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Most Bioactive Metabolite

Discovering Thoughts, Inventing Future

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Isolation and Partial Characterization of the Most Bioactive Metabolite from the Hexane Extract of the Aerial Part of *Hydrocotyle Verticillata* (WHORLED MARSHPENNYWORTH)

By Nathalie L. Daminar & Lydia M. Bajo
Caraga State University-Cabadbaran Campus

Abstract - Several of thousands of bioactive compounds have been mined from the natural product resources both from the marine and terrestrial environment all over the world. Natural product has proved potentiality of many plants for diverse clinical conditions. Many plants were found to contain compounds, the bioactive components which are used in natural medicine. In this study, the plant of interest is *Hydrocotyle verticillata*, mistakenly identified to be "Gotu ko la" *(Centella asiatica)* by local folks of Iligan City and Butuan City, Philippines. The natural components of the aerial part of *H. verticillata* were extracted using solvent extraction. The crude ethanolic extract of the aerial part of *Hydrocotyle verticillata* was further subjected to solvent partitioning according to increasing polarity: n-hexane, ethyl acetate (EtOAc), dichloromethane (DCM) and water. Bioassays like Brine Shrimp Lethality Test was then conducted to test which of the extracts consist of bioactive components. Among the four partitioned solvent extracts, result showed that n – hexane extract showed to be the most bioactive since it has the lowest LC50 .

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Isolation and Partial Characterization of the Most Bioactive Metabolite from the Hexane Extract of the Aerial Part of *Hydrocotyle Verticillata* (WHORLED MARSHPENNYWORTH)

Nathalie L. Daminar [α] & Lydia M. Bajo [σ]

Abstract - Several of thousands of bioactive compounds have been mined from the natural product resources both from the marine and terrestrial environment all over the world. Natural product has proved potentiality of many plants for diverse clinical conditions. Many plants were found to contain compounds, the bioactive components which are used in natural medicine. In this study, the plant of interest is *Hydrocotyle verticillata*, mistakenly identified to be "Gotu ko la" (*Centella asiatica*) by local folks of Iligan City and Butuan City, Philippines. The natural components of the aerial part of *H. verticillata* were extracted using solvent extraction. The crude ethanolic extract of the aerial part of *Hydrocotyle verticillata* was further subjected to solvent partitioning according to increasing polarity: n-hexane, ethyl acetate (EtOAc), dichloromethane (DCM) and water. Bioassays like Brine Shrimp Lethality Test was then conducted to test which of the extracts consist of bioactive components. Among the four partitioned solvent extracts, result showed that n – hexane extract showed to be the most bioactive since it has the lowest LC_{50} .

Fractionation and purification of the hexane extract was done through series of Gravity Column Chromatography (GCC). Four isolates (HF5B1, HF5B2, HF5B3 and HF5B4) were obtained. Analysis using High Performance Liquid Chromatography (normal phase HPLC) showed three of the isolates (HF5B2, HF5B3 and HF5B4) to have similar profile, two peaks of closely similar retention time. HF5B2 was further purified in HPLC and yielded a subfraction HF5B2a with a single sharp peak on the HPLC chromatogram and with a retention time of 8.135 min.

This subfraction was partially characterized using Perkin Elmer FTIR Spectrometer. The spectrum showed peaks for the following functional groups: NH, C=O, C-C(O)-C, and CN stretch (aliphatic amine). Considering the presence of these functional groups particularly the amine, the isolated metabolite HF5B2A from hexane extract could be an alkaloid derivative. Alkaloids containing basic nitrogenic compounds were known as DNA binders.

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I. Introduction

Medicinal plants are regularly used to treat ailments because of minimal side effect and cost effectiveness. The potential for developing antimicrobial, high toxicity property from higher plants appears rewarding as it may lead to the development of phytomedicine. Hence, there is a worldwide interest to isolate biologically active compounds from higher plant species that are possible sources of medicine. The bioactive components of these plants are great sources for new therapeutic agents. These therapeutic agents are of great importance nowadays because these pose new hope for disease prevention.

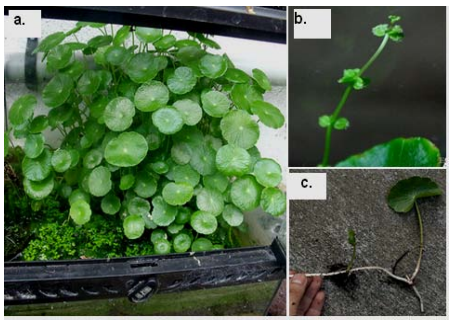


Figure 1 : Hydrocotyle verticillata plant shows its (b) flower and (c) roots

The plant of interest in this study is the *Hydrocotyle verticillata* (Figure 1) which has been mistakenly identified to be **"Gotu kola"** (*Centella asiatica*) by local folks of Iligan City and Butuan City. *Hydrocotyle verticillata* is one of the 100 species of genus hydrocotyle.[1] This is a flowering plant found in South and North America, West Indies and Bangladesh.[2] People claim that *H. verticillata* can effectively treat inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly associated to *C. asiatica*.[3]

Toxicity test using Brine Shrimp Lethality Test was performed to obtain the bioactive components of

this plant, *H. verticillata* which is great source for new therapeutic agents. To isolate the most bioactive metabolite different chromatographic techniques were used in this study such as gravity column chromatography (GCC), thin-layer chromatography (TLC), and high performance liquid chromatography (HPLC). The most pure isolate then was partially characterized using the Perkin Elmer Fourier Transform Infrared Spectrometer.

a) Objective of the Study

The primary aim of this study is to isolate the bioactive metabolites from the plant sample *Hydrocotyle verticillata* and conduct phytochemical screening on the most bioactive extract.

This attempts to achieve the following specific objectives:

1. to extract the bioactive components possibly present in the aerial part of the plant sample *H. verticillata* using hexane, ethyl acetate, dichloromethane and distilled water;
2. to determine cytotoxicity of the sample using brine shrimp lethality test on the extracts;
3. to further fractionate the most bioactive crude extract of the plant sam H. verticillata by Thin Layer Chromatography (TLC), Gravity Column Chromatography (GCC), High Performance Chromatography (HPLC);
4. to partially characterize the purified extract by Fourier Transform Infrared (FTIR) Spectroscopy

II. Materials and Methods

a) Chemicals and Reagents

The chemicals used in this study were: 95% ethanol (EtOH), hexane (Hex), ethyl acetate (EtOAc), dichloromethane (DCM), methanol (MeOH), chloroform ($CHCl_3$). All chemicals were purchased locally and signed out from Chemistry stockroom of Mindanao State University – Iligan Institute of Technology (MSU-IIT), Philippines.

b) Test Organisms

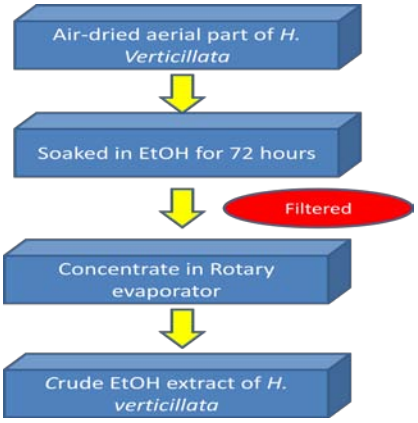
The brine shrimp, *Artemia salina* Leach were used as test organism in Brine Shrimp Lethality Test (BSLT). These test organisms were purchased as eggs in the local pet shop.

c) Plant Material

Fresh samples of aerial part of *Hydrocotyle verticillata* were collected by hand from a local area of Iligan City, Philippines. The samples were washed with water several times to remove dirt and other contaminants. These aerial parts were then air-dried for about three weeks.

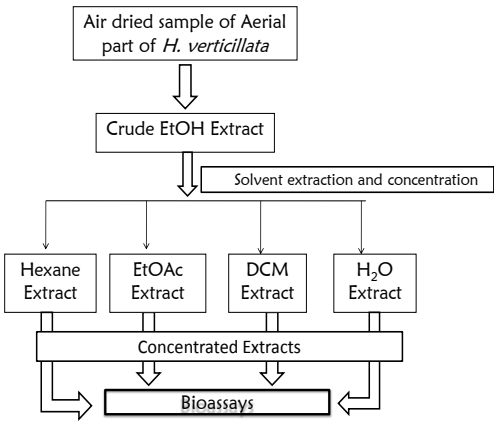
d) Extraction

The air – dried aerial part of the plant sample *H. verticillata* were ground using mechanical grinder. Six hundred sixty-eight grams of the ground sample were extracted with 95% ethanol for 72 hours. The resulting mixture was filtered and the solvent removed by rotary evaporator at 40^0C to yield the crude ethanolic extract as shown in Scheme 1.



Scheme 1 : Preparation of Crude Extract

The crude ethanolic extract was separated by solvent partitioning according to increasing polarity: n-hexane, EtOAc, DCM and water to obtain four extracts (Scheme 2). Hex, EtOAc and DCM extracts were dried under a *vacuo* while the aqueous extract was freeze-dried. The concentrated extracts were subjected to Brine Shrimp Lethality Test (BSLT). The extracts with the highest toxicity or lowest LC_{50} were considered as bioactive extracts.



Scheme 2 : Schematic Diagram for Solvent Extraction

e) Fractionation and Purification

The crude hexane extracts (H) having the highest bioactivity was subjected to gravity column chromatography (GCC) using silica gel (silica gel 60, mesh 70 – 230) as adsorbent. The column used in this study was packed using the slurry method which has an

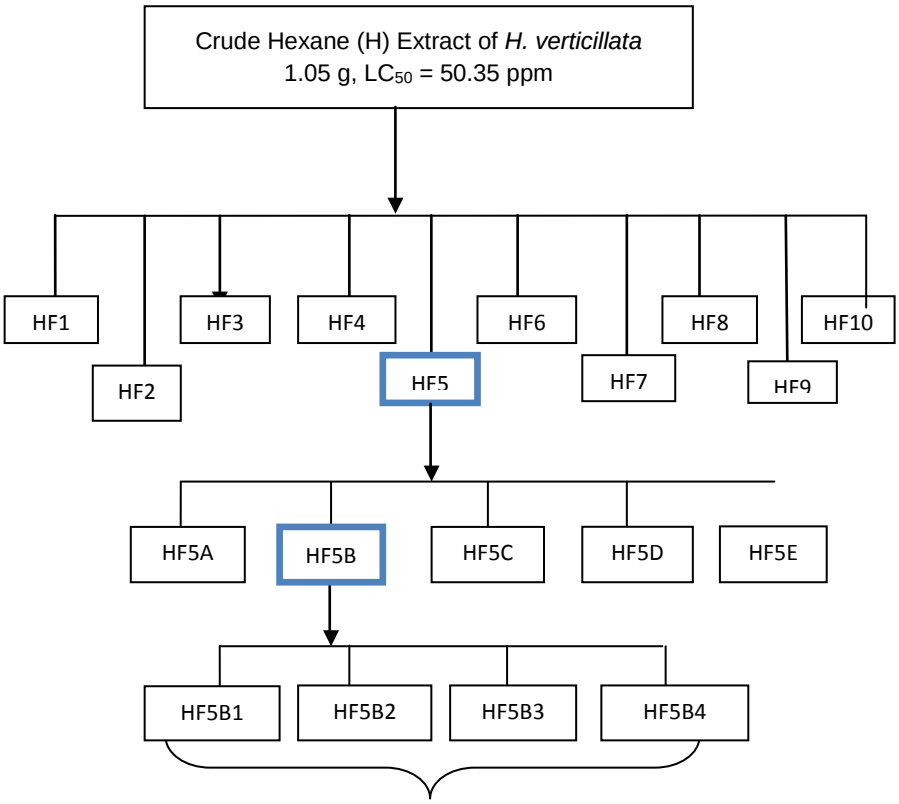
internal diameter of 25 mm and a length of 350 mm. A gradient step elution was required to elute the compounds from the column and was previously equilibrated with pure hexane. Hexane and ethyl acetate were first used as eluant . Ethyl acetate in methanol was next to be used as an eluant starting with 5-100% EtOAc in MeOH (increment of 5% in each step). The fractions obtained were subjected to Thin Layer Chromatography using Fluka TLC plate. Detection of the spots was done using UV lamp (short and long wave) and iodine vapour. Fractions with the same TLC profile were pooled together as one fraction. There were 10 fractions obtained from the first column chromatographic separation. All of these fractions were subjected to BSLT at LC_{50} of 50 ppm and antimicrobial assay to obtain the highest bioactivity. Fraction 5 (HF5) was selected for further purification because of its highest toxicity among the fractions (Scheme 3).

Second GCC was performed for further purification of HF5 using a column with smaller internal diameter of 15mm. EtOAc in Hex was used as eluting solvent starting with 35 mL of 10%, 20%, 25%, 30% of the mobile phase followed by 70% - 30% of EtOAc in Hex (increment of 2% in each step). There were five pooled fractions obtained. Pooling was done based on the result of TLC.

From among the five fractions obtained in the second GCC, the second was further purified (HF5B) for third gravity column chromatography since it has the greatest weight and highest toxicity. The column used in the third GCC had the smallest diameter of 7 mm. Four pooled fractions using 20% - 40% of EtOAc in Hex (increment of 1% in each step) as mobile phase.

