Chapter One

Introduction and literature review

1.1 Natural dyes: An overview

Natural dyes are dyes or colorants derived from plant sources(roots, berries, bark, leaves, and woods) and other organic sources such as(fungi and lichens). Natural dyes are mostly employed for dyeing of natural fiber textiles to enhance their eco-friendly characteristics. They are usually applied to textiles by dyeing. Apart from indigo, other natural dyes are usually not used for printing directly. For producing printed fabrics, the printing is usually done with mordant and the whole material is dyed whereby only the area printed with mordant picks up the color [1].

Natural dyes, like synthetic dyes, can also be used to dye textiles at all stages such as fiber, yarn, or fabric. Fiber dyeing has the advantage that any shade variation can be easily adjusted by blending and therefore has been practiced at industrial scale also but is costly due to problems in spinning and loss of dyed fibers. Wool is generally dyed in yarn form and traditional dyers prefer yarn dyeing for all material as it offers versatility in designing during weaving [1].

1.2 Curcuma Longa L

1.2.1 Taxonomicclassification[2]

Kingdom: Plantae
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Order: Zingiberales
Family: Zingiberaceae
Genus: Curcuma L
Species: Curcuma Longa L

1.2.2 General introduction

The origin of the plant *Curcuma Longa L*, which belongs to *Zingiberaceae* family, is India. The plant is distributed throughout tropical regions of the world, being widely cultivated in south-east Asian countries. Turmeric, i.e. the ground *rhizomes of curcuma longa L* has a long history of use in food as a spice, mainly as an ingredient in many of varieties of curry powders and sauces, where curcumin from Turmeric is a main coloring substance. Curcumin is the principal curcuminoids of the popular Indian spice turmeric [3].

1.2.3 Properties of curcuminoids Physicochemical properties

The three principal colouring components of curcuminoids that are present in various proportions are all di-cinnamoyl methane derivatives:

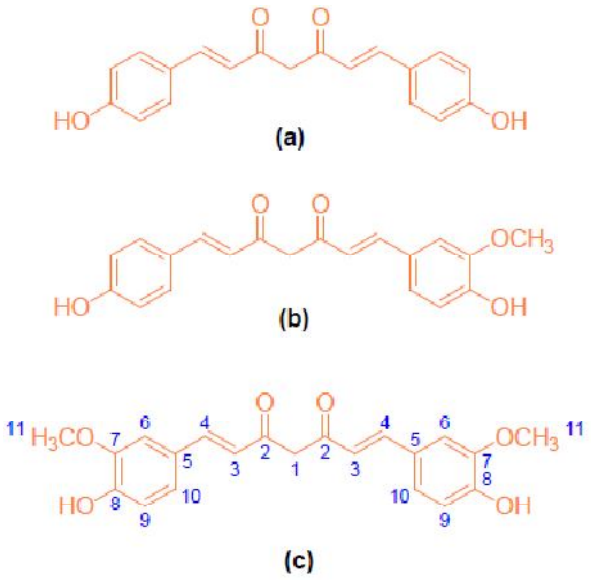
(i) 1,7- (Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dion (di-feruloyl methane)

(ii)1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-dien-3,5-dione(p-hydroxyl cinnamoyl feruloyl methane

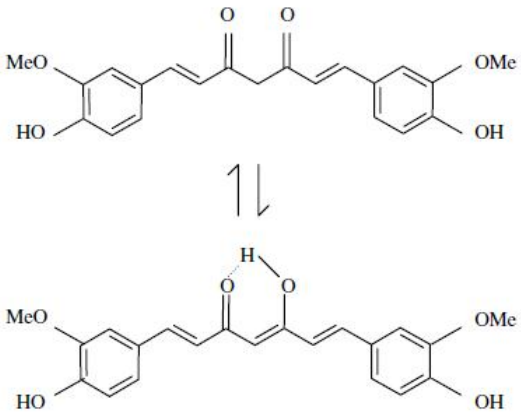
(iii)1,7-Bis-(4-hydroxyphenyl)-hepta-1,6-diene-3,5-dione(p,p dihydroxydicinnamoylmethane

Curcumin is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, and soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (e.g. sodium dodecyl sulfate, acetylpyridinium bromide, gelatin, polysaccharides, poly ethyl englycol, and cyclodextrins) have been reported[3] .

