



## WATER SOLUBLE FRACTION OF METHANOLIC EXTRACTS OF *CURCUMA LONGA* (L.) ON THE ABSORPTION SPECTRUM OF DEOXYRIBONUCLEIC ACID

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### ABSTRACT

This study was carried out to examine the staining of fractions prepared from defatted methanolic extract of *Curcuma longa* (L.) as a natural product in interacting with nucleic acid of Calf Thymus DNA to ascertain the DNA binding property of the fractions using Thin Layer Chromatography. Dry powdered sample of *Curcuma longa* was subjected to cold maceration using 95% methanol. The cold liquid extract was concentrated with rotary evaporator and subsequently evaporated to dryness in water bath at 50°C. The water soluble portion of the dark brown crude extract was partitioned subsequently in a separating funnel using solvents of varying polarity (chloroform, ethyl acetate, n-butanol.) Three dry fractions obtained from separating funnel system were further subjected to thin layer chromatography from where a total of 3 fractions were obtained. Each thin layer fraction ( $6.6 \times 10^3 \mu\text{g/ml}$ ) were mixed with  $13.5 \mu\text{g/ml}$  of DNA and the absorption spectrum where compared with control. Of the three fractions obtained, three (100%) showed fluorescence when viewed under UV light. The effect of Thymus DNA observed for fraction 1A seems to be unique and may perhaps bind to Calf Thymus DNA. Fractions 2A and 2B showed loss of some peaks and bands which may be probably due to toxic effect on DNA or other mechanisms not presently clear. Thus we propose that fractions 1A, from preparative TLC of defatted methanolic extract of *Curcuma longa*, had effect on absorption spectrum of Calf Thymus DNA and could probably serve as basis for search of alternative DNA binding stain.

**Keywords:** *Curcuma longa*; DNA; Methanolic extracts; Thin layer chromatography (TLC).

### INTRODUCTION

*Curcuma longa* known popularly as Turmeric is a rhizomatous herbaceous perennial herb of the ginger family, Zingiberaceae (Avwioro *et al.*, 2007; Chan *et al.*, 2009; Akramet *et al.*, 2010) which is categorized under the class Monocotyledoneae (Hashimiet *et al.*, 2013). It is native to South-East India and needs temperatures between 20 °C and 30 °C and a considerable amount of annual rainfall to thrive (Prasad *et al.*, 2011). The plant is known to be the major source of Curcuminoids. Curcuminoids are group of compounds considered as major active compound of *Curcuma longa* (Turmeric) and is known to be responsible for its yellow color. Curcuminoid complex consist of three main components namely: curcumin, demethoxycurcumin and Bisdemethoxycurcumin (Mishra, 2009). *Curcuma longa* is a plant with high nutraceutical, medicinal and cultural significance. Nutraceutically, it is one of the key ingredients in many Asian dishes.

Medicinally, *Curcuma longa* (Turmeric) has been used traditionally for thousands of years as a remedy for stomach and liver ailments, as well as topically to heal sores, basically for its supposed antimicrobial property (Chaturvedi, 2009). In the Siddha system (1900 BC) *Curcuma longa* (Turmeric) was

a medicine for a range of diseases and conditions including those of the skin, pulmonary and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders. A fresh juice is commonly used in many skin conditions, including eczema, chicken pox, shingles, allergy, and scabies (Khalsa, 2013). Recently, *Curcuma longa* (Turmeric) is being evaluated for its potential efficacy against several human diseases in clinical trials, including kidney and cardiovascular diseases, Arthritis, several types of cancer and irritable bowel disease. *Curcuma longa* (Turmeric) is also being investigated for potential treatment of Alzheimer's disease (Mishra and Palanivelu, 2008), diabetes (Boaz *et al.*, 2011) and other clinical disorders (Gregory *et al.*, 2008; Henrotin *et al.*, 2010). This paper aimed at studying the effect of fractions of water soluble portion of methanolic extracts of *Curcuma longa* (L.) on the absorption spectrum of deoxyribonucleic acid (DNA)

The objective of this paper is to determine the effect of fractions of water soluble portion of methanolic extracts of *Curcuma longa* (L.) on the absorption spectrum of deoxyribonucleic acid (DNA).

## MATERIALS AND METHODS

### Sample Collection

Fresh rhizomes of *Curcuma longa* were collected from Agricultural field in Kano, Kano State and transported to the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria for identification. The Voucher specimen number is 473.

### Preparation of the Crude Methanolic Extract of *Curcuma longa*

The extraction procedure was carried out according to the modified procedure of Tandon and Rane (2008). Plant materials were air dried in the laboratory at room temperature (25-28°C). The dried rhizomes were ground into fine powder, sieved and kept in an air-tight container at room temperature (25-28°C). Four hundred grams of the powdered sample was weighed and transferred into 1 litre extraction bottle containing 800ml of petroleum ether and shaken thoroughly. The shaking was repeated at intervals for three days. The mixture was sieved through a layer of muslin cloth and subsequently filtered through two layers of NO 1 Whatmann filter paper. The resultant residue was allowed to dry and re-extracted in 400 ml of petroleum ether and processed as above. After processing, the residue was allowed to dry and suspended in 600 ml of 95% methanol in an extraction bottle and shaken thoroughly. The shaking was repeated at intervals for three days. The mixture was sieved through a layer of muslin cloth and the liquid filtered through two layers of NO 1 Whatmann filter paper. The resultant residue was re-extracted in 400 ml of 95% methanol. The filtrate was concentrated using rotary evaporator at 62°C, 100 rpm for 2 hours as described by Fatope *et al.* (1997). The concentrate was transferred into a beaker and evaporated to dryness at 50°C using a water bath to obtain a final dark brown methanol extract.