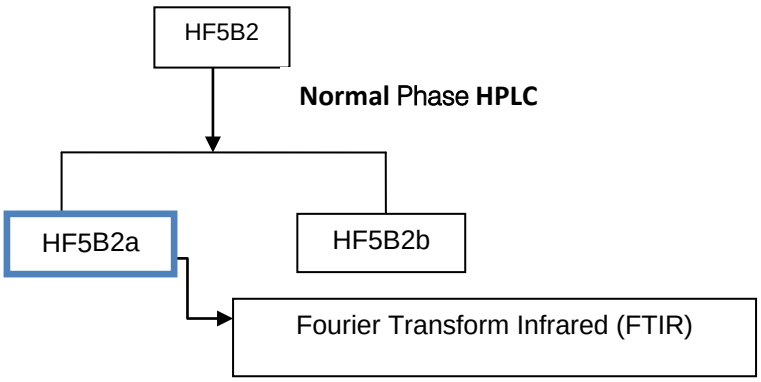
The profile of each of the four fractions (HF5B1, HF5B2, HF5B3, HF5B4) was examined from the resulting peaks produced by high performance liquid chromatography (normal phase HPLC) at the Chemistry Department, Xavier University, Ateneo de Cagayan. Twenty microliters of each fraction was injected to the HPLC (Perkin Elmer) at 254 nm using a wakopak wakosil column (normal phase) with a flow rate of 1 mL per minute as stationary phase, ethyl acetate as mobile phase, and UV detector (254 nm).

Among four fractions were subjected to HPLC HF5B2, HF5B3, HF5B4 showed similar retention time. The three fractions had similar HPLC profile, thus, they had similar composition and bioactivity. HF5B2 was chosen to be further purified to represent the entire fraction by means of HPLC instrument to yield a subfraction isolate with a single sharp peak in its chromatogram (Scheme 4). Collection of the subfraction from the HPLC was guided by the appearance of the chromatogram on the computer screen. This was collected in vial and labeled as HF5B2a and partially characterized using FTIR.

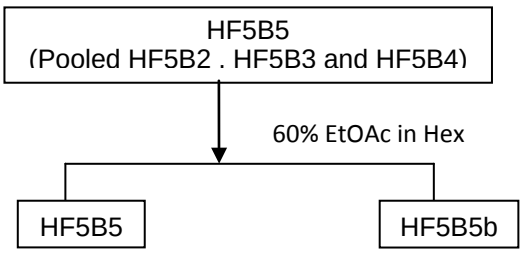


Scheme 3 : Purification of Crude Hexane Extract

Moreover, melting point determination was then conducted to purified isolate HF5B5a corresponds to HPLC isolate (HF5B2a which was partially characterized using FTIR) to determine its property. Melting point determination was done using Fisher John Melting Point Apparatus



Scheme 4 : HPLC Purification of fraction HF5B2



Scheme 5 : TLC Purification of HF5B5 (Pooled HF5B2, HF5B3 and HF5B4 Fractions)

f) Partial Caharacterization

The HPLC subfraction labeled HF5B2a was analyzed using a Perkin Elmer FTIR spectrometer. The IR spectrum produced was examined to identify the functional groups present in the compound. This was done by making a portion of the liquid sample using KBR window, spacer of 0.015 mm and loading it into the FTIR instrument thru its injection port. Analysis of the sample was done at the Analytical Laboratory Services of Mindanao University of Science and Technology, Philippines.

From the 668 g of air dried sample, 99.765 g of ethanolic crude extract was obtained. The crude ethanolic extract was separated by solvent partitioning according to increasing polarity and four solvent extracts were achieved. These were hexane (Hex), ethyl acetate (EtOAc), dichloromethane (DCM) and aqueous (Aq) extracts. Table 1 shows the consistency and appearance of each extract. Hex and EtOAc extracts were observed to be dark green and sticky. DCM was light green and has oily consistency while the aqueous was light orange and solid.

III. Results and Discussion

a) Solvent Extraction

Table 1 : The Appearance and Consistency of the Solvent Extracts from the Aerial Part of Plant Sample *H. verticillata*

Organic Extracts	Appearance	Consistency	Weight (grams)
Hex	Dark green	sticky	28.4594
EtOAc	Dark green	sticky	1.6624
DCM	Light green	oily	12.5324
Aq	Light orange	solid	19.7625

The colored appearance observed for each extract was due to the presence of pigment found in plants, example chlorophyll. The great abundance of chlorophyll in leaves and its occasional presence in other plant tissues, such as stems, caused these plant parts to appear green. In addition to chlorophyll, leaf cells also may contain other pigments such as yellow *xanthophylls,* yellowish-orange carotenes, and red and purple *anthocyanins.* Leaves also may contain which give them a golden-yellow color.[4]

b) Brine Shrimp Lethality Test

The toxicity of all crude extracts was evaluated by brine shrimp lethality test.[5] Toxicities of test extracts were tested at 10, 100, 1000 ppm and LC_{50} value was determined from the 24-hour death counting using probit analysis. The results of the assay are presented in Table 2.

Table 2 : Brine Shrimp Lethality Test Results of the Crude Extracts from Aerial Part of *H. verticillata*

Extraction Solvent	LC_{50} values (ppm) from the different crude extracts
EtOH	94.34
Hexane	50.35
EtOAc	76.98
DCM	223.87
Aq	377.45

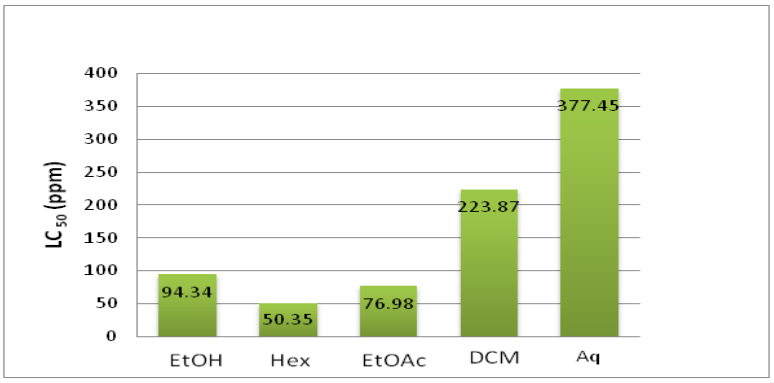


Figure 2 : Graphical Comparison of LC_{50} Values of Crude Extracts

Crude extracts of EtOH, Hex and EtOAc gave lower LC_{50} compared to DCM and the Aq extracts as shown clearly in Figure 2. But the hexane extract gave the lowest LC_{50} value of 50.35 ppm thus, it is the most active. Further- more, the bioassay indicates that the aqueous extract showed very little toxicity at LC_{50} value of 377.45 ppm.

c) Bioassay-Guided Fractionation of Hexane Crude Extracts

The most bioactive extract, the crude hexane extract which gave the lowest LC_{50} value was subjected to further purification and partial characterization.

Table 3 : The Appearance and Weight of Pooled Fractions Obtained from First GCC

Crude hex extract pooled fractions	Color	Weight (mg)
HF1 HF2 HF3	Colorless Orange Yellow	31.1 202.13 30.7
HF4	Dark green	119
HF5	Dark green	79.9
HF6	Dark reddish brown	49.6
HF7	Dark green	41.4

HF8	Dark green	44.3
HF9	Yellow	79.8
HF10	Pale yellow	72.4

- *bold most bioactive fraction and further purified*

There were 10 pooled fractions (Table 3) obtained from the first column chromatographic separation of the crude hexane extract. All of these were subjected to BSLT at LC_{50} of 50 ppm. Results are tabulated in Table 4.

Table 4 : Percent mortality of 10 Pooled Fractions (Crude Hex Extract)

Fraction	Mean % mortality±SD	*Corrected mean % mortality at 50 ppm
HF1	3.33 ± 0.58	0.00
HF2	6.67 ± 1.15	3.45
HF3	6.67 ± 0.58	3.45
HF4	3.33 ± 0.58	0.00
HF5	80.00 ± 1.00	79.31
HF6	23.33 ± 2.08	20.69
HF7	16.67 ± 2.08	13.80
HF8	6.67 ± 0.58	3.45
HF9	13.33 ± 1.15	10.35
HF10	13.33 ± 1.15	10.35
Blank	3.33 ± 0.58	

** calculated using Schneider-Orelli's Formula*

Out of the 10 fractions only fraction 5 (HF5) showed the highest toxicity at 79.31 % mortality rate at 50 ppm. HF5 was further purified and subjected to second GCC. There were five pooled fractions obtained from the second gravity column chrom- atography. Pooling was done based on the result of TLC. Solvent EtOAc in Hex was used as eluant starting with 35mL of 10%, 20%, 25%, 30% of the mobile phase followed by 70% - 30% of EtOAc in Hex (increment of 2% in each step). To test for toxicity, BSLT assay was again done to all of these fractions using LC_{50} of 50ppm.

The results of the five fractions obtained from second GCC and their weight in milligrams (mg) are shown in Table 5. Percent mortality of these pooled fractions is also tabulated on Table 6.

Table 5 : The Appearance and Weight of Pooled Fractions Obtained from Second GCC

Crudehexextract pooled fractions	Color	Weight (mg)
HF5A	Colorless	10.5
HF5B	Dark green	30.6
HF5C	Dark green	10.2
HF5D	Dark green	11.8
HF5E	Yellow	9.98

Table 6 : Percent Mortality of 5 Pooled Fractions

Fraction #	Mean % mortality at 50 ppm ± SD
HF5A	23.33 ± 0.58
HF5B	83.33 ± 1.15
HF5C	50.00 ± 1.00
HF5D	33.33 ± 1.53
HF5E	10.00 ± 1.00
Blank	0

Results reveal that HF5B has the highest mortality rate of 83.33%; coupled to its greater weight among the five fractions, it was further purified and subjected to third GCC using the smaller diameter of a pipette serving as the column. This time 20% - 40% of EtOAc in Hex (increment of 1% in each step) was used as the eluting solvent. The results revealed four pooled fractions which are presented in Table 7. Due to the very small quantity of the samples collected in the third GCC, weights were not measured.

Again, all of these fractions were subjected to TLC detection of the shade of spots. It was done under the UV lamp (254 nm and 365nm). The results revealed one spot for each fraction.

The profile of each four fractions (HF5B1, HF5B2, HF5B3, HF5B4) was examined also through the resulting peaks obtained using high performance liquid chromatography (normal phase HPLC at 254 nm) employing wakosil wakopak column with a flow rate of 1mL/minute.

Table 7 : HPLC Results of the Pooled Fractions in the Third GCC

Isolate	#of prominent peaks	Retention time for the prominent peaks (respectively)
HF5B1	2	8.130 , 8.417
HF5B2	2	8.400 , 9.122
HF5B3	2	8.450 , 9.165
HF5B4	2	8.257 , 9.003

HPLC results showed that HF5B1 had two shouldered peaks. For fractions HF5B2, HF5B3, HF5B4 two peaks (Appendix E. Fig.,10,11,12,) were also observed which interestingly showed closely similar retention times (Table 7). Hence, the molecules were eluted nearly all at the same time. Moreover, the appearance of first eluted peak was long and followed by a shorter peak. These results suggested that all the fractions were of similar composition and bioactivity.

Among four fractions, HF5B2, HF5B3 and HF5B4 were decided to be chosen to be further purified. This does not suggest that HF5B1 did not possess biological activity. However, due to time constraints and large number of variables that could be explored when using solvent system, thus, investigation would be focused to the three fractions mentioned above. As had been discussed similar HPLC profiles of HF5B2, HF5B3 and HF5B4 suggested that all the fractions were of similar composition and bioactivity. The fraction H5B2 was chosen to be subjected to normal phase HPLC for further purification since it represents already the entire fraction. Moreover, the two peaks of HF5B2 were both sharp which indicates that the components of this fraction were much more concentrated. Purification was done using normal phase HPLC instrument to yield a subfraction, HF5B2a with a sin- gle sharp peak on its chromatogram and with a retention time of 8.135 min. Collection of the subfraction from the HPLC was guided by the appearance of the chromatogram on the computer screen. The HPLC isolate was collected in a vial and was partially characterized using FTIR to analyze the functional groups present in this sample.

Melting point determination was then conducted to purified TLC isolate HF5B5a which corresponds to HPLC isolate HF5B2a (the first eluted peak done by HPLC and this was partially characterized using FTIR). The melting point of the isolate obtained 102-103^{0}C. It was determined by using Fisher-Johns Melting Apparatus. The short range of melting point implies its possibility of being pure.

d) Partial Characterization

IR spectrum of subfraction HF5B2a is shown in Figure 3. Absorption peaks and the possible functional groups present are tabulated below in Table 8.