In solutions the principal coloring components of curcuminoid exhibit keto-enol tautomerism and, depending on the solvent, up to 95 percent are in the enol form. The principal coloring components of curcuminoids are relatively stable at acidic pH, but they rapidly decompose at pHs above neutral [3].



Scheme 1.1: Structure of curcuminoids**:(a)**Bisdemethoxycurcumin **(b)** Demethoxycurcumin and **(c)** Curcumin [4]



Scheme 1.2: Tautomerism of curcumin under physiological conditions [5]

1.2.4 Uses of curcuminoids

Curcumin is mainly used as an ingredient in many varieties of curry powders and sauces, where curcumin from turmeric is a main coloring substance. Turmeric is used extensively in foods for both its flavor and color, as well as having a long tradition of use in the (Chinese and Ayurvedic) systems of medicine. Curcumin has antioxidant, anti-inflammatory, anti-carcinogenic, antiviral and antifungal actions. In addition, curcumin was used in cardiovascular disease and gastrointestinal disorders. Studies have shown that curcumin is not toxic to humans. It exerts anti-inflammatory activity by inhibition of a number of different

molecules that play an important role in inflammation. Turmeric is effective in reducing post-surgical inflammation. Turmeric helps to prevent atherosclerosis by reducing the formation of bloods clumps. Curcumin inhibits the growth of Helicobacter pylori, which causes gastric ulcers and has been linked with gastric cancers. Curcumin can bind with heavy metals such as cadmium and lead, thereby reducing the toxicity of these heavy metals [3,5,8,9].

1.3 Objective

The main objective of this research was to isolate and characterize curcuminoids pigment from *Curcuma Longa L* (turmeric).

Chapter Two

Experimental

2.1 Sample collection and pretreatments

The *Curcuma Longa* (Turmeric) was collected and kindly supplied from Ngazidja (Grand Comoros), Comoros.

Figure 2.1: Photograph of freshly collected *Curcuma Longa* (Turmeric)

The fresh sample was washed with tape water and the outermost layer was sliced and the remaining flesh was cut into small pieces and left to dry under ambient temperature in a dark place. The dry sample was ground into a fine powder and stored in a cupboard for further analysis.

Figure 2.2: Photograph of the powdered *Curcuma Longa* (Turmeric)

2.2 Chemicals

Dichloromethane(minimum assay = 98%), was purchased from Alpha Chemika. Toluene(Assay = 99.8%) was purchased from Alpha Chemika. Methanol(minimum assay =99.9%),was purchased from Alpha Chemika.Ethanol(minimum assay=95%),was purchased from LOBAChemie.Ethyl acetate (minimum assay =99%),was purchased from Alpha Chemika**.**

2.3 Solvent extraction of curcuminoids

(i)5 grams of the powdered turmeric sample were placed into a round bottomed flask and 100 mL of dichloromethane was added. The flask was connected to a condenser and placed in a hot plate with stirrer and heated under reflux for one hour. The heating process was stopped and the stirring was continued for 24 hours. The solution was decanted and60mL of dichloromethane were added to the remaining solid precipitate and heated for one hour and left to stir for three days. The supernatant was decanted and added to the previous one. The process was repeated again by addition of 30mL of dichloromethane and refluxed for one hour and decanted afterward. Finally the extracts of the three steps were collected and

transferred into a beaker and left open for complete evaporation of the solvent in ambient conditions. The residue of the samplewas collected, dried and reweighed.The amount of curcuminoids was calculated using the following equation:

Weight of crude curcuminoids(g/5g) = W_{org} - W_{res} ……(1)

Where W_{org} is the weight of the original sample and W_{res} is the weight of the residue after extraction processes.

(ii) The curcuminoids pigment was recrystallized by adding 50mL of methanol to the pigment and heated in a water bath at 60 ˚C until complete dissolution. 10 mL of distilled water was added to the methanolic solution and cooled in ice-bath for 2 hours. The precipitate was separated from the solution by decantation and hot methanol was added again to the precipitate which followed by decantation for the second time. Finally the precipitated yellow orange pigment was collected, dried and reweighed. The pigment was kept in a dark place for further characterization. The weight of the purified curcuminoids was calculated as follows:

Weight of pure curcuminoids (g/5g) = W_{recry} ……..(2)

Where W_{recry} is the weight of curcuminoids after recrystallization.