### Partitioning of Methanolic Extract of *Curcuma Longa*

The extract was soaked in water and left for three days to dissolve. This was found to be largely insoluble in water and thereafter, the extract was heated at 50 °C and decanted to get the water soluble portion. The water soluble portion was transferred into 250ml separating funnel. Fifty (50) ml Chloroform was added to it and shaken thoroughly and allowed to separate into two phases. The Chloroform was tapped out into a beaker. Another 50 ml of Chloroform was transferred into the separating funnel to re-extract the Chloroform soluble component. The procedure above was repeated using Ethyl acetate and n-Butanol. Each fraction was left to evaporate at room temperature and the percentage weight measured.

### Preparative Thin Layer Chromatography of the Partitioned Fractions of Water Soluble portion of Methanolic Extract of *Curcuma longa*

Preparative thin layer chromatography of the partitioned fractions was carried out as follows.

The chloroform water soluble fraction was dissolved with 10ml of methanol and a capillary tube was used to spot the TLC plate with it, 2.5cm from the base. The plate was allowed to dry for five minutes. The solvent system (hexane/ethyl acetate in the ratio of 5:1) was transferred into the gel tank

and allowed to saturate for four minutes after which the TLC plate was carefully placed in the gel tank and covered. The solvent system was monitored for its movement from TLC plate base to the solvent front, after which the plate was removed from the tank. The plate was then viewed under visible and ultra violet light to check for fluorescence ability. Plate picture was taken. The major bands were scraped separately using a spatula, each band was purified by centrifuging with methanol at 1 revolution per minute for 10 minutes until the gel is clear. The solvent was left to evaporate at room temperature (25-28°C) in order to recover the fraction. The procedure above was repeated for ethyl acetate water soluble and n-butanol water soluble, using chloroform ethyl-acetate (3:2), n-butanolacetic acid and water (4:1:5), respectively.

### Collection of Sample for DNA Extraction

Thymus gland was obtained from slaughtered Calf from Kaduna Abattoir (Kwata-Kawo Kaduna) and transported on ice in thermox box to Department of Biochemistry, Kaduna State University Kaduna. The gland was kept on at 0°C in a freezer until used for DNA extraction.

### DNA Extraction from Animal Tissue and Gland using Salting out DNA Extraction (Sode) Method

The extraction procedure was carried out according to the procedure described by Brice *et al.* (2005). DNA sample was extracted from liver and thymus gland of a calf. One hundred mg and two hundred mg of tissue was weighed using analytical weighing balance, macerated with surgical blade and transferred into a new micro centrifuged tube. Two hundred microliter of Phosphate Buffered Saline (PBS) X1, (pH 7.2) was added into the tubes and centrifuged at 12000 rpm for one minute. The procedure was repeated twice. 50 µl of lysis buffer X1 (pH 7.2) and 5µl (200 µg/ml) of Proteinase K were added using a micro pipette and incubated in a regulatory water bath at 55°C for 2 hours. After incubation, the samples were transferred into a refrigerator and allowed to cool at 4°C for 10 minutes. 250 µl of cold 5M NaCl was added into the tubes. The samples were then centrifuged at 14000 rpm for 30 minutes. The supernatant was aspirated out and transferred into a new micro centrifuged tube. The DNA was precipitated by adding 500µl of ice cold absolute ethanol and swirled gently by several inversions. The tubes were then centrifuged at 12000 rpm for 10 minutes in order to bring the DNA thread to stick to the surface of the centrifuge tube. The supernatants were then discarded and the tubes were sucked up with clean tissue paper by placing and removing the mouth of the tubes upside down for 2-3 times. The DNA pellets were then washed with 500 µl of DNA washing solution by centrifuging the tubes at 12000 rpm for 10 minutes. The supernatants were then discarded and the tubes were sucked up with clean tissue paper by placing and removing the mouth of the centrifuge tube upside down for 2-3 times. The tubes containing the DNA pellets were then dried at 95 °C for five minutes to evaporate the remaining ethanol in the tubes. The DNA pellets were then dissolved in 50 µl of dissolution buffer pH 7.2 and was vortexed briefly using a Vortexer

### Agarose-Gel Electrophoresis of DNA Sample

This was carried out according to the standard laboratory procedure as described by the Centre for Biotechnology Research and Training (CBRT), AhmaduBello University, Zaria. Briefly, 0.8% Agarose was prepared by weighing 0.8g of Agarose in a conical flask and adding 100ml of 1X TAE buffer. The Agarose was solubilized by heating on a hot plate for 10 minutes. The Agarose was cooled to about 45 degrees Celsius and 4 microliter of ethidium bromide was added. Combs were assembled in the gel tray before pouring the gel. The gel was poured into the gel tray and allowed to solidify. Combs were removed from the gel and 0.5µl of loading dye was mixed with 10 microliter of Deoxyribonucleic acid and loaded into the wells. A 100 base pair DNA ladder was also loaded into two wells. Nuclease free water was used as a control. The gel was run at 75V for 40 minutes. Gel was removed and observed in the gel documentation unit. DNA bands were observed and documented.

#### UV-Spectroscopic Analysis of Preparative Thin Layer Fractions from Methanolic Extract of *Curcuma longa*

The UV-analysis was carried out according to the procedure described by Dipita and Ramamoorthy (2012). The absorption spectrum of each preparative thin layer fraction at concentration 0.0066mg/ml, Calf thymus DNA at concentration 13.5µg/ml and a combination of 1.25mg/ml of each fraction from preparative TLC with 13.5µg/ml of Calf thymus DNA were determined by recording the absorbance at various wave length (200-720 nm). The absorbance was taken as follows:

- A constant concentration (0.0066mg/ml) of each fraction
- 1.25mg/ml of each fraction and (13.5 µg/ml) DNA.

## RESULTS AND DISCUSSION

### Extract of Methanol

The methanol was observed to have turned to dark brown during the methanolic extraction of *Curcuma longa*. Seventeen grams of dark brown methanolic extract of *Curcuma longa* was obtained from four hundred grams of defatted *Curcuma longa* powder [Table 1].