Table 8 : IR Absorption Peaks for HF5B2a and Inferences[6]

Wavenumber, cm^{-1}	Inferences
3464.15 2994.38	NH secondary stretch C-H stretch
1762.44 , 1722.93	C=O stretch
1466.10	CH_2 bend
1447.66, 1382.69	CH_3 bend
1276.76	C-C(O)-C stretch
1213.41	CN stretch, aliphatic
1098.55,1062.07, 1034.99	C-C stretch
938.54	Out of plane CH bend
786.92	NH secondary wag

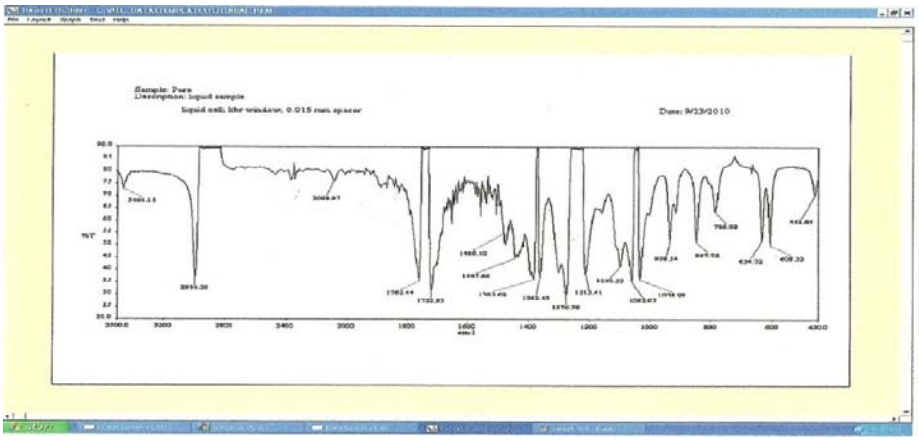


Figure 3 : FTIR Spectrum of HF5B2a

IV. Conclusion

Partial characterization of HF5B2a the most purified bioactive component of the Hexane Extract of *H. verticillata* was done using the Perkin Elmer FTIR. The following are the possible functional groups present that was deduced from the IR spectrum: NH secondary stretch, C=O stretch, CH_2 and CH_3 bend, C-C(O)-C stretch, CN stretch (secondary aliphatic amine), out of plane CH bend and NH wag. Considering the presence of these functional groups particularly the amine, the isolated metabolite HF5B2a from hexane extract could be an alkaloid derivative since alkaloid is composed of amines or nitrogen-containing compound. Alkaloid which was a DNA binder, play an important role in cancer therapy.[7]

V. Recommendations

1. HF5B2a will be subjected to reverse-phase HPLC
2. Frractions HF5B1, HF3B2 and HF5B3 must be further purified and the results will be tested for DNA-binding property

3. HF5B2a must be further recovered to characterize and eventually elucidate their structures that may also have a pharmacological significance.
4. Subfraction HF5B2a be further characterized by EIMS, 1D and 2D-NMR, H-NMR, ^{13}C-NMR and various correlations by ^{1}H-^{1}H HMQC and HMBC.

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Food Properties of Hull, Dehulled and Whole Seed Samples of Bambara Groundnut (*Vigna Subterranea* L. Verdc)

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Abstract - The functional properties of the testa, dehulled and whole seed samples of *Vigna subterranea* were examined under different neutral salt concentrations. Parameters examined for were: foaming capacity (FC), foaming stability (FS), water absorption capacity (WAC), emulsion capacity (EC), lowest gelation concentration (LGC) and protein solubility (PS). Results showed that the highest FC was recorded for NaCl at 0.5 % salt concentration, the lowest FC was recorded for Na_2SO_3 at 10 % salt concentration. Most of the present values of FS were lower than those reported for some legumes in literature. WAC values ranged between 250- 400 % (testa), 140-240 % (dehulled) and 100-240 % (whole seeds) in the various salt solutions; high WAC values could make the samples useful replacement in various food formulations such as baked goods and custards. The EC results showed that EC depended on salts concentrations and the type of salts under consideration. LGC results were in the range of 2.0-8.0 % which were mostly lower or within the range of most literature values for leguminous seeds.

Keywords : *functional properties, bambara groundnut seed anatomical parts.*

GJSFR-B Classification : *FOR Code: 259999p*

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E.I. Adeyeye [α], A.A. Olaleye [σ] & A.J. Adesina [ρ]

Abstract - The functional properties of the testa, dehulled and whole seed samples of *Vigna subterranea* were examined under different neutral salt concentrations. Parameters examined for were: foaming capacity (FC), foaming stability (FS), water absorption capacity (WAC), emulsion capacity (EC), lowest gelation concentration (LGC) and protein solubility (PS). Results showed that the highest FC was recorded for NaCl at 0.5 % salt concentration, the lowest FC was recorded for Na_2SO_3 at 10 % salt concentration. Most of the present values of FS were lower than those reported for some legumes in literature. WAC values ranged between 250-400 % (testa), 140-240 % (dehulled) and 100-240 % (whole seeds) in the various salt solutions; high WAC values could make the samples useful replacement in various food formulations such as baked goods and custards. The EC results showed that EC depended on salts concentrations and the type of salts under consideration. LGC results were in the range of 2.0-8.0 % which were mostly lower or within the range of most literature values for leguminous seeds. For the five salts used in the analysis (NaCl, Na_2CO_3, Na_2SO_3, $NaNO_3$ and CH_3COONa), the proteins in the samples were more soluble in the basic region of pH. The PS figures mostly showed two distinct peaks meaning that the bambara groundnut samples might be having two major proteins.

Keywords : functional properties, bambara groundnut seed anatomical parts.

I. Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc) is a seed crop of African origin. It is cultivated principally by farmers as a famine culture crop because of its agronomic values and the ability to produce in soils considered insufficiently fertile for cultivation of other more favoured species such as common beans and groundnuts *(Arachis hypogaea*) (Anchirinah*et al.,* 2001). It is very adaptable to hot temperatures but it is also tolerant to rainfall (Wrigley, 1981). Bambara seeds may be consumed in various forms for food. Fresh seeds may be consumed raw, boiled, grilled or dry seeds made into a powdery form to make cakes (Adebowale and Lawal, 2002).

The nutritional potentials of bambara groundnut were documented. The seed is regarded as a balanced food because when compared to most food legumes, it is rich in iron and the protein contains high lysine and methionine (Adu-Dapaah and Sangwan, 2004). In addition, it is known to contain 63% carbohydrates, 18% oil and the fatty acid content is predominmatly linoleic, palmitic and linolenic acids (Minka and Bruneteau, 2000). It was reported also that it is richer than groundnut in essential amino acids such as leucine, isoleucine, lysine, methionine, phenylalanine, threonine and valine (Ihekoronye and Ngoddy, 1985).

Soils of medium or low fertility, with a pH of 5.0 – 6.5 will produce satisfactory crops. Yields of bambara groundnut on low – fertility soils are generally higher than those of groundnut grown on similar soils. Bambara groundnut will often yield well in environments that may be too hostile for more favoured legumes (Collinson*et al.,* 1996).

Recently, Pasquet*et al.* (1999), using isozyme analysis, found high genetic identities between wild and domesticated bambara groundnut accessions and concluded that the wild bambara is the progenitor of the domesticated form, both being characterized by low total genetic diversity.

An evenly distributed rainfall in the range 600-1000mm encourages optimum growth but satisfactory yield can be obtained in areas with pronounced dry season since the crop is relatively drought resistant. It is tolerant to periods of heavy rainfall except during the flowering period.

It has been scientifically declared that bambara bean is high in protein quotient, particularly in methionine which makes its protein more complete than any other bean. The proximate composition of the bambara groundnut was reported to be 9.7% moisture, 16.6% protein, 5.9 % fat, 2.9% ash, 4.9% crude fibre and 64.9% carbohydrate (Enwere and Hung, 1996).

The high concentration of soluble fibre than any other bean also makes it one step ahead of other beans. This further enhances its quality as nutritious food which reduces the incidence of heart disease and certain types of cancer. Also, bambara beans being nitrogen fixers themselves and along with providing the soil with essential nutrients do not require any artificial fertilizer. The use of artificial flavours or preservatives during the food processing is greatly discarded.

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Bambara groundnut was reported to have been fairly well supplied with calcium and iron although poor in phosphorus. It contains thiamine, riboflavin, niacin and carotene but very low in ascorbic acid (Oyenuga, 1968).

Several other reports have been made on bambara nut. The swelling capacity increases with increase in temperature (Adebowale*et al*., 2002). Bambara bean is higher in water absorption capacity than that of great Northern bean (Sathe and Salunkhe, 1981).

The water absorption capacity (WAC) of bambara groundnut flour was higher than that of starch (Piyarat, 2008). This result shows that the flour is more hydrophilic due to a higher protein and carbohydrate contents. Also, the higher oil absorption capacity (OAC) in flour could be due to its higher protein and fat contents which can entrap more oil.

The objectives of this research work therefore are to: find out the functional properties of bambara groundnut, reveal the food properties of the samples and hence, their industrial applications in the food industry and provide useful information that can further suggest the consideration of bambara groundnut as an alternative source of nutrients, especially for populations of the developing countries such as Nigeria.

II. Resources and Techniques

a) Sample collection and preparation

The sample (bambara groundnut) was obtained from the Department of Plant Science, Ekiti State University, Ado-Ekiti. The seeds were screened to eliminate the defective ones, washed and rinsed with distilled water. The seeds were divided into two parts. One part was soaked with distilled water overnight while the other part was dried without soaking. The soaked ones were removed after 24 h and were manually dehulled. Both the cotyledon and the testa were dried in an oven at 45 °C. All the three samples (whole seed, cotyledon and testa) were dry milled separately to fine powder and stored in a dry, cool place prior to use. The three samples were used for various analyses as described below.

b) Preparation of salt solutions

0.5, 1, 2, 5 and 10% (w/v) concentrations of various salts used were prepared by weighing 0.5, 1, 2, 5 and 10g of salts (NaC1, Na_2CO_3, $NaNO_3$ CH_3COONa and Na_2SO_3) which are respectively added to 99.5, 99, 98, 95 and 90 ml of distilled water for each solution to make up to 100g.

Biuret solution was prepared by weighing (30g of NaOH dissolved in 300 ml of distilled water) + (1.5g $CuSO_4.4H_2O$+6.0g potassium sodium tertarate dissolved in 500ml distilled water) and made up to 1dm^3 with distilled water.

c) Determination of foaming capacity and stability

1g of the sample was whipped with 50ml of distilled water for 5 min in a blender at speed set at "max" and was transferred into a 100ml graduated cylinder. Total volume at time interval of 0.0, 0.05, 0.1, 0.2, 0.3 and 1.0 hour was noted to study the foaming stability. Volume increase (%) was calculated according to the equation of Coffman and Garcia (1977) to obtain the foaming capacity.

d) Determination of emulsion activity

2g of the sample, 20ml distilled water, 20ml executive chef vegetable oil was prepared in a calibrated centrifuge tube. The emulsion was centrifuged for 5 minutes. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as the emulsion activity expressed in percentage (Inklaar and Fortuin, 1969).

e) Determination of water and oil absorption capacity

The water and oil absorption capacity of the sample was determined as described by Beuchat (1977). 10cm^3 of water or salt solution was added to 1.0g sample in a centrifuge tube. The suspension was mixed vigorously using vortex mixer. This was then centrifuged for 25 min and the volume of the supernatant left after centrifuging was noted. Water bound was calculated from the difference in volume of the initial volume of the solvent used and the final volume after centrifuging. The same procedure was used for oil absorption capacity (Inklaar and Fortuin, 1969).

f) Determination of least gelation concentration

Appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16% were prepared in 5ml of distilled water. The test tubes containing these suspensions were heated for 1 hour in boiling water followed by rapid cooling under running tap water. The test tubes were then cooled for 2 hours at 4^0C. The least gelation concentration was determined as concentration when the sample from the inverted test tube did not fall down or slip (Coffman and Garcia, 1977).

g) Protein solubility determination

The protein solubilities of the samples at different salts concentration were studied using the Biuret method (Wiechselboven, 1946).

0.5g of the sample was suspended in 10ml of different salt concentrations. The solubility at natural pH was first determined, that is no acid or alkali was added and so solubility in the case was based on the normal pH of the sample in solution. The suspension was centrifuged at room temperature for 30 min at 3,500 rpm. The supernatant obtained was filtered and the protein of filtrate was determined by Biuret method with standard Bovine Serum Albumin (BSA) (Wiechselboven, 1946).

The Biuret method is a convenient assay for large number of samples of relatively soluble proteins. Kjeldahl method is not a rapid and convenient assay although useful for the determination of the amount of protein in crude mixtures.

For the determination of standard protein using Biuret method, 1g of BSA was dissolved in 100ml distilled water in a volumetric flask. Five tubes were set up containing fractions of the BSA solution in the order: 0.0ml, 0.5ml, 1.0ml, 1.5ml, 2.0ml and they were made up to 2ml by adding 2.0ml, 1.5ml, 1.0ml, 0.5ml and 0.0ml of distilled water. The tubes were left to stand for 30 min. The solution from the tube containing 2.0ml distilled water and 8.0ml Biuret solution was used as the blank to standardize the UV spectrophotometer at 450nm. The absorbance of each of the other tubes was also taken. A standard curve was drawn for absorbance against concentration.

The determination of protein of the filtered supernatant in each sample was carried out as follows: 1.0ml of filtrate was pipetted into a test tube and 8ml of Biuret solution was added. The tube was left to stand for 30 min after which the absorbance was taken. The corresponding protein concentration was obtained. The obtained protein concentrations for the various salts were plotted (Oshodi and Ekperigin, 1989).