2.4 Characterization methods of curcuminoids

2.4.1 Melting point

The melting point of curcuminoids was determined using a melting apparatus. Small amount of curcuminoids was placed in the bottom of a narrow capillary tube which had been closed at one end and heated gradually from room temperature until melting. The melting point was registered.

2.4.2 Ultraviolet/Visible analysis

UV/Visible analysis was carried out using JENWAY 6505 UV/Vis spectrophotometer.Small amount of curcuminoids was dissolved in ethanol and the solution was placed in a cuvette.

Blank solution was also examined. Measurements were done in the range between 200 to 800 nmand the wavelength at maximum absorption was determined (λ_{max}).

2.4.3 Infrared measurement

FT-IR transmittance spectrum of the curcuminoids was obtained using a Shimadzu FT-IR spectrophotometer in the wave number range between 4000 to600cm^{-1}. The powdered sample was thoroughly mixed with potassium bromide, pressed to make a pellet and then scanned under FTIR.

2.4.4 Then layer chromatography

Small amount of curcuminoidswas dissolved in a toluene and spotted by using capillary tube in a precoated silica gel TLC plate. The plate was developed using a solvent system composed of toluene and ethyl acetate in ratio80:20% (v/v).Visualization of the separated components was done underUV lampand directly from the spots of the colored components. The retardation factors were calculated.

Chapter Three

Results and discussion

3.1 Percentage of curcuminoids

Table3.1: Shows the grams of curcuminoids per 100 grams of sample

Weight of sample (grams)	Weight of recrystallized curcuminoids (grams)	g of curcuminoids/100g sample
5	1.09	21.8

3.2 Melting point

The melting point of the extracted curcuminoids was found to be equal to 173 ˚C. Typical value was reported in a number of articles in the literature [4,7].

3.3 IR measurement

FTIR analysis was carried out to characterize the obtained product. The main characteristics functional groups of curcuminoids involve carbonyl group, C=C aromatic, C=C alkenic, C-H aromatic, OH group, and C-O group. As can be seen from the spectrum (Figure 3.1), the broad intense peak in3410 cm^{-1} is due to the stretching vibration of-OH group. The strong sharp peak at 1630 $cm^{-1}$is attributed to absorption ofconjugated carbonyl groups.In addition, the two absorption peaks at 1585 cm^{-1} and 1514 $cm^{-1}$are both due to carbon-carbon stretching vibration of the aromatic ring. The carbon-hydrogen (-C-H) of the aromatic ring gave weak stretching band which appears as a small shoulder in the region above 3000 cm^{-1}. The two stretching vibration at 2923 and 2854 $cm^{-1}$are attributed to absorption bands of sp^3 hybridized -CH group. Finally, the absorption bands in the region between 1260 and 1200 $cm^{-1}$are due to –C-O stretching vibrations. It could be noticed that this spectrum clearly domenstrated the presence of the characteristics absorption bands of curcuminoids and hence it confirms that the obtained product is curcuminoids.