### Partitioning of Methanolic Extract of *Curcuma longa*

Result of column fractionation of crude methanolic extraction of *Curcuma longa* is presented in Table 1. The highest

percentage yield of 1.06%, was obtained as water soluble portion when partitioned between ethyl acetate. This was followed by chloroform water soluble portion with 0.49% and the least was obtained as water soluble portion when partitioned with n-butanol.

Extraction of Methanol and Partitioning of Methanolic extract of *Curcuma longa* were presented in Table 1. *Curcuma longa* contains several coloring compounds, chief of which is "Curcumin" as reported by Evans (1998). In this study, the petroleum ether used as solvent for the defatting turned yellow, this could probably means the chromophore group in the non-polar components are yellow in colour, while methanol used as solvent for extraction produced a dark brown extract, which might probably be due to the chromophore polar components or groups present.

Due to the low boiling point of methanol (65°C), extraction and concentration of bioactive compounds is easy by Soxhlet extraction and Rotavapour respectively. Methanol is also said to be an amphiphilic solvent which proves to extract various chemical groups with high fidelity. The ability of methanol as a good solvent for *Curcuma longa* dye extraction was supported by the work of Ravathy *et al.* (2012) and Kulkarni *et al.* (2012) who independently reported that methanol can be used as an extracting solvent for curcumin extraction. Previous work by Avwioro *et al.* (2007) reported 18.6% yield (percentage yield) of ethanolic extract of *Curcuma longa*. In our study, methanolic extract of *Curcuma longa* yielded 4.25%, the variation might be as a result of defatting that took place and probably the difference in solvents used.

Methanolic extract of *Curcuma longa* was previously reported to be soluble in both water and organic solvent. Hence, solvents used here were chloroform, ethyl acetate and n-butanol for partitioning. The water-soluble portions (ethyl-acetate>chloroform>n-butanol) have percentage solubility of (0.56%). The lower solubility of water-soluble portions is in contrast with the work of Braide *et al.* (2011). However, Anand *et al.* (2008) reported that Curcumin, a major compound in Turmeric has the disadvantage of low solubility in water.

**Table 1: Fractionation of Crude Defatted Methanolic Extract of *Curcuma longa* Rhizome**

Weight of Methanolic extract (g)	Solubility in water	Solvents used in partitioning	Yield	
			(g)	(%)
14.2	Soluble	Chloroform	0.07	0.49
		Ethyl Acetate	0.15	1.06
		n-butanol	0.02	0.14

### Thin Layer (Preparative) Chromatography of the Partitioned Fractions of Defatted Methanolic Extract of *Curcuma longa*

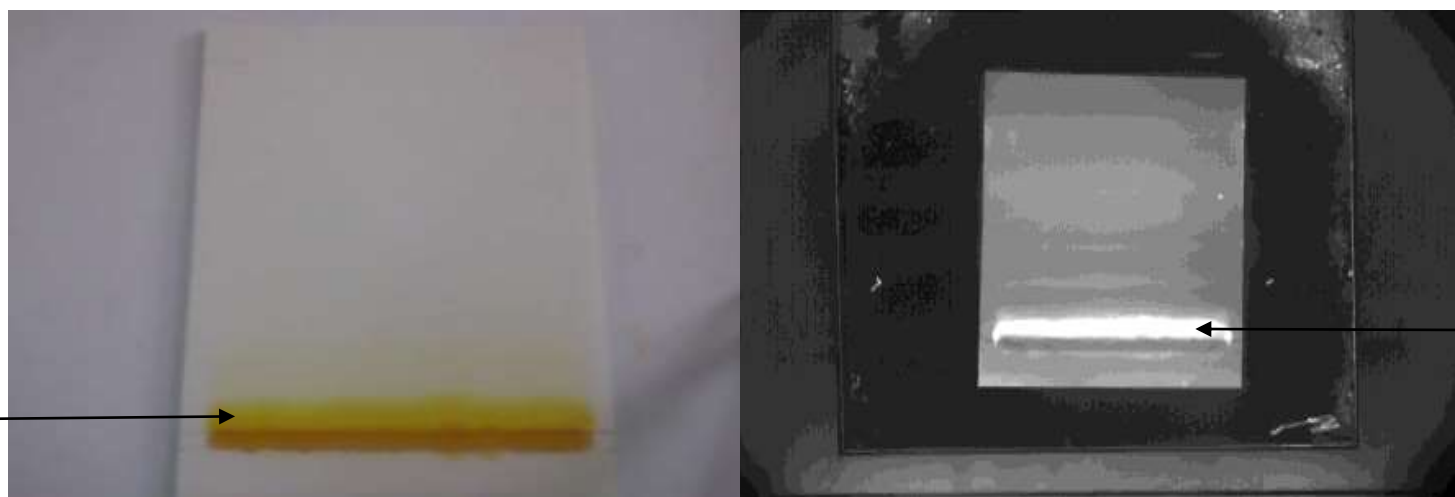
Table 2 and plates I & II present the result of preparative thin layer chromatography of column fractions obtained from wet partitioning of methanolic extract of *curcuma longa*. One band that fluoresced under UV light was obtained from chloroform water soluble portion when hexane/ethyl acetate (5:1) was used as the solvent. Two bands that both fluoresced under UV light were obtained from ethyl acetate water soluble portion when chloroform/ethyl acetate (3:2) was used as the solvent. No separation was seen from n-butanol water soluble portion when n-butanol, acetic acid and water in the ratio of 4:1:5.

From the result obtained from thin layer chromatography of the fractions obtained from partitioning of defatted methanolic extract of *Curcuma longa* in Table 2, the percentage of fluorescence fractions in water-soluble portions is 86.67%. This means that the water soluble portion of defatted methanolic extract of *Curcuma longa* has fluorescent compounds.