III. Results and Discussion

a) Foaming Capacity (FC)

Tables 1-3 present the foaming capacity (FC) of bambara groundnut: testa, dehulled and whole seeds under various salts and salt concentrations. The Tables revealed that foaming capacity depends on concentration and types of salts used. There was a decrease in the foaming capacity with increase in concentration of salt from 0.5 to 10%. The FC values in Table 1 showed that the FC was 9.0 cm^3 (18.0%) at zero percent concentration (water only). The FC ranged from 2.0cm^3 (4.0%)-7.0cm^3 (14.0%) for NaCl, 2.0cm^3 (4.0%) – 6.50cm^3 (13.0%) for Na_2CO_3, 2.0cm^3 (4.0%) – 4.0cm^3 (8.0%) for $NaNO_3$, 2.0cm^3 (4.0%) – 5.0cm^3 (10.0%) for CH_3COONa and 2.0cm^3 (4.0%) – 5.50cm^3 (11.0%) for Na_2SO_3. Highest foaming capacity was reported for NaCl at 0.5% salt concentration whereas lowest foaming capacity was reported for NaCl at 5% and 10% salt concentrations, Na_2CO_3 at 10%, $NaNO_3$ at 5%, CH_3COONa at 10% and Na_2SO_3 at 5 and 10% salt concentrations in testa. In dehulled (Table 2), highest FC was recorded for Na_2SO_3 at 1.0% salt concentration and NaCl at 0.5% and the lowest for NaCl and Na_2CO_3 at 10% and CH_3COONa at 2% and 10% salt concentrations. In Table3, for whole seeds, most of the FC values were fairly higher than those recorded for testa and dehulled samples. While the highest FC was recorded for NaCl at 0.5% salt concentration, the lowest FC was recorded for Na_2SO_3 at 10% salt concentration. Fairly high variation in foaming capacity existed within the salt concentrations in all the samples as shown by the CV%. The values of foaming capacity in all the three samples were lower than the values earlier reported for hulled seed flours of African yam bean (AYB) (39.9-55.4%) and dehulled AYB seeds (21.3-48.4%) (Adeyeye and Aye, 1998). The low foaming capacities will reduce the functionality of bambara groundnut in its uses for the production of some foods where foaming is an important factor like cakes (Johnson *et al.,* 1979; Lee *et al.,* 1993).

b) Foaming Stability (FS)

The foaming stability (FS) values for testa are presented in Tables 4 (NaCl), 5 (Na_2CO_3), 6 ($NaNO_3$), 7 (CH_3COONa) and 8 (Na_2SO_3). The order of increasing foaming stability among the salts were Na_2CO_3 (rate= 0.0-3.75% min^{-1})< Na_2SO_3 (rate = 0.0-10.0%min^{-1}) <NaCl (rate =1.19-10.0% min^{-1}) < $NaN0_3$ (rate = 2.5-10.0 % min^{-1}) < CH_3COONa (rate = 2.67-10.0 % min^{-1}). The best concentration of NaCl was 0.5% (w/v), 1.0% (w/v) in Na_2CO_3, 1.0% (w/v) in $NaNO_3$, 0.5% (w/v) in CH_3COONa and 0.5% (w/v) in Na_2SO_3.

The foaming stability of dehulled bambara groundnut in Tables 9-13 revealed that the best NaCl concentration was 1.0% (w/v), 2.0% (w/v) for Na_2CO_3,1.0% (w/v) for $NaNO_3$, 5.0% for CH_3COONa and 1.0% (w/v) for Na_2SO_3. Also, in whole seeds (Tables 14-18), the best concentration of NaCl, Na_2CO_3, $NaNO_3$ and Na_2SO_3 was 0.5% (w/v) whereas, 1.0% (w/v) was best in CH_3COONa. Most of the present values of FS% were lower than those reported for AYB seeds (Adeyeye and Aye, 1998), pigeon pea (Oshodi and Ekperigin, 1989) and raw cowpea flour (Padmashree*et al.,* 1987). Foaming stability is important since success of a whipping agent depends on its ability to maintain the whip as long as possible.

c) Water absorption capacity (WAC)

The water absorption capacity (WAC) values of bambara groundnut samples: testa, dehulled and whole seeds are shown in Tables 19 (testa), 20 (dehulled) and 21 (whole seeds). The WAC in distilled water was 280%. The values ranged between 250-400% in testa, 140-240% in dehulled and 100-240% in whole seeds in the various salt solutions. The WAC values in various salt solutions were close in all the samples as seen in the coefficients of variation percent (CV %). In testa, the best salts for WAC property were NaCl particularly at 1.0% (w/v) and Na_2CO_3 at 2.0% (w/v) salt concentrations. In dehulled, the best salt was CH_3COONa particularly at 5.0% (w/v) whereas in whole seeds, the best salt for WAC property was Na_2SO_3. All the values in the testa and most of the values in dehulled and whole seeds were comparatively higher than the WAC of 138% reported for pigeon pea flour (Oshodi and Ekperigin, 1989), 130% for soy flour, 107% for sunflower and 60.2%

for wheat flour (Lin *et al.,* 1974) but compared favourably with that of cowpea flour (246%) (Olaofe *et al.,*1993). The high values of WAC in bambara groundnut could make it a useful replacement in viscous food formulations such as baked goods and custards.

d) Emulsion Capacity (EC)

Tables 22–24 present the emulsion capacity (EC) of bambara groundnut testa, dehulled and whole seeds. The results showed that emulsion capacity depended on salt concentrations and the types of salts under consideration. A gradual increase in EC was observed with increase in salt concentrations in NaCl solution in all the samples and CH_3COONa in testa. There was a progressive increase up to 2.0% generally in Na_2CO_3 solution; in Na_2SO_3 solution (testa and whole seeds) and CH_3COONa (dehulled) after which it decreased up to 5.0% and later increased up to 10.0%. Low levels of the coefficients of variation showed that the emulsion capacity values in the various salt concentrations were close in the present report. The present report was fairly better than the 18.0% reported for soy flour and 11.0% for wheat flour (Lin *et al.,* 1974).

e) Lowest gelation concentration (LGC)

The variation in lowest gelation concentration with increase in concentration of salts of bambara groundnut samples: testa, dehulled and whole seeds are indicated in Tables 25, 26 and 27 respectively. The salt free values ranged from 2.0-8.0% while the various salt concentration values ranged from 4.0- 8.0% (NaCl), 4.0-8.0% (Na_2CO_3), 4.0-6.0% ($NaNO_3$), 4.0-8.0% (CH_3COONa) and 4.0-8.0% (Na_2SO_3) (for testa); 2.0-4.0% (NaCl), 2.0-4.0% (Na_2CO_3), 2.0-6.0% ($NaNO_3$),2.0-4.0% (CH_3COONa) and 2.0-6.0% (Na_2SO_3) (for dehulled) and 6.0-8.0% (NaCl), 6.0-8.0% (Na_2CO_3), 6.0-8.0% ($NaNO_3$), 6.0-8.0% (CH_3COONa) and 6.0-8.0% (Na_2SO_3) (for whole seeds). Most of these values were lower or within the range of most literature values for leguminous seeds (Oshodi and Ekperigin, 1989; Adeyeye and Aye, 1998). The variation in the gelation concentration of the samples under different salt concentrations and anions might be due to their different effects on the relative ratios of different constituents: proteins, lipids and carbohydrates (Sathe*et al.,* 1982). The low CV% of lowest gelation concentration among the various salts showed that the results were very close.

f) Protein Solubility (PS)

The solubility of proteins is greatly influenced by pH, as might be expected from their amphoteric behaviour; solubility is at a minimum at the isoelectric point and increases with increasing alkalinity or acidity. It is worthy of note that each protein has a definite solubility in a solution of a fixed salt concentration and pH. The effect of salts in increasing the solubility of globulins is called the "salting-in" effect. The solubility is a function of the ionic strength, which is usually calculated from the molar concentrations of the ions and their charge using the expression: $\mu = \frac{1}{2} \varepsilon m z^2$ where μ is the ionic strength, m is the molarity and z, the charge of the ion, the ε denotes that mz^2 terms are added for each of the ions (White *et al.,* 1973) (Table 28). Neutral salts are known to exert striking effects on the solubility, the association-dissociation equilibrium, the enzyme activity, the stability of natural and fibrillar structures and the rate of conformational change of proteins, polypeptides and nucleic acids (Oshodi and Ojokan, 1997). The dependence of protein solubility with pH in the presence of salts is presented in Figures 1-.2.5 Figures 1-5 depict the protein solubility (PS) in different salts/pH of testa. The minimum solubility pH (considering just one or two concentrations) was pH 3 (10%) in Na_2SO_4, pH 3 (10%) in Na_2CO_3;pH 10 (2%) in Na_2SO_3, pH 8 (10%) in CH_3COONa, pH 3 or 5 (2%) and pH 4 (5%) in $NaNO_3$ .In dehulled sample (Figures 6 -10), the maximum solubility pH (taking the best two concentrations) was 12 (0.5%) and 11 (2%) in Na_2SO_4, pH 12 (10%) and 9 (2%) in Na_2CO_3, pH 9 (10%) and 7 (2%) in Na_2SO_3, pH 1 (2%) and 1 (1%) in CH_3COONa and pH 12 (2%) and 7 (10%) in $NaNO_3$. In whole seeds (Figures 11-15), the maximum solubility pH was pH 9 (1.0%) in Na_2SO_4, pH 1 or 10 (5.0%) in Na_2CO_3, pH 10 (0.5%) and pH 7 (10.0%) in Na_2SO_3, pH 12 (5.0%) in CH_3COONa and pH 9 (10.0%).

The minimum solubilities (pI) were recorded for various salts at various concentrations, viz: Na_2CO_3, 2.31% ($8.5x10^{-3}$M or 10%); $NaNO_3$, 6.94 ($2.3x10^{-3}$M or 2.0% and $5.6x\ 10^{-3}$M or 5.0%); Na_2SO_3 3.7 ($7.1x\ 10^{-3}$M or 10.0%); CH_3COONa, 4.63 ($1.1x10^{-2}$M or 10.0%) [testa], Na_2CO_3, 5.41 ($9.3x10^{-4}$ M or 1.0% and $1.8x10^{-3}$M or 2.0%); Na_2SO_3, 1.27 ($3.8x10^{-3}$ M or 2.0%); $NaNo_3$, 0.64 ($5.9\ x10^{-4}$ M or 0.5%); $NaNO_3$, 0.64 ($5.9x10^{-4}$ M or 0.5%); Na_2SO_3, 1.27 ($3.8\ x\ 10^{-3}$ M or 5.0%); CH_3COONa, 1.59($1.1\ x\ 10^{-2}$ M or 10.0%) [dehulled] and Na_2CO_3, 7.75 ($4.7\ x\ 10^{-4}$ M or 0.5%); $NaNO_3$, 6.81 ($5.9\ x\ 10^{-4}$ M or 0.5%); Na_2SO_3, 9.84 ($7.9\ x10^{-4}$ M or 1.0%, $1.6x10^{-3}$ M or 2.0%. and $3.8\ x\ 10^{-3}$ M or 5.0%); CH_3COONa, 6.05 ($6.1x10^{-4}$ M or 0.5%w/v) [whole seeds]. Maximum solubility values varied similarly viz: Na_2CO_3 16.2 ($4.7x10^{-4}$ M or 0.5%); $NaNO_3$, 12.0 ($1.2x10^{-3}$M or 1.0%); Na_2SO_3 10.7 ($7.9x10^{-4}$ M or 1.0%); CH_3COONa, 11.6 ($2.4x10^{-3}$ M or 2.0% and $5.8x10^{-3}$ M or 5.0% w/v) [testa], Na_2CO_3, 10.8 ($8.5x10^{-3}$ M or 10.0%); $NaNO_3$, 7.64 ($2.3x10^{-3}$ M or 2.0% and $1.1x10^{-2}$ M or 10.0%); Na_2SO_3 6.36 ($7.1x10^{-3}$ M or 10.0%); CH_3COONa, 8.0 ($2.4x10^{-3}$ M or 2.0% w/v) [dehulled] and Na_2CO_3, 12.1 ($4.5x10^{-3}$ M or 5.0%); $NaNO_3$, 13.2 ($1.1x10^{-2}$ M or 10.0%); Na_2SO_3 12.9 ($3.9x10^{-4}$ M or 0.5% and $7.1x10^{-3}$ M or 10.0%); CH_3COONa, 11.4 ($5.8x10^{-3}$ M or 5.0% w/v) [whole seeds]. The lyotropic series is therefore in the order: $CO_3^{2-} > NO_3^- > CH_3COO^- > SO_3^{2-}$ (testa), $CO_3^{2-} > CH_3COO^- > NO_3^- > SO_3^{2-}$ (dehulled) and $NO_3^- > SO_3^{2-} > CO_3^{2-} > CH_3COO^-$ (whole seeds).

It was generally observed that for the five salts used in the analysis, the proteins in the bambara

groundnut samples were more soluble in the basic region of pH. The electrostatic interactions (ionization of interior non-polar groups) are more important in hydration of proteins than the surface charge. This might contribute to the improved protein solubility in the alkaline region. Also, most of the figures showed two distinct peaks meaning that the bambara groundnut samples: testa, dehulled and whole seeds might be having two major proteins.

IV. Conclusion

The samples were good in water absorption capacity and emulsion capacity but foaming capacity and stability are not favourable. It could therefore be concluded that bambara groundnut should be dehulled when it is being used as food supplement especially for infants.