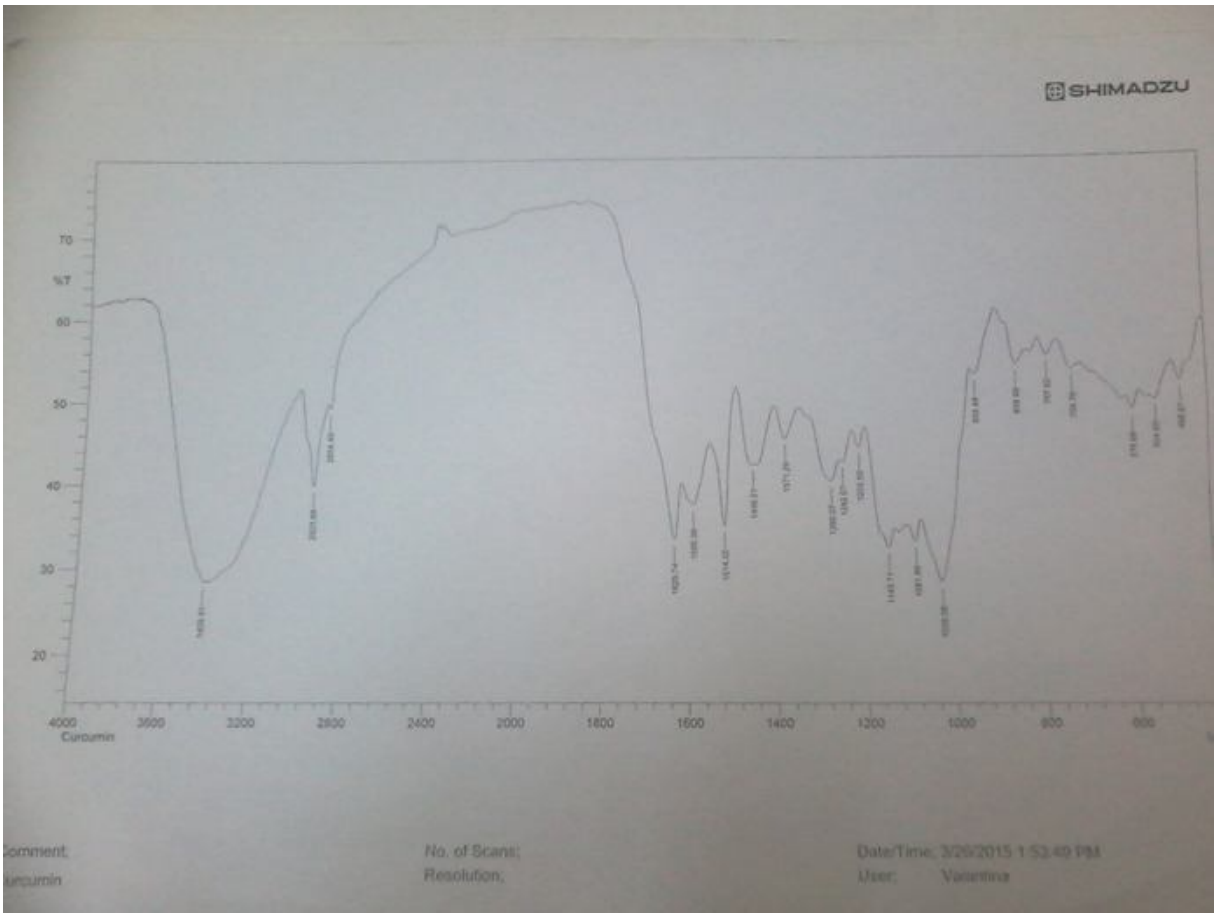


Figure 3.1: Shows the FT-IR spectrum of the separated curcuminoids pigment.

3.4 UV/Visible measurement

The solution of curcuminoids ethanol has given characteristics absorption bands in the region between 240 and 430nm with maximum absorption at 420 nm. Similar results were reported by other researchers [4,5,10].

3.5 Thin layer chromatography

Thin layer chromatography was used to separate the different components of the separated curcuminoids pigment. As can be seen from Figure 3.2 and 3.3 and table 3.2, three distinct color-zones were observed which are overlapped with each other. The same order of the

presence of the different colored zones (orange first which followed by yellow and red) was observed in the cases. Based on the findings of other research articles the first separated spot (orange) is curcumin, the second (yellow) is di-methoxycurcumin and finally (red) is bis-dimethoxy curcumin [8].