**Table 2: Thin Layer (preparative) Chromatographic Fractions obtained from Defatted Methanolic Extracts of *Curcuma longa***

Solubility	Thin layer Chromatography Fraction	Solvent system	Ratio	No. of band(pate Id)	no. of UV fluorescence band	Weight(mg)	Yield(%)
Water soluble	Chloroform	H:E	5:1	1 (1A)	1A +ve	0.05	71.45
	Ethyl acetate	C:E	3:2	2(2A, 2B)	2A +ve	0.12	80.00
					2B +ve	0.04	26.70
	n-butanol	NB:AC:W	4:1:5		No separation		

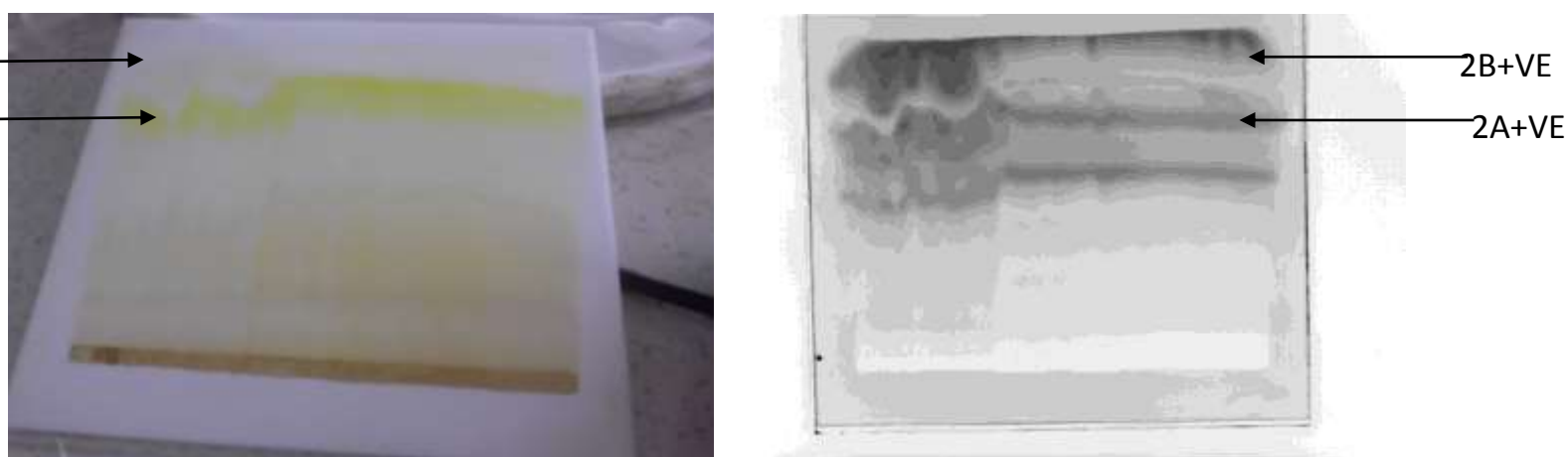
Note: H:E-Hexane/Ethyl acetate, C:E-Chloroform/Ethyl acetate, N:AC:W-n-butanol/acetic acid/water, +ve-fluorescences under UV, -ve-no fluorescence under UV



A

B

**Plate I: Thin layer chromatogram of chloroform: Water soluble fraction of methanolic extract of *Curcuma longa* under visible light(A) and as revealed by ultraviolet light(B). Solvent mixture:hexane:ethyl acetate (5:1).**



A

B

**Plate II: Thin layer chromatogram of ethyl acetate: Water soluble fraction of methanolic**

**Plate II: Arrow points to separated fractions of *Curcuma longa* under visible light(A) and as revealed by ultraviolet light (B) Solvent mixture:chloroform:ethyl acetate (3:2).Arrow points to separated fractions extract.**

#### DNA Extraction from Thymus Gland and Liver

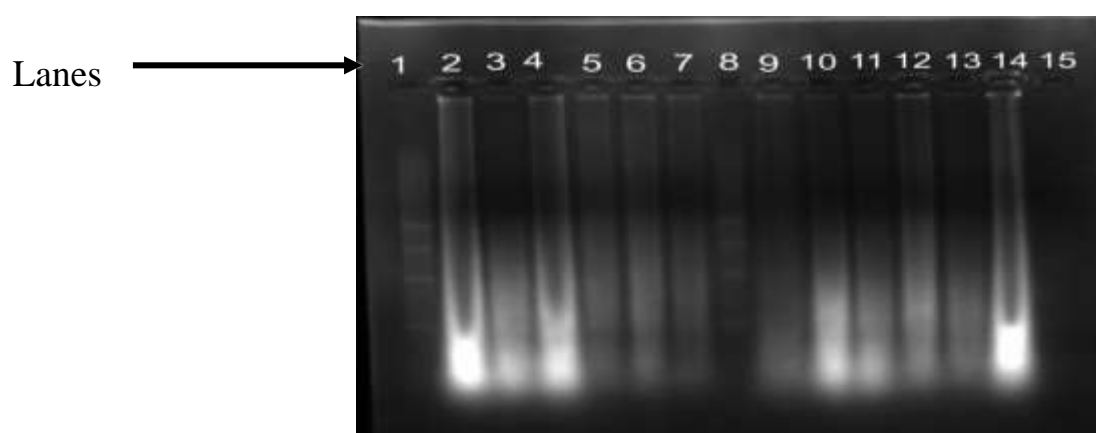
Table 3 shows the result of DNA extraction from thymus gland and liver using salting out DNA extraction (SODE) method. The highest DNA yield was obtained from 100 mg of thymus gland (5865mg/ml) and the lowest was obtained from 200mg of Liver. DNA extracted from 200mg of thymus gland was found to have the highest purity ( $A_{260}/A_{280}=1.983$ ) followed by ( $A_{260}/A_{280}=1.98$ ) from 100mg of thymus gland and the least was obtained when DNA was extracted from 200mg of Liver ( $A_{260}/A_{280}=1.267$ )

Genomic DNA was successfully extracted from calf thymus and liver (Table 3, Figure 1& Plate I) using SODE method of DNA extraction, while the  $A_{260}/A_{280}$  describes the purity of DNA. The ratio should be in the range of 1.8-1.9 to ensure that the DNA is sufficiently free from protein (Benesi and Hildebrand, 1949). In this study, the DNA extracted from calf thymus gland (Ab. 1.87 and 1.896) was found to be purer and higher in concentration than DNA extracted from liver (Ab.