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Table 1 : Foaming capacity (cm^3) of bambara groundnut testa in var ous salticoncentrations

	Sal concentrations (%)						Mean	SD	CV%
Salt	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
NaCl	9.0(18.0)	7.0(14.0)	5.0(10.0)	3.0(60)	2.0(4.0)	2.0(4.0)	4.67	2.88	61.7
Na_2CO_3	9.0(18.0)	6.50(13.0	6.0(12.0)	4.0(8.0)	3.50(7.0)	2.0(4.0)	5.17	2.50	48.4
$NaNO_3$	9.0(18.0)	3.0(6.0)	4.0(8.0)	3.50(7.0)	2.0(4.0)	4.0(8.0)	4.25	2.44	57.4
CH_3COONa	9.0(18.0)	5.0(10.0)	5.0(10.0)	4.0(8.0)	3.0(6.0)	2.0(4.0)	4.42	2.46	55.7
Na_2SO_3	9.0(18.0)	5.50(11.0)	4.0(8.0)	3.0(6.0)	2.0(4.0)	2.0(4.0)	4.25	2.68	63.1

Note: The values inside the brackets represent the corresponding percentage values. Mean, SD and CV% were calculated based on volume.

Table 2 : Foaming capacity (cm^3) of dehulledbambaragroundnut in various salt concentrations

	Salt concentrations (%)						Mean	SD	CV%
Salt	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
NaCl	5.0(10.0)	6.0(12.0)	5.50(11.0)	3.0(6.0)	3.0(6.0)	2.0(4.0)	4.08	1.63	40.0
Na_2CO_3	5.0(10.0)	3.0(6.0)	4.0(8.0)	5.0(10.0)	3.50(7.0)	2.0(4.0)	3.75	1.17	31.2
$NaNO_3$	5.0(10.0)	3.0(6.0)	3.50(7.0)	4.0(8.0)	3.0(6.0)	2.50(5.0)	3.50	0.89	25.4
CH_3COONa	5.0(10.0)	4.0(8.0)	3.0(6.0)	2.0(4.0)	3.0(6.0)	2.0(4.0)	3.17	1.17	36.9
Na_2SO_3	5.0(10.0)	5.0(10.0)	6.0(12.0)	4.0(8.0)	3.0(6.0)	2.50(5.0)	4.25	1.33	31.3

Table 3 : Foaming capacity (cm^3)of bambara groundnut wholeseeds in various salt concentrations

	Salt concentrations (%)						Mean	SD	CV%
Salt	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
NaCl	13.0(26.0)	12.0(24.0)	10.0(20.0)	11.0(22.0)	8.0(16.0)	6.0(12.0)	10.0	2.61	26.1
Na_2CO_3	13.0(26.0)	7.0(14.0)	6.0(12.0)	4.0(8.0)	3.0(6.0)	2.50(5.0)	5.92	3.88	65.5
$NaNO_3$	13.0(26.0)	10.0(20.0)	8.0(16.0)	6.0(12.0)	7.0(14.0)	4.0(8.0)	8.0	3.16	39.5
CH_3COONa_3	13.0(26.0)	6.0(12.0)	5.0(10.0)	3.0(6.0)	3.0(6.0)	2.0(4.0)	5.33	4.03	75.6
Na_2SO_3	13.0(26.0)	5.0(10.0)	3.50(7.0)	4.0(8.0)	2.0(4.0)	1.50(3.0)	4.83	4.20	87.0

Table 4 : Foaming stability (%) of bambara groundnut testa using NaCl

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(9.0)	100(7.0)	100(5.0)	100(3.0)	100(2.0)	100(2.0)	100	0.0	0.0
5	88.9(8.0)	85.7(6.0)	80.0(4.0)	66.7(2.0)	50.0(1.0)	100(2.0)	78.6	17.8	22.6
10	77.8(7.0)	71.4(5.0)	60.0(3.0)	66.7(2.0)	0.0(0.0)	50.0(1.0)	54.3	28.3	52.1
20	66.7(6.0)	4.0(57.1)	40.0(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	27.3	31.1	114
30	55.6(5.0)	42.9(3.0)	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	19.8	24.5	125
60	44.5(4.0)	28.6(2.0)	0.0(0.0)	0.0(0.0)	0.0(.0)	0.0(0.0)	12.8	19.5	152
90	223(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	3.7	9.1	246
Rate	0.86	1.19	2.67	3.33	10.0	5.0	3.84	3.37	87.8

Table 5 : Foaming stability (%) of bambara groundnut testa using Na_2CO_3

Time (min)	Salt concentrations (%) 0.0	0.5	1.0	2.0	5.0	10.0	Mean	SD	CV%
0	100(9.0)	100(6.5)	100(6.0)	100(4.0)	100(3.5)	100(2.0)	100	0.0	0.0
5	88.9(8.0)	76.9(5.0)	83.3(5.0)	75.0(3.0)	57.1(2.0)	100(2.0)	80.2	14.5	18.1
10	77.8(7.0)	61.5 (4.0)	83.3(5.0)	50.0(2.0)	28.6(1.0)	0.0(0.0)	50.2	31.5	62.7
20	66.7(6.0)	46.2(3.0)	66.0(4.0)	25.0(1.0)	28.6(1.0)	0.0(0.0)	38.9	26.1	67.1
30	55.6(5.0)	30.8(2.0)	50.0(3.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	22.7	26.2	115
60	44.5(4.0)	30.8(2.0)	33.3(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	18.1	20.4	113
90	22.3(2.0)	15.4(1.0)	33.3(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	11.8	14.2	120
Rate	0.86	0.94	0.74	3.5	3.57	0.0	1.64	1.60	97.6

Table 6 : Foaming stability (%) of bambara groundnut testa using $NaNO_3$ salt concentrations

Time (min)	Salt concentrations (%) 0.0	0.5	1.0	2.0	5.0	10.0	Mean	SD	CV%
0	100(9.0)	100(3.0)	100(4.0)	100(3.50)	100(2.0)	100(4.0)	100	0.0	0.0
5	88.9(8.0)	66.7(2.0)	75.0(3.0)	57.1(2.0)	50.0(1.0)	75.0(3.0)	68.8	14.0	20.3
10	77.8(7.0)	33.3(1.0)	50.0(2.0)	28.6(1.0)	0.0(0.0)	50.0(2.0)	40.0	26.1	65.3
20	66.7(6.0)	0.0(0.0)	50.0(2.0)	0.0(0.0)	0.0(0.0)	25.0(1.0)	23.6	29.1	123
30	55.6(5.0)	0.0(0.0)	25.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	13.4	23.0	172
60	44.5(4.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	7.42	18.2	245
Rate	0.93	6.67	2.50	7.14	10.0	3.75	5.17	3.36	65.0

Table 7 : Foaming stability (%) of bambara groundnut testa using CH_3COONa

Time (min)	Salt concentrations (%) 0.0	0.5	1.0	2.0	5.0	10.0	Mean	SD	CV%
0	100(90)	100(5.0)	100(4.0)	100(3.0)	100(3.5)	100(2.0)	100	0.0	0.0
5	88.9(8.0)	80.0(4.0)	75.0(3.0)	66.7(2.0)	85.7(3.0)	50.0(1.0)	74.4	14.3	19.2
10	77.8(7.0)	60.0(3.0)	50.0(2.0)	33.3(1.0)	57.1(2.0)	0.0(0.0)	46.4	26.9	85.0
20	66.7(6.0)	40.0(2.0)	25.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	22.0	27.5	125
30	55.6(5. 0)	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	12.6	22.5	179
60	44.5(4.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	7.42	18.2	68.0
Rate	0.93	2.67	3.75	6.67	4.29	10.0	4.72	3.21	68.0

Table 8 : Foaming stability (%) of bambara groundnut testausing Na_2SO_3

Time (min)	Salt concentrations (%) 0.0	0.5	1.0	2.0	5.0	10.0	Mean	SD	CV%
0	100(9.0)	100(5.5)	100(4.0)	100(3.0)	100(2.0)	100(2.0)	100	0.0	0.0
5	88.9(8.0)	72.7(4.0)	75.0(3.0)	66.7(2.0)	100(2.0)	50.0(1.0)	75.6	17.4	23.0
10	77.8(7.0)	54.5(3.0)	50.0(2.0)	33.3(1.0)	0.0(0.0)	0.0(0.0)	35.9	31.3	87.2
20	66.7(6.0)	36.4(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	17.2	28.3	165
30	55.6(5.0)	18.2(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	12.8	22.4	175
60	44.5(4.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	7.42	18.2	245
Rate	0.93	2.73	5.0	6	0 .0	10.0	4.22	3.76	89.1

Table 9 : Foaming stability (%) of dehulledbambara groundnut using NaCl

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(5.0)	100(6.0)	100(5.50)	100(3.0)	100(3.0)	100(2.0)	100	0.0	0.0
5	80.0(4.0)	83.3(5.0)	90.9(5.0)	66.7(2.0)	66.7(2.0)	50.0(1.0)	72.9	14.7	20.2
10	60.0(3.0)	66.7(4.0)	90.9(5.0)	33.3(1.0)	66.7(2.0)	0.0(0.0)	52.9	31.8	60.1
20	40.0(2.0)	50.0(3.0)	72.7(4.0)	0.0(0.0)	33.3(1.0)	0.0(0.0)	32.7	28.6	87.5
30	20.0(1.0)	33.3(2.0)	54.5(3.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	18.0	22.5	125
60	0.0(0.0)	0.0(0.0)	36.4(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	6.07	14.9	245
Rate	2.67	2.22	1.06	6.67	3.34	10.0	4.33	3.36	77.6

Table 10 : Foaming stability (%) of dehulledbambara groundnut using Na_2CO_3

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(5.0)	100(3.0)	100(4.0)	100(5.0)	100(3.5)	100(2.0)	100	0.0	0.0
5	80.0(4.0)	66.7(2.0)	75.0(3.0)	80.0(4.0)	85.7(3.0)	50.0(1.0)	72.9	13.0	17.8
10	60.0(3.0)	33.3(1.0)	50.0(2.0)	60.0(3.0)	57.1(2.0)	50.0(1.0)	51.7	10.1	19.5
20	40.0(2.0)	0.0(0.0)	25.0(1.0)	40.0(2.0)	28.6(1.0)	0.0(0.0)	22.3	18.3	82.1
30	20.0(1.0)	0.0(0.0)	0.0(0.0)	40.0(2.0)	0.0(0.0)	0.0(0.0)	10.0	16.7	167
60	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0	0.0	0.0
Rate	2.67	6.67	3.75	2.0	3.57	5.0	3.94	1.69	42.9

Table 11 : Foaming stability (%) of dehulledbambara groundnut using $NaNO_3$

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(5.0)	100(3.0)	100(3.50)	100(4.0)	100(3.0)	100(2.50)	100	0.0	0.0
5	80.0(4.0)	66.7(2.0)	85.7	75.0(3.0)	66.7(2.0)	80.0(2.0)	75.7	7.74	10.2
10	60.0(3.0)	33.3(1.0)	57.1(2.0)	50.0(2.0)	33.3(1.0)	40.0(1.0)	45.6	11.8	25.9
20	40.0(2.0)	0.0(0.0)	28.6(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	11.4	18.1	159
30	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	3.33	8.16	245
60	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0	0.0	0.0
Rate	2.67	6.67	3.57	5.0	6.67	6.0	5.10	1.67	32.7

Table 12 : Foaming stability (%) of dehulledbambara groundnut usingCH_3COONa

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(5.0)	100(4.0)	100(3.0)	100(2.0)	100(3.0)	100(2.0)	100	0.0	0.0
5	80.0(4.0)	75.0(3.0)	66.7(2.0)	50.0(1.0)	83.3(2.50)	50.0(1.0)	67.5	14.7	21.8
10	60.0(3.0)	50.0(2.0)	0.0(0.0)	0.0(0.0)	66.7(2.0)	50.0(1.0)	37.8	30.0	79.4
20	40.0(2.0)	25.0(1.0)	0.0(0.0)	0.0(0.0)	33.3(1.0)	0.0(0.0)	16.4	18.6	113
30	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	3.33	8.16	245
60	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0	0.0	0.0
Rate	2.67	3.75	6.66	10.0	3.34	5.0	5.24	2.73	52.1

Table 13 : Foaming stability (%) of dehulledbambara groundnut using Na_2SO_3

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(5.0)	100(5.0)	100(6.0)	100(4.0)	100(3.0)	100(2.50)	100	0.0	0.0
5	80.0(4.0)	80.0(4.0)	83.3(5.0)	75.0(3.0)	66.7(2.0)	80.0(2.0)	77.5	5.92	7.6
10	60.0(3.0)	60.0(3.0)	66.7(4.0)	50.0(2.0)	66.7(2.0)	40.0(1.0)	57.2	10.4	18.2
20	40.0(2.0)	40.0(2.0)	50.0(3.0)	25.0(2.0)	33.3(1.0)	0.0(0.0)	31.4	17.5	55.7
30	20.0(1.0)	20.0(1.0)	33.3(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	12.2	14.2	116
60	0.0(0.0)	0.0(0.0)	33.3(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	5.55	13.6	245
Rate	2.67	2.67	1.11	3.57	3.34	6.0	3.26	1.62	49.7