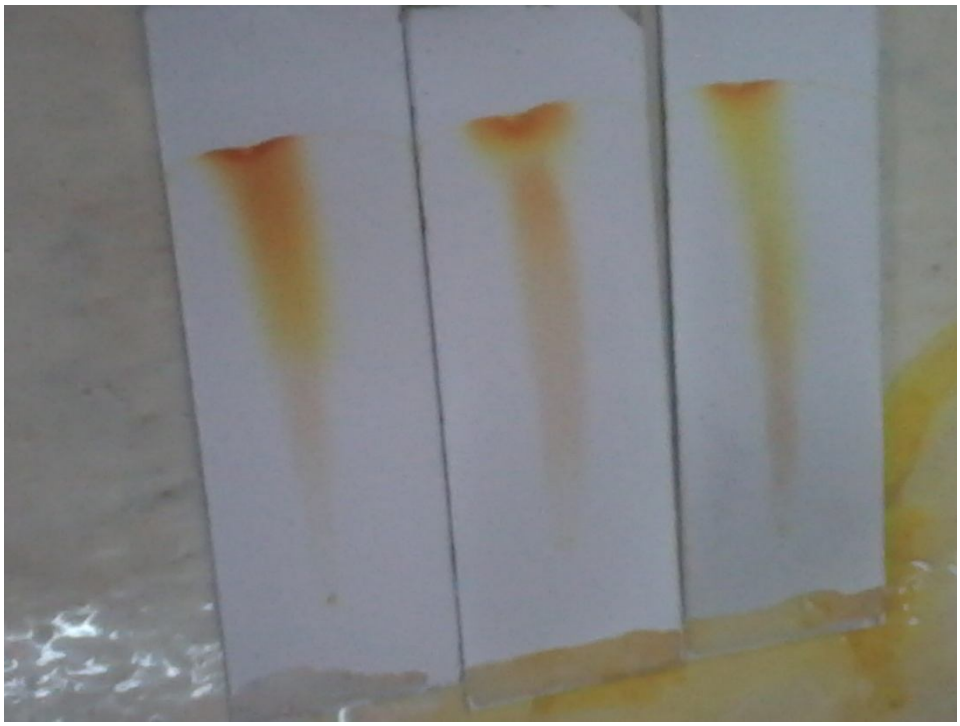


Figure 3.2: Shows the TLC chromatogram of the curcuminoids pigments using Ethyl acetate/methanol 80:20)

Table3.2: Shows the solvent system and retardation factors of the separated components

Solvent system	**Number and color of separated spots**	**Retardation factor (R_f)**
Ethyl acetate/Toluene (20:80)	Three overlapped spots (pale yellow, pale red and orange)	0.08, 0.29, 0.41 respectively
Ethylacetate/Methanol (20:80)	Three overlapped spots (pale yellow, pale red and orange)	0.06, 0.38, 0.56 respectively

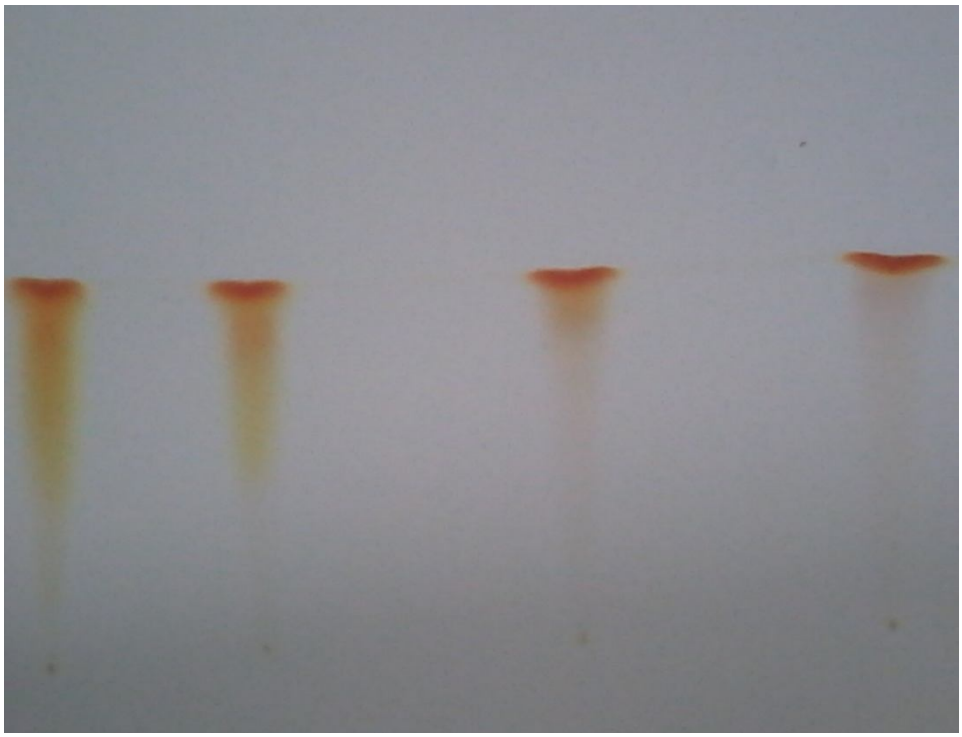


Figure 3.3: Shows the TLC chromatogram of the curcuminoids pigments using ethyl acetate/Toluene 80:20)

Conclusion

The main characterization pigment of the essential curcuminoids are curcumin, bis dimethoxy curcumin and dimethoxy curcumin .

The TLC technique showed the presence of three different components (pigment)of curcuminoids by three different colored zone orange ,red, yellow respectively, however using different solvent system.

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