1.764 and 1.60 ) as shown in Table 3. This could probably be as a result of numerous T-lymphocytes (disease fighting T-cell) secreted by Thymus gland. In a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample. Using the Beer Lambert Law, it is possible to relate the amount of light absorbed to the concentration of the absorbing molecule. At a wavelength of 260 nm, the average extinction coefficient for double-stranded DNA is  $0.020 (\mu\text{g/ml})^{-1} \text{cm}^{-1}$ , for single-stranded DNA it is  $0.027 (\mu\text{g/ml})^{-1} \text{cm}^{-1}$ , for single-stranded RNA it is  $0.025 (\mu\text{g/ml})^{-1} \text{cm}^{-1}$  and for short single-stranded oligonucleotides; it is dependent on the length and base composition. Thus, an Absorbance (A) of 1 corresponds to a concentration of 50  $\mu\text{g/ml}$  for double-stranded DNA. This method of calculation is valid for up to an A of at least 2 as reported by Sambrook and Russell (2001).

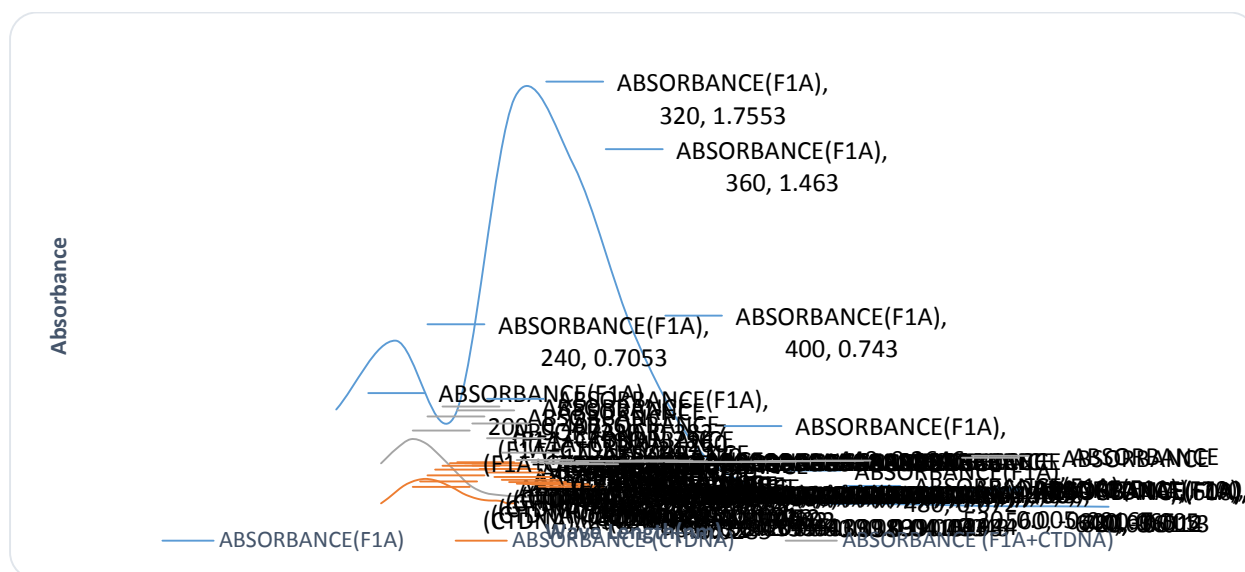
**Table 3: Comparison of DNA yield and purity between Thymus Gland and Liver using salting out DNA Extraction (SODE) Method**

Calf Tissue	Weight (mg)	$A_{260}/A_{280}$	DNA (mg/ml)
Thymus	100	1.89	4200
Thymus	100	1.85	5865
Liver	100	1.772	585
Liver	100	1.756	395
Thymus	200	1.97	1180
Thymus	200	1.821	2020
Liver	200	1.932	715
Liver	200	1.267	95



**Plate III: Agarose gel electrophoresis of genomic DNA extracted from Calf Liver and Thymus tissue.**

Lanes 1 and 8 100 bp ladder; 2,3 and 4 100 mg Liver; 5,6 and 7 200 mg Liver; 9,10 and 11 100 mg Thymus gland; 12,13 and 14; 200 mg Thymus gland 15; DNase - free water.



**Figure 1: The effect of interaction between Calf Thymus DNA (CTDNA) and Thin Layer Chromatographic Fraction (1A) of Chloroform fraction of the Water Soluble part of Methanolic Extract of *Curcuma longa* on Absorption Spectrum of DNA.**

F1A: (0.0066 mg/ml) of Thin Layer Chromatography Fraction 1A of Chloroform Fraction of the Water Soluble part of MECl, CTDNA: 13.5 µg/ml of Calf Thymus DNA. F1A+CTDNA: 1.25 mg/ml of F1A + CTDNA.