Table 14 : Foaming stability (%) of bambara groundnut whole seed using NaCl

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(13.0)	100(12.0)	100(10.0)	100(11.0)	100(8.0)	100(6.0)	100	0.0	0.0
5	92.3(12.0)	91.7(11.0)	90.0(9.0)	72.7(8.0)	87.5(7.0)	83.3(5.0)	86.3	7.41	8.6
10	84.0(11.0)	83.3(10.0)	80.0(8.0)	63.6(7.0)	75.0(6.0)	66.7(4.0)	75.5	8.75	11.6
20	76.9(10.0)	66.7(8.0)	70.0(7.0)	54.5(6.0)	62.5(5.0)	50.0(3.0)	63.4	9.96	15.7
30	69.2(9.0)	58.3(6.0)	60.0(6.0)	45.5(5.0)	50.0(4.0)	33.3(2.0)	52.7	12.6	23.9
60	61.5(7.0)	41.7(5.0)	40.0(4.0)	27.3(3.0)	25.0(2.0)	16.7(1.0)	36.8	16.9	45.9
90	53.8(7.0)	41.7(6.0)	30.0(3.0)	18.2(2.0)	12.5(1.0)	0.0(0.0)	26.0	19.8	76.2
Rate	0.51	0.65	0.78	0.91	0. 97	1.39	0.87	0.31	35.6

Table 15 : Foaming stability (%) of bambara groundnut whole seed using Na_2CO_3

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(13.0)	100(7.0)	100(6.0)	100(4.0)	100(3.0)	100(2.50)	100	0.0	0.0
5	92.3(12.0)	85.7(6.0)	83.3(6.0)	75.0(3.0)	66.7(2.0)	40.0(1.0)	73.8	18.8	25.5
10	84.6(11.0)	71.4(5.0)	66.7(4.0)	50.0(2.0)	33.3(1.0)	0.0(0.0)	51.0	30.7	60.2
20	76.9(10.0)	57.1(4.0)	50.0(3.0)	25.0(1.0)	0.0(0.0)	0.0(0.0)	34.8	31.7	90.1
30	69.2 (9.0)	42.9(3.0)	33.3(2.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	24.2	29.0	120
60	61.5(8.0)	28.6(2.0)	16.7(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	17.8	24.4	137
90	53.8(7.0)	14.3(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	11.4	21.6	189
Rate	0.51	0.95	1.39	3.75	6.67	12.0	4.21	4.45	106

Table 16 : Foaming stability (%) of bambara groundnut whole seedusing $NaNO_3$

	Salt concentrations (%)						Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(13.0)	100(10.0)	100(8.0)	100(6.0)	100(7.0)	100(4.0)	100	0.0	0.0
5	92.3(12.0)	90.0(9.0)	87.5(7.0)	83.3(5.0)	85.7(6.0)	75.0(3.0)	85.6	6.09	7.1
10	84.6(11.0)	80.0(8.0)	75.0(6.0)	66.7(6.0)	71.4(5.0)	50.0(2.0)	71.3	12.2	17.1
20	76.9(10.0)	70.0(7.0)	62.5(5.0)	50.0(3.0)	57.1(4.0)	50.0(2.0)	61.1	10.9	17.8
30	69.2(9.0)	60.0(6.0)	50.0(4.0)	33.3(2.0)	42.9(3.0)	25.0(1.0)	46.7	16.5	35.3
60	61.5(8.0)	60.0(6.0)	37.5(3.0)	16.7(1.0)	28.6(2.0)	0.0(0.0)	34.1	24.2	71.0
90	53.8(7.0)	50.0(5.0)	25.0(2.0)	0.0(0.0)	14.3(1.0)	0.0(0.0)	23.9	23.7	99.2
Rate	0.15(0.07)	0.56	0.83	1.39	0.95	2.50	1.12	0.75	67.0

Table 17 : Foaming stability (%) of bambara groundnut whole seedusing CH_3COONa

Salt concentrations (%)							Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(13.0)	100(6.0)	100(5.0)	100(3.0)	100(3.0)	100(2.0)	100	0.0	0.0
5	92.3(12.0)	83.3(5.0)	80.0(4.0)	66.7(2.0)	83.3(2.50)	50.0(1.0)	75.9	15.2	20.0
10	84.6(11.0)	66.7(4.0)	60.0(3.0)	33.3(1.0)	66.7(2.0)	0.0(0.0)	51.9	30.4	58.6
20	76.9(10.0)	50.0(3.0)	40.0(2.0)	0.0(0.0)	33.3(1.0)	0.0(0.0)	33.4	29.8	89.2
30	69.2(9.0)	33.3(2.0)	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	20.4	27.6	135
60	61.5(8.0)	16.7(1.0)	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	16.4	24.0	146
90	53.8(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	8.97	22.0	245
Rate	0.51	1.39	1.33	6.67	3.34	10.0	3.87	3.73	96.4

Table 18 : Foaming stability (%) of dehulledbambara groundnut using Na_2SO_3

Salt concentrations (%)							Mean	SD	CV%
Time (min)	**0.0**	**0.5**	**1.0**	**2.0**	**5.0**	**10.0**			
0	100(13.0)	100(5.0)	100(3.50)	100(4.0)	100(2.0)	100(1.50)	100	0.0	0.0
5	92.3(12.0)	80.0(4.0)	85.7(3.0)	87.5(3.5)	100(2.0)	66.7(1.0)	85.4	11.4	13.3
10	84.6(11.0)	60.0(3.0)	57.1(2.0)	75.0(3.0)	50.0(1.0)	0.0(0.0)	54.5	29.5	54.1
20	76.9(10.0)	40.0(2.0)	28.6(1.0)	50.0(2.0)	0.0(0.0)	0.0(0.0)	32.6	30.0	92.0
30	96.2(9.0)	40.0(2.0)	0.0(0.0)	25.0(1.0)	0.0(0.0)	0.0(0.0)	22.4	28.3	126
60	61.5(8.0)	20.0(1.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	13.6	24.8	182
90	53.8(7.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	8.96	22.0	245
Rate	0.51	1.33	3.57	2.50	5.0	6.66	3.26	2.30	70.6

Table 19 : Water absorption capacity (g/100 g) of bambara groundnuttesta

Concentrationof salts (%)	Water absorption capacity					Mean	SD	CV%
	NaCl	**Na_2CO_3**	**$NaNO_3$**	**CH_3OONa**	**Na_2SO_3**			
0.0	280.0	280.0	280.0	280.0	280.0	280.0	280.0	0.0
0.5	300.0	300.0	300.0	260.0	270.0	286	19.5	0.07
1.0	400.0	350.0	290.0	270.0	250.0	312	61.8	0.19
2.0	290.0	400.0	320.0	300.0	280.0	318	48.2	0.15
5.0	280.0	380.0	350.0	320.0	270.0	320	46.4	0.15
10.0	270.0	390.0	330.0	280.0	280.0	310	505	0.16

Table 20 : Water absorption capacity (g/100 g) of dehulled Bambara groundnut

Concentration	Water absorption capacity					Mean	SD	CV%
of salts(%)	**NaCl**	**Na_2CO_3**	**$NaNO_3$**	**CH_3OONa**	**Na_2SO_3**			
0.0	280.0	280.0	280.0	280.0	280.0	280.0	0.0	0.0
0.5	170.0	150.0	170.0	180.0	140.0	162.0	16.4	10.0
1.0	200.0	170.0	160.0	200.0	160.0	178.0	20.5	11.5
2.0	180.0	180.0	180.0	220.0	150.0	182.0	24.9	13.7
5.0	190.0	200.0	200.0	240.0	170.0	200.0	24.5	12.3
10.0	220.0	190.0	170.0	200.0	160.0	188.0	23.9	12.7

Table 21 : Water absorption capacity (g/100 g) of whole seed ofbambara groundnut

Concentration of salts(%)	Water absorption Capacity					Mean	SD	CV%
	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	280.0	280.0	280.0	280.0	280.0	280.0	0.0	0.0
0.5	100.0	120.0	150.0	160.0	220.0	150.0	45.8	30.5
1.0	110.0	140.0	160.0	180.0	230.0	164.0	45.1	27.5
2.0	150.0	110.0	180.0	220.0	150.0	162.0	40.9	25.2
5.0	120.0	150.0	160.0	160.0	210.0	160.0	32.4	20.3
10.0	140.0	150.0	210.0	170.0	240.0	182.0	42.1	23.1

Table 22 : Emulsion capacity (g/100g) of bambara groundnut testa in various salt concentrations

Concentration of salt (%)	Emulsion capacity of salt					Mean	SD	CV%
	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	13.0	15.0	15.0	15.3	15.0	14.7	0.94	6.4
0.5	14.3	14.3	23.0	16.0	21.4	17.8	4.12	23.1
1.0	15.0	16.0	22.0	18.4	22.0	18.7	3.27	17.5
2.0	16.3	18.0	24.0	20.0	23.0	20.3	3.25	16.0
5.0	20.0	16.0	25.0	22.2	21.0	20.9	3.18	15.2
10.0	21.0	22.0	24.0	23.0	24.0	22.8	1.30	5.7

Table 23 : Emulsion capacity (g/100g) of dehulledbambara groundnut in various salt concentrations

Concentration of salts (%)	Emulsion capacity of salt					Mean	SD	CV%
	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	13.0	15.0	15.0	15.3	15.0	14.7	0.94	6.4
0.5	15.0	19.0	23.0	20.4	20.4	19.6	2.93	14.9
1.0	16.2	20.0	22.0	22.2	30.9	22.3	5.40	24.2
2.0	20.0	21.4	25.5	23.0	27.0	23.4	2.88	12.3
5.0	21.0	18.8	26.3	21.0	28.0	23.0	3.92	17.0
10.0	23.5	22.0	25.3	24.7	29.3	25.0	2.73	10.9

Table 24 : Emulsion capacity (g/100 g) of bambara groundnut whole seed in various salt

Concentration Of salt (%)	Emulsion capacity of salts					Mean	SD	CV%
	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	13.0	15.0	15.0	15.3	15.0	14.7	0.94	6.4
0.5	22.0	25.0	24.0	24.0	32.0	25.4	3.85	15.2
1.0	23.0	26.0	26.5	23.5	33.0	26.4	3.99	15.1
2.0	22.5	28.3	25.0	25.0	29.0	26.0	2.67	10.3
5.0	24.0	27.0	28.0	26.5	35.0	28.1	4.13	14.7
10.0	25.3	28.6	29.6	28.0	34.3	29.2	3.29	11.3

Table 25 : Least gelation concentration of bambara groundnut testa in salt concentrations

Concentration of	Least gelation concentration (%)					Mean	SD	CV%
salts in water (%)	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	0.0
0.5	6.0	6.0	6.0	6.0	8.0	6.4	0.89	0.14
1.0	4.0	6.0	6.0	8.0	6.0	6.0	1.41	0.24
2.0	8.0	8.0	6.0	4.0	6.0	6.4	1.67	0.26
5.0	4.0	6.0	4.0	6.0	4.0	4.8	1.09	0.23
10.0	6.0	4.0	4.0	4.0	6.0	4.8	1.09	0.23

Table 26 : Least gelation concentration of dehulledbambaragroundnut in salt concentrations

Concentration of	Least gelation concentration(%)					Mean	SD	CV%
salts in water (%)	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0	0.0
0.5	2. 0	2.0	6.0	4.0	6.0	4.0	2.0	0.5
1.0	4.0	4.0	4.0	2.0	2.0	3.2	1.09	0.34
2.0	2.0	2.0	6.0	4.0	4.0	3.6	1.67	0.46
5.0	4.0	4.0	4.0	4.0	2.0	3.6	0.89	0.25
10.0	4.0	2.0	4.0	2.0	2.0	2.8	1.09	0.39

Table 27 : Least gelation concentration of whole bambara groundnut in salt concentrations

Concentration of	Least gelation concentration (%)					Mean	SD	CV%
salts in water (%)	NaCl	Na_2CO_3	$NaNO_3$	CH_3OONa	Na_2SO_3			
0.0	8.0	8.0	8.0	8.0	8.0	8.0	0.0	0.0
0.5	8.0	8.0	6.0	6.0	8.0	6.8	1.09	0.16
1.0	6.0	6.0	8.0	8.0	6.0	6.8	1.09	0.16
2.0	6.0	6.0	8.0	8.0	6.0	6.8	1.09	0.16
5.0	6.0	6.0	6.0	8.0	8.0	6.8	1.09	0.16
10.0	8.0	6.0	8.0	6.0	6.0	6.8	1.09	0.16

Table 28 : Various salts concentrations [percentage, molarity and ionic strength (μ)]

Salt	Percentageconcentration	Molairty	Ionic strength
NaCl	0.5	8.5×10^{-4}	8.5×10^{-4}
NaCl	1.0	1.7×10^{-3}	1.7×10^{-3}
NaCl	2.0	3.4×10^{-3}	3.4×10^{-3}
NaCl	5.0	8.1×10^{-3}	8.1×10^{-3}
NaCl	10.0	1.5×10^{-2}	1.5×10^{-2}
Na_2CO_3	0.5	4.7×10^{-4}	1.4×10^{-3}
Na_2CO_3	1.0	9.3×10^{-4}	2.8×10^{-3}
Na_2CO_3	2.0	1.8×10^{-3}	5.4×10^{-3}
Na_2CO_3	5.0	4.5×10^{-3}	1.35×10^{-2}
Na_2CO_3	10.0	8.5×10^{-3}	2.6×10^{-2}
$NaNO_3$	0.5	5.9×10^{-4}	5.9×10^{-4}
$NaNO_3$	1.0	1.2×10^{-3}	1.2×10^{-3}
$NaNO_3$	2.0	2.3×10^{-3}	2.3×10^{-3}
$NaNO_3$	5.0	5.6×10^{-3}	5.6×10^{-3}
$NaNO_3$	10.0	1.1×10^{-2}	1.1×10^{-2}

