4. Although the compound could move along with the non-polar solvent (BEA), it could not properly separate. These observations were not expected since the extracting solvent, dichloromethane is of low polarity. This might be due to sample dissolution by

DMSO solvent which could have unlocked some polar functional groups from the extract resulting in better separation and mobility in polar mobile phase. The polarity of the compound is also supported by the ability of the extract to mix with the polar ammonium buffer solution.

Characterisation by chromatographic methods

In an attempt to identify the active curling compound(s), the D_2 was subjected to column chromatography. The fractions were analysed using UV light and vanillin-sulphuric acid reagent The compound degraded to give other compounds which were active under UV light but when column chromatography was performed with the column covered with opaque paper, there was no evidence of compound degradation.

TLC plates were subjected to UV light soon after elution to determine compounds that are active under UV light. The dominating compound likely contains aromatic rings [14]. Compounds containing aromatic rings absorb UV light at 254nm and therefore quench the fluorescence of the pigment present in the silica gel as shown in Figure 4.

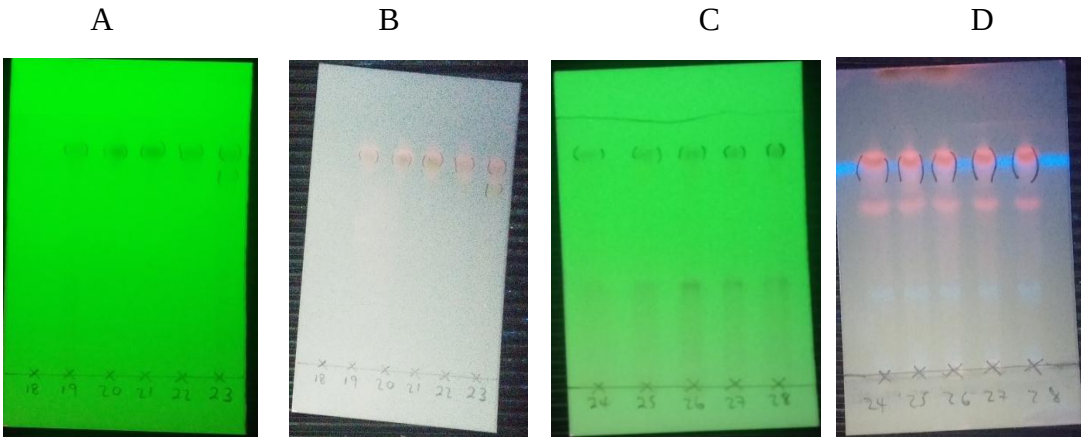

Figure 4: TLC chromatograms of fractions 18-23 observed under UV light at 254 nm (A) and visible light at 366 nm (B). TLC chromatograms of fractions 24-28 under UV light at 254 nm (C) and under visible light at 366 nm (D)

F_{254} silica gel TLC plates were used because they fluoresce with 254nm wavelength absorption. Compounds that typically appear in UV light are aromatic compounds, α, ß unsaturated compounds and any of such groups with extensive conjugation [14]. Conjugated compounds appear as dark sports because they block the fluorescence by absorbing the UV light on a green background.

CONCLUSION

The dichloromethane extract of *Dicerocaryum senecioides* proved to be able to curl hair more predictably than can be done by the hexane and water extracts. The curling performance depends on pH, the higher the pH the greater the curling ability. The best curling performance was achieved at a buffered pH of 9.5. Ring tests performed on Asian and African hair revealed that Asian hair was easily curled by the D_2 extract compared to African hair. Metal ions were found to reduce the curling strength of the D_2 extract as evidenced by an increase in percentage perm set when the ions were removed by ion exchange. The D_2 extract was found to be a strong reducing agent comparable to vitamin C. Characterisation of column chromatography fractions by thin layer chromatography under UV light revealed the presence of an aromatic and moderately polar compound which is very unstable on exposure to light.

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