Note: CT – Calf thymus

F1A- Fraction (1A)

MECl- Methanolic Extract of *Curcuma longa*

#### Effects of DNA binding on Absorption Spectrum of various Fractions of preparative Thin Layer Chromatography of Methanolic Extract of *Curcuma longa*

Effects of DNA binding on absorption spectrum of various fractions of preparative thin layer chromatography of methanolic extract of *Curcuma longa* was presented in Table 4. The three most common mechanisms undertaken by molecules in the quest of binding with DNA are: external binding with DNA-helix, intercalation between base pairs and binding with minor or major grooves (Vijayalakshmi *et al.*, 2000; Vaidyanathan *et al.*, 2005; and Cate and Doudna, 1996). The binding efficacy as a function of assorted spectroscopic studies remain a major focus of the present investigation. The thin layer chromatographic fractions of the defatted methanolic extract of *Curcuma longa* fractions gave different absorption spectrum and behave differently under Visible and UV light, the fractions interacted differently with Calf thymus DNA. The results of UV-VIS spectral analysis of binding phenomenon of thin layer chromatographic fraction with of *Curcuma longa* is represented in Figures 2 & 3. Drug-DNA interactions can be studied by comparison of UV-Visible absorption spectra of the free drug and drug-DNA complexes,

which are usually different. Compounds binding with DNA through intercalation usually results in hypochromism and bathochromism (red shift). (Muhammad *et al.*, 2013). Pattern of absorption of Fractions 1A show hypochromism (blue shift) indicating that it contains a compound that binds to DNA. Dipita and Ramamoorthy (2012) also reported the binding of Morindone dye to DNA. In their study, one of the usual peaks of Morindone at 216nm get shifted to 260nm on addition of DNA, the occurrence is an indicator of bathochromic or red shift (Muhammad *et al.*, 2013). In this study, the occurrence of hypochromic or blue shift is a primary testimonial to the binding of Fractions 1A to DNA as it is a regular phenomenon particularly in case of small molecules when they bind rather than intercalate with DNA (Bi *et al.*, 2008). Interaction of molecules with DNA persists to be an extremely vital parameter. In particular, owing to the hazards posed by synthetic derivatives, the search for natural products in interacting with nucleic acid remains a viable interest in this study. Fraction 2A and 2B showed loss of peak probably due to toxic effect on DNA.

Baker and Silverton (1976) and Carleton (1976) reported that, the ability of a dye to stain specific tissue structures is determined by certain factors, one of which is the acidity of

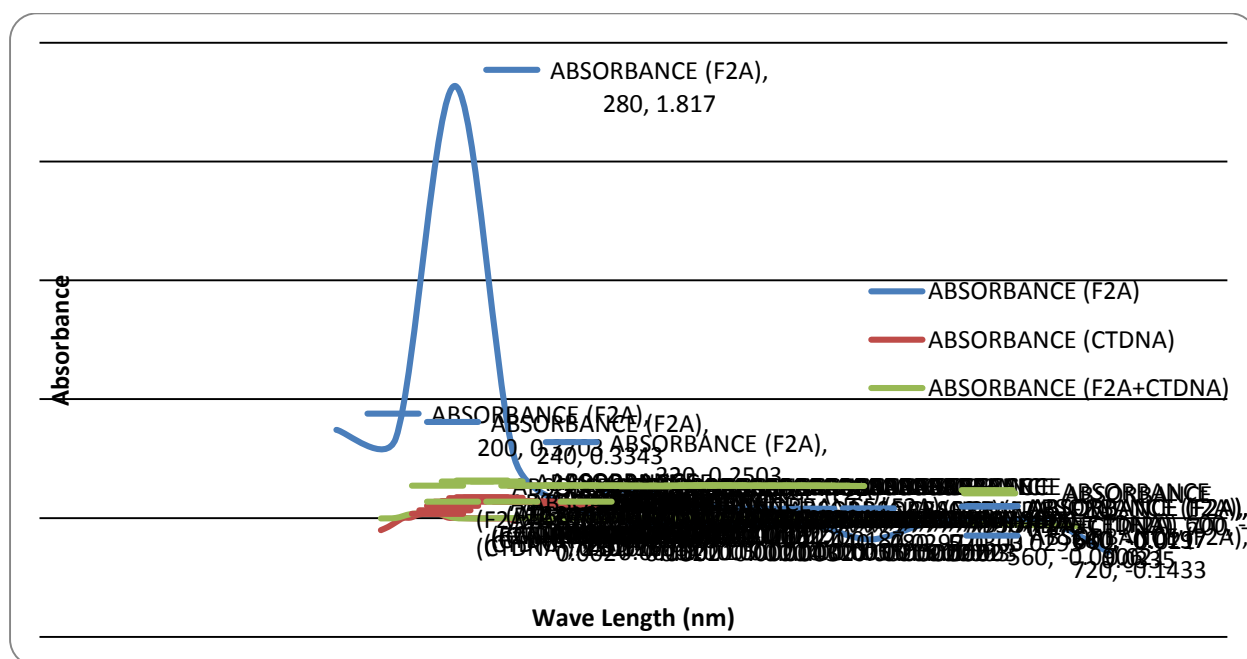
the stain. Acidic structures would be stained by basic dyes while basic structures would be stained by acidic dyes. *C. longa* has affinity to nucleus which is an acidic structure, it can be said that methanolic extract of *Curcuma longa* is basic in nature. This supported the report of Hoffman and Banknocht (1999), that pH of staining solution often affect staining reactions.

In Nigeria, many researchers have extracted a number of dyes from a variety of local plants. According to Akpuakaet al.(1998) and Osabohienet al.(2002), the local plants - Camwood, Redwood, Henna, Annato, Rothmania, Terminalia, Indiqovine,Kola, Banana, Turmeric, Roselle and Ginger all contain different types of dyes which are used for various purposes. Turmeric powder is used as food coloring agent and also as a natural dye (FAO, 1995), apart from being a major ingredient in culinary. Avwioroet al.(2007) reported

the use of ethanolic extract of *Curcuma longa* in staining collagen fibers, red blood cells and cytoplasm. In his study, ethanolic extract of *Curcuma longa* stained collagen fibers, red blood cells and cytoplasm yellow. Egbujoet al. (2008) reported the use of Roselle (*Hibiscus sabdariffa*) in differential staining of testicular tissue sections to find out it's staining effect on nuclear, cytoplasmic, and other structure. In his study, he reported that Roselle extract shows reasonable potential as a candidate nuclear stain especially when mordanted with iron alum or mordantedwith potassium alum and acidified with acetic acid. Abubakaret al. (2012) also reported the use of same plant in mycological staining, where he reported the extract to have affinity to stain fungal mycelia as well as the fungal sporangia. The use of Henna leaves extract as a counter stain in gram staining reaction has also been reported by Hafiz et al. (2012).