CH_3COONa	0.5	6.1×10^{-4}	6.1×10^{-4}
CH_3COONa	1.0	1.2×10^{-3}	1.2×10^{-3}
CH_3COONa	2.0	2.4×10^{-3}	2.4×10^{-3}
CH_3COONa	5.0	5.8×10^{-3}	5.8×10^{-3}
CH_3COONa	10.0	1.1×10^{-2}	1.1×10^{-2}
Na_2SO_3	0.5	3.9×10^{-4}	1.2×10^{-3}
Na_2SO_3	1.0	7.9×10^{-4}	2.4×10^{-3}
Na_2SO_3	2.0	1.6×10^{-3}	4.8×10^{-3}
Na_2SO_3	5.0	3.8×10^{-3}	1.14×10^{-2}
Na_2SO_3	10.0	7.1×10^{-3}	2.14×10^{-2}

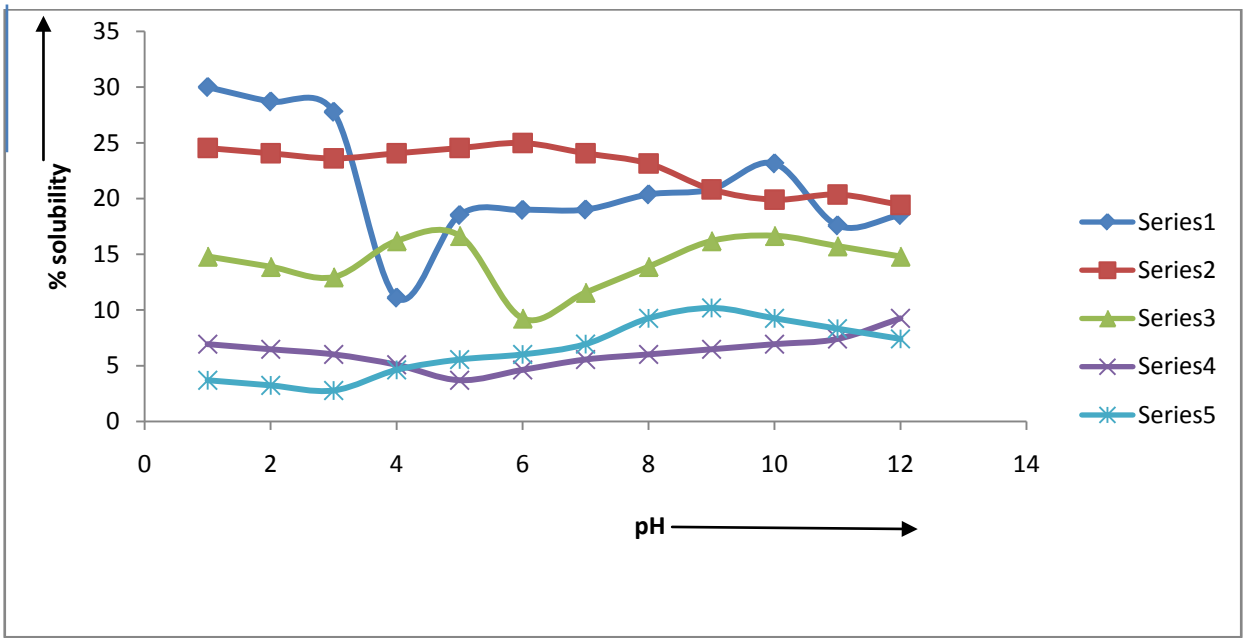

Figure 1 : Protein solubility (%) vs pH at different Na_2SO_4 concentrations in testa

Series 1 - 0.5% salt concentration
Series 2 - 1.0% salt concentration
Series 3 – 2.0% salt concentration
Series 4 – 5.0% salt concentration
Series 5 – 10.0% salt concentration

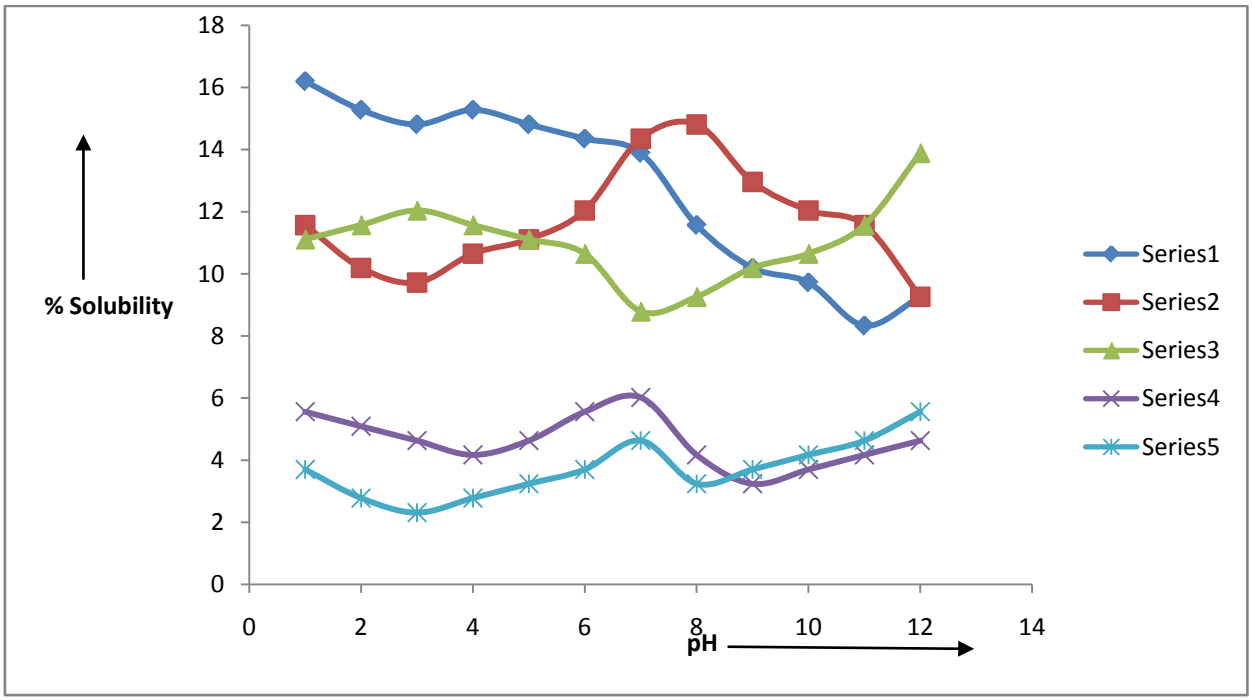

Figure 2 : Protein solubility (%) vs pH at different Na_2CO_3 concentrations in testa

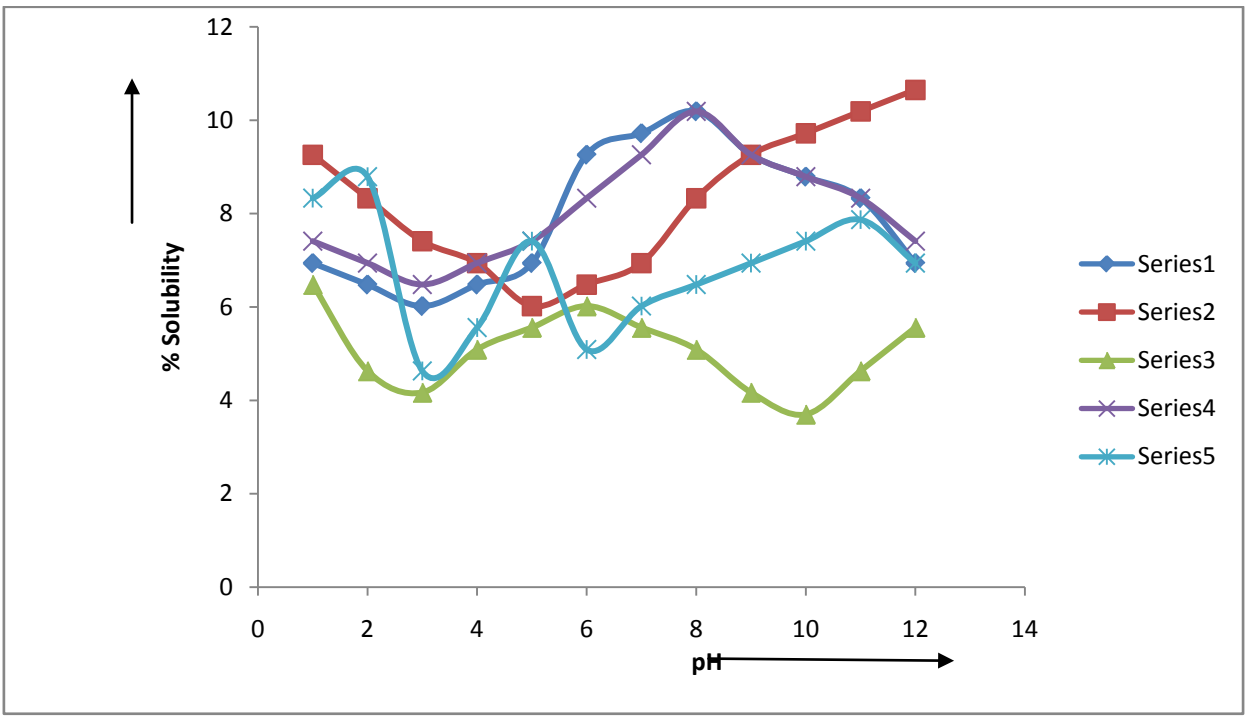

Figure 3 : Protein solubility (%) vs pH at different Na_2SO_3 concentrations in testa

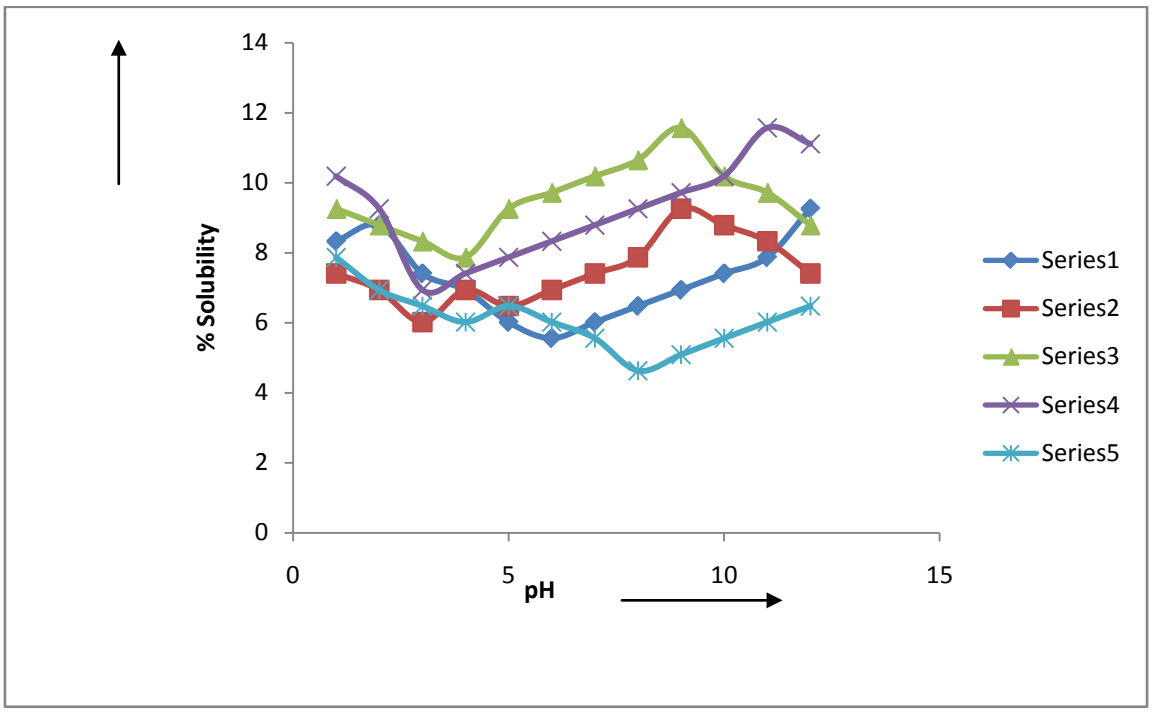

Figure 4 : Protein solibility (%) vs pH at different CH_3COOH concetrations in testa