**Table 4: Effects of DNA binding on Absorption Spectrum of various Fractions ofpreparative Thin Layer Chromatography of Methanolic Extract of *Curcuma longa***

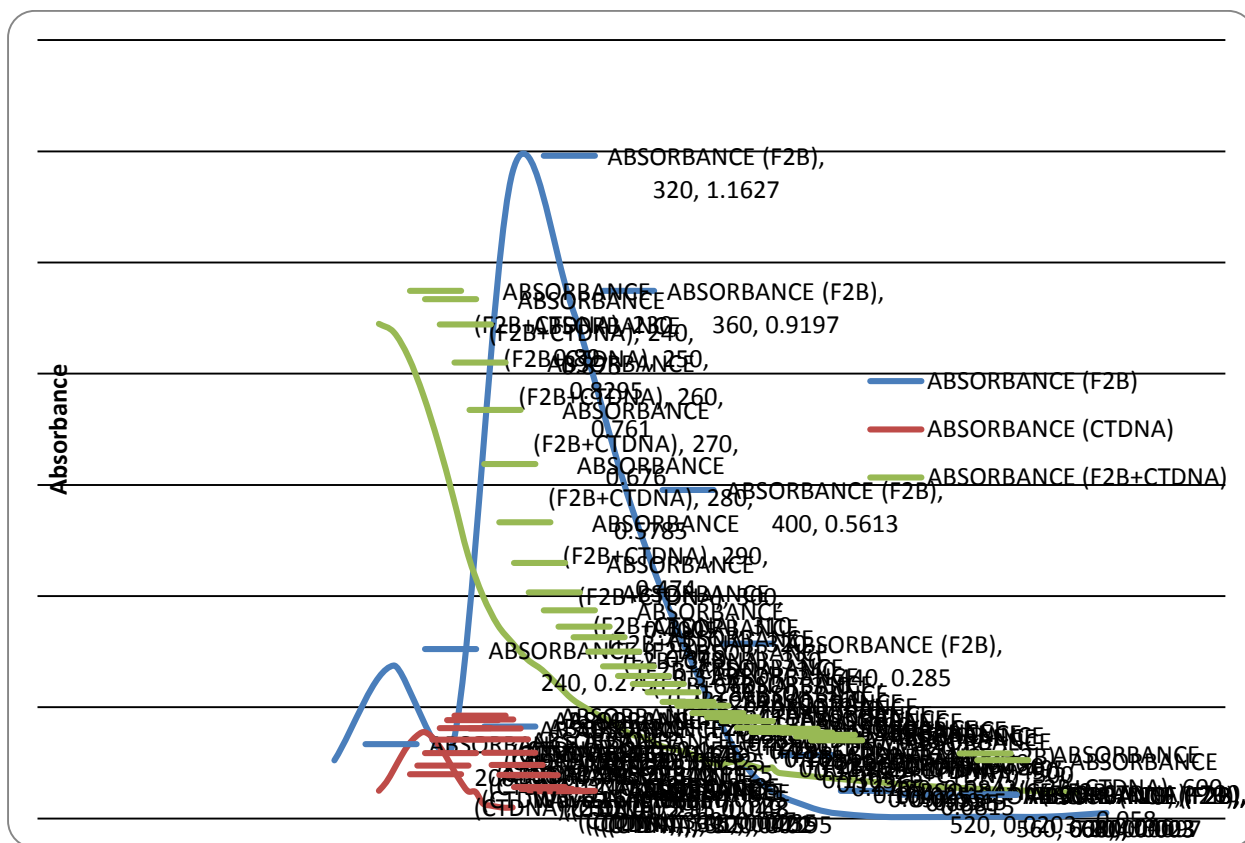
Thin Layer Fraction	Position of peaks (nm)			Effects of fraction on DNA
	Fraction		DNA	
	Minor peak	Major peak		
1A	240	320	260	Position of the minor peak has been shifted to 250
2A	-	280	260	Lost of peak
2B	240	320	260	Lost of minor peak



**Figure 2: The effect of interaction between Calf Thymus DNA (CTDNA) and Thin Layer Chromatographic Fraction (2A) of Ethyl Acetate Fraction of the Water Soluble part of Methanolic Extract of *Curcuma longa* on Absorption Spectrum of DNA.**

F2A: (0.0066 mg/ml) of Thin Layer Chromatography Fraction 2A of Ethyl Acetate Fraction of the Water Soluble part of MECl. CTDNA: 13.5µg/ml of Calf Thymus DNA. F2A+CTDNA:1.25 mg/ml of F2A + CTDNA.

Note: CT – Calf thymus  
 F2A- Fraction 2A  
 MECl- Methanolic Extract of *Curcuma longa*



**Figure 3: The effect of interaction between Calf Thymus DNA (CTDNA) and Thin Layer Chromatographic Fraction (2B) of Ethyl Acetate Fraction of the Water Soluble part of Methanolic Extract of *Curcuma longa* on Absorption Spectrum of DNA.**

F2B: (0.0066 mg/ml) of Thin Layer Chromatography Fraction 2B of Ethyl Acetate Fraction of the Water Soluble part of MECl. CTDNA: 13.5µg/ml of Calf Thymus DNA. F2B+CTDNA:1.25 mg/ml of F2B + ctDNA.

Note: CT – Calf thymus



F2B- Fraction 2B

MECl- Methanolic Extract of *Curcuma longa*

## CONCLUSION

From the results obtained, it was concluded that preparative Thin Layer Chromatography (TLC) of defatted methanolic extract of *Curcuma longa* (Turmeric) had effect on absorption spectrum of Calf Thymus DNA especially Fraction 1A and could probably serve as basis for search of alternative DNA binding stain.

## CONFLICT OF INTEREST

There is no conflict of interest.

## FUNDING FOR THE WORK

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## REFERENCES

- Abubakar, S., Usman, A. B., Etim, V., Nnadi, O. and Ajaku, C. (2012). Application of Organic Dyes from Roselle Calyx (*Hibiscus sabdariffa* Linn) for Mycological staining. *Indian Journal Innovations Development*, **1**(9):2277-5390.
- Akpuaka, M. U., Chukwunke, C. and Agbo, G. (1998). The dyeing of textile fabrics with natural dyes from some local trees. *Journal of Chemical Society of Nigeria*, **23**: 47-52.
- Akram, M., Shahab-Uddin, A. A., Khan, U., Abdul, H., Mohiuddin, E. and Asif, M. (2010). *Curcuma longa* and curcumin: A review article. *Rom. Journal of Biology – Plant Biology*, **55** (2): 65–70.
- Avwioro, O. G., Onwuka, S. K., Moody, J. O., Agbedahunsi, J. M., Oduola, T., Ekpo, O. E. and Oladele, A. A. (2007). *Curcuma longa* extract as a histological Dye for Collagen Fibres and Red Blood Cells. *Journal of Anatomy*, **210**(5): 600–603.
- Avwioro, O. G., Aloamaka, P. C., Ojiana, N. U., Oduola, T. and Ekpo, E. O. (2005). Extracts of *Pterocarpus* as a histological stain for collagen fibres. *African Journal Biotechnology*, **4**(5): 460-462.
- Brice, R., Audrey, G. and Bernard, C. (2005). Trypanosomatid Protozoa: A simplified DNA isolation procedure. *Journal of experimental parasitology*, **111**:207-209.
- Cate, J. H. and Doudna, J. A. (1996). Metal binding sites in the major groove of a large ribozyme domain. *Structure*, **4**: 1221–1229.
- Chan, E. W. C., Lim, Y. Y., Wong, S. K., Lim, K. K., Tan, S. P., Lianto, F. S., Yong, M. Y. (2009). Effects of different drying methods on the antioxidant properties of Leaves and Tea of Ginger species. *Food Chemistry*, **113** (1): 166–172.
- Chaturvedi, T. P. (2009). Uses of *Curcuma longa* (Turmeric) in dentistry: an update. *Indian Journal of Dental Research*, **20** (1): 107–109.
- Dipita, B. and Ramamoorthy, S. (2012). Morindone, an Anthraquinone, Intercalates DNA Sans Toxicity: a spectroscopic and Molecular Modeling Perspective. *Journal of Applied Biochemistry and Biotechnology*, **167** (4): 0273-2289.
- Egbujo, E. C., Adisa, O. J. and Yahaya A. B. (2008). A study of the staining effect of Roselle (*Hibiscus sabdariffa*) on the Histologic section of the testis. *International Journal of Morphology*, **26**(4):927-930.
- Evans, A. (1998). *Pharmacopoealand Related Drugs of Biological Origin*. Pharmacognosy, London: W.B. Saunders.
- F.A.O. (1995). Natural colourants and dyestuffs. Non-Wood Forest Products 4. *Food and Agriculture Organization of the United Nations*, Rome, Italy.
- Fatope, M. O., Adoun, O. A. and Dabo, N. T. (1997). Bioactivities of some savannah plants in the brine shrimp lethality test an invitro antimicrobial assay. *International Journal of Pharmacognosy*, **35**(35):334-337.
- Gregory, P. J., Sperry, M. and Wilson, A. F. (2008). Dietary supplements for osteoarthritis. *American Family Physician*, **77**(2): 177–84.
- Hafiz, H., Chukwu, O. O. C. and Nura, S. (2012). The potential of henna (*Lawsonia inamis* (L.)) Leaves extracts as counter stain in gram staining reaction. *Bayero Journal of pure and Applied Sciences*, **5**(2):56-60.
- Hashimi, F. J., Muayad, S. S. and Aljewari, H. (2013). Anti-cancer effect of *Curcuma longa* on leukemic cell lines evaluated by apoptosis and comet assay. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5** (3): 671-674.
- Henrotin, Y., Clutterbuck, A. L. and Allaway, D. (2010). Biological actions of curcumin on articular chondrocytes. *Osteoarthritis and Cartilage*, **18** (2): 141–9.
- Mishra, P. (2009). Isolation, spectroscopic characterization and molecular modeling studies of mixture of *Curcuma longa*, ginger and seeds of fenugreek. *International Journal of Pharmacology and Technology Research*, **1**(1): 79-95.
- Mishra, S. and Palanivelu, K. (2008). The effect of curcumin (turmeric) on Alzheimer's disease: An overview. *Annals of Indian Academy of Neurology*, **11** (1): 13–9.

- Mishra, S., and Palanivelu, K. (2008). The effect of curcumin(turmeric) on Alzheimer's disease: An overview. *Annalof Indian Academy of Neurology*, **11** (1): 13–9.
- Muhammad, S., Saqib, A. and Amin, B. (2013). Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltammetry. *Journal of Photochemistry and Photobiology B: Biology*, **124** : 1–19
- Osabohien, E. and Ukpumwan, D. O. (2002). Extraction of natural dyes from some local plants. *Journal of Chemical Society*, **27**(2): 139-142.
- Prasad, S., Aggarwal, B. B., Benzie, I. F. F. and Wachtel-Galor, S. (2011). *Curcuma longa (Turmeric), the Golden Spice: From Traditional Medicine to Modern Medicine*. Biomolecular and clinical aspect 2<sup>nd</sup> Edition.
- Sambrook, J. and Russel, D. (2001). *Molecular Cloning: A Laboratory Manual*, vol. 3, Cold Spring Harbor Laboratory Press, New York, NY, USA, 3rd edition.
- Vijayalakshmi, R., Kanthimanthi, M. and Subramanian, V. (2000). DNA cleavage by a Chromium(III) complex. *Biochemical and Biophysical Research Communications*, **271**: 731734.