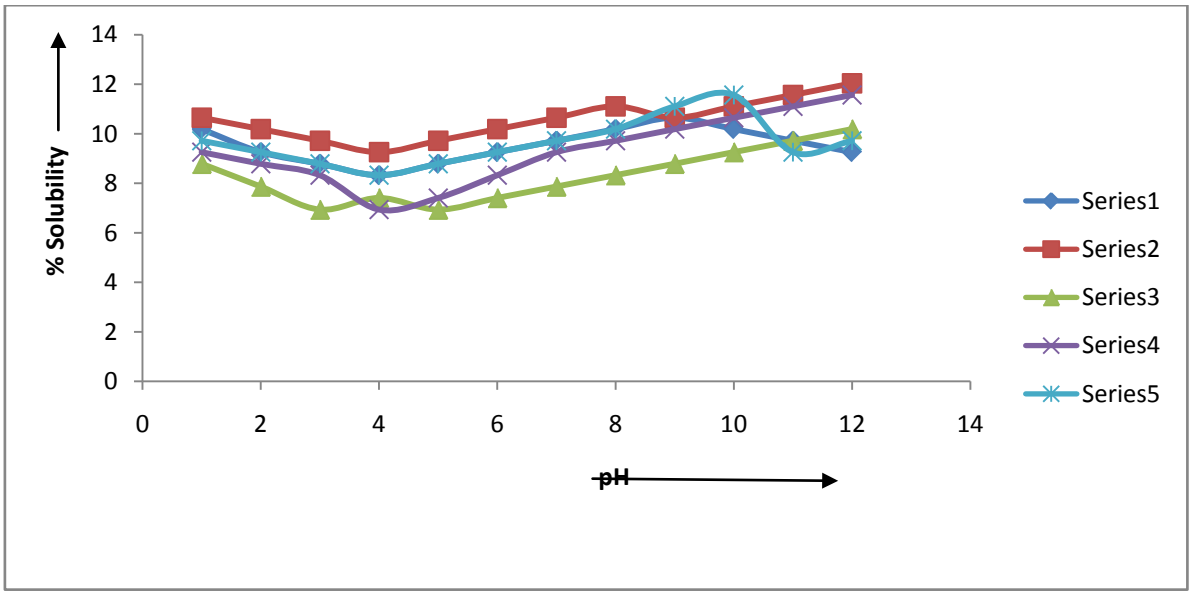

Figure 5 : Protein solubility (%) vs pH at different $NaNO_3$ concentrations in testa

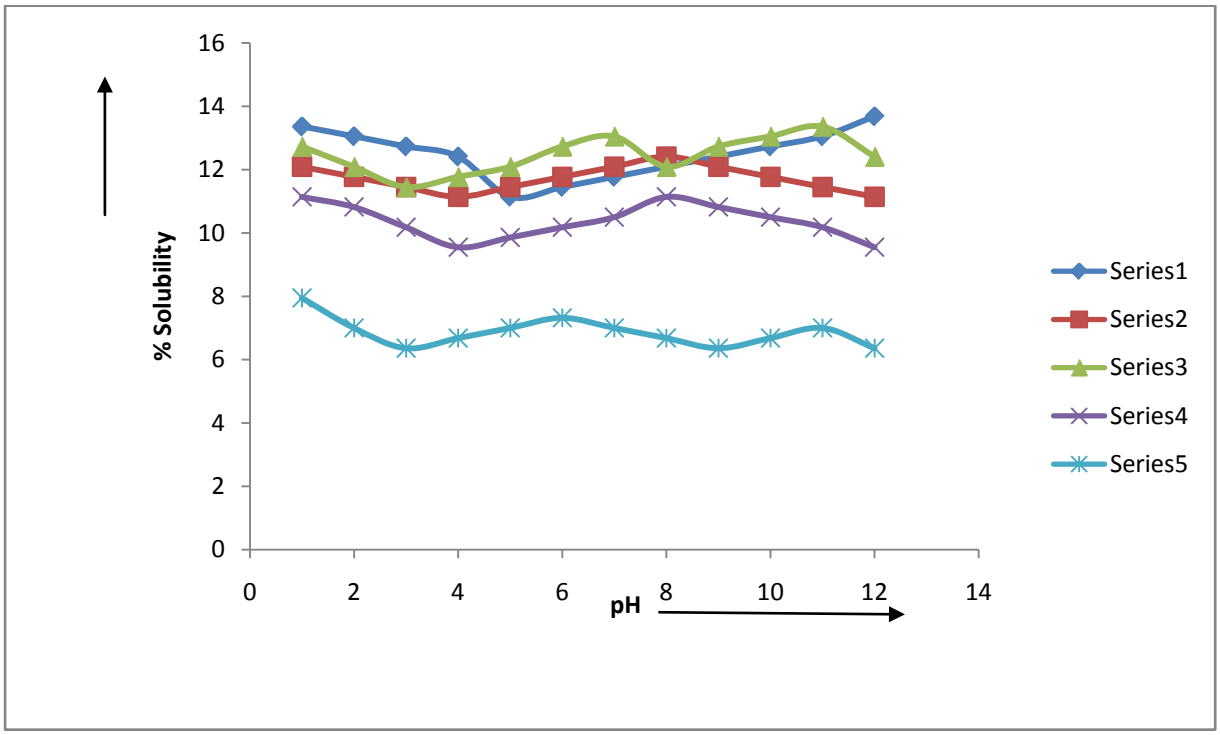

Figure 6 : Protein solubility (%) vs pH at different Na_2SO_4 concentrations in dehulled sample

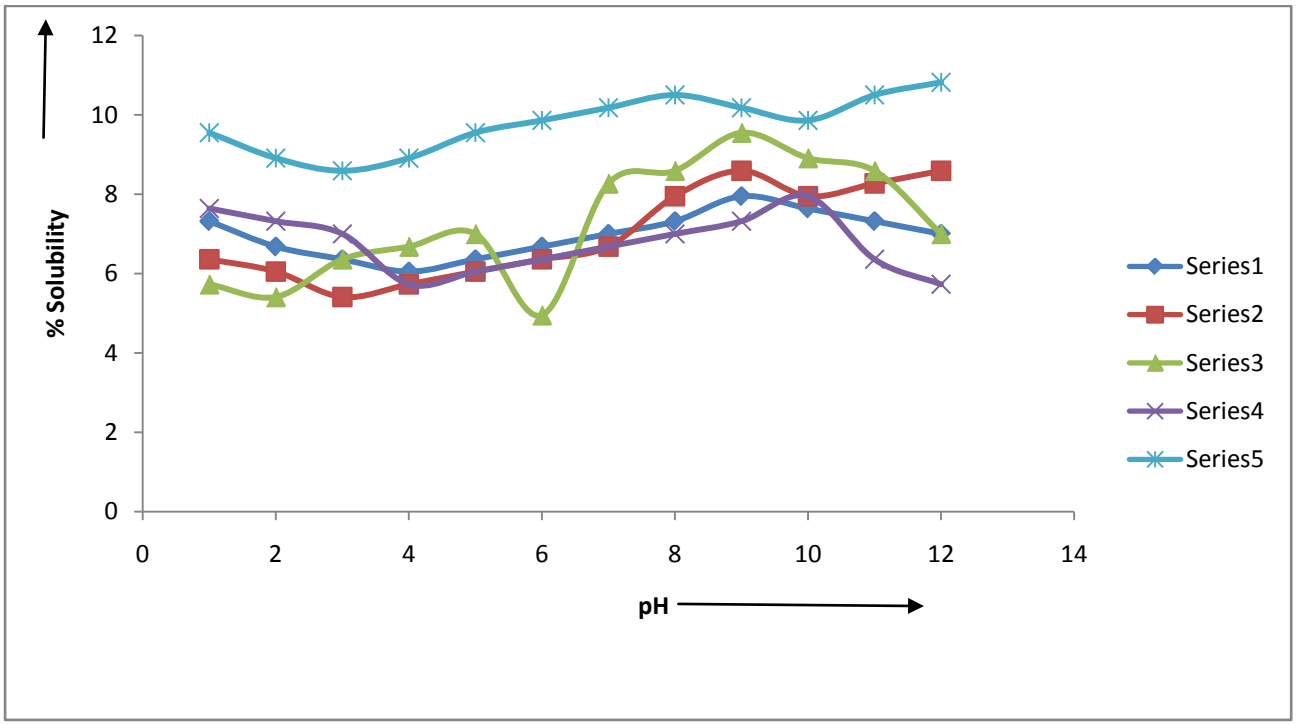

Figure 7 : Protein solubility (%) vs pH at different Na_2CO_3 concentrations in dehulled sample

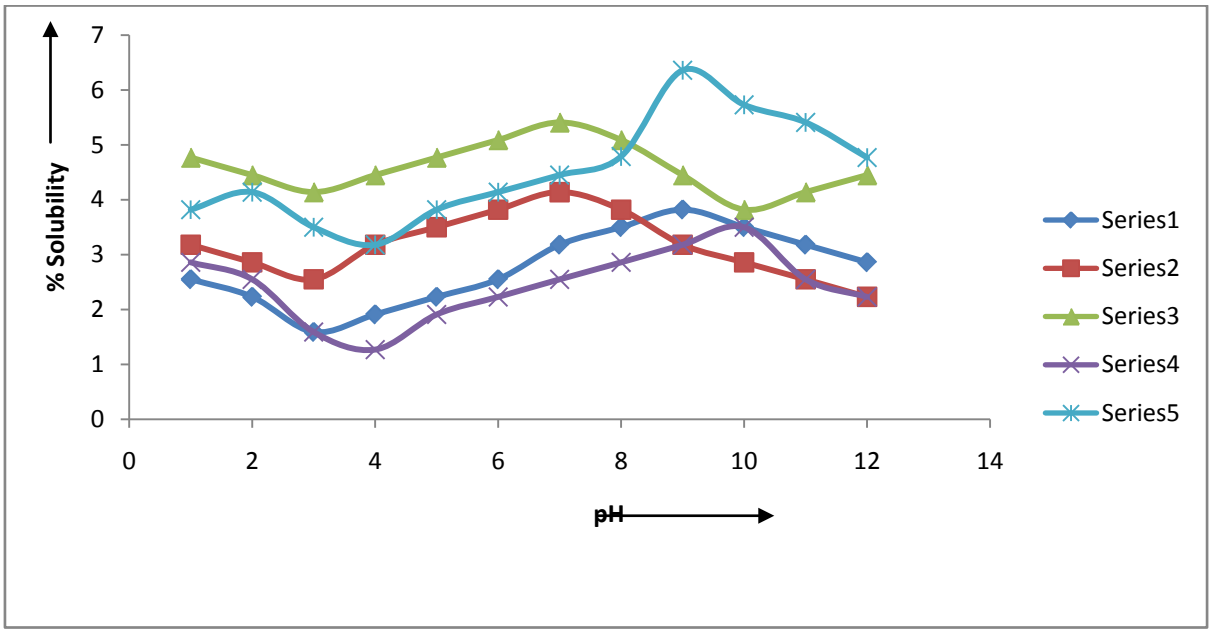

Figure 8 : Protein solubility (%) at different Na_2SO_3 concentrations in dehulled sample

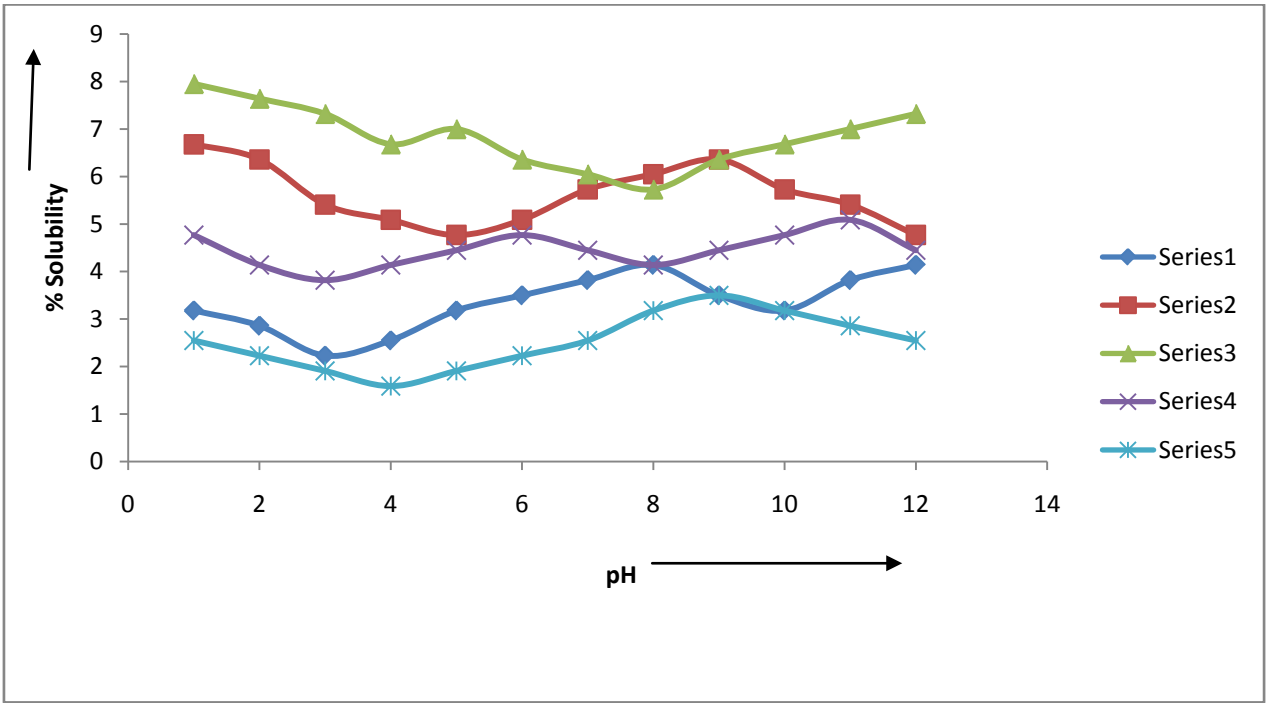

Figure 9 : Protein solubility (%) vs pH at different CH_3COONa concentrations in dehulled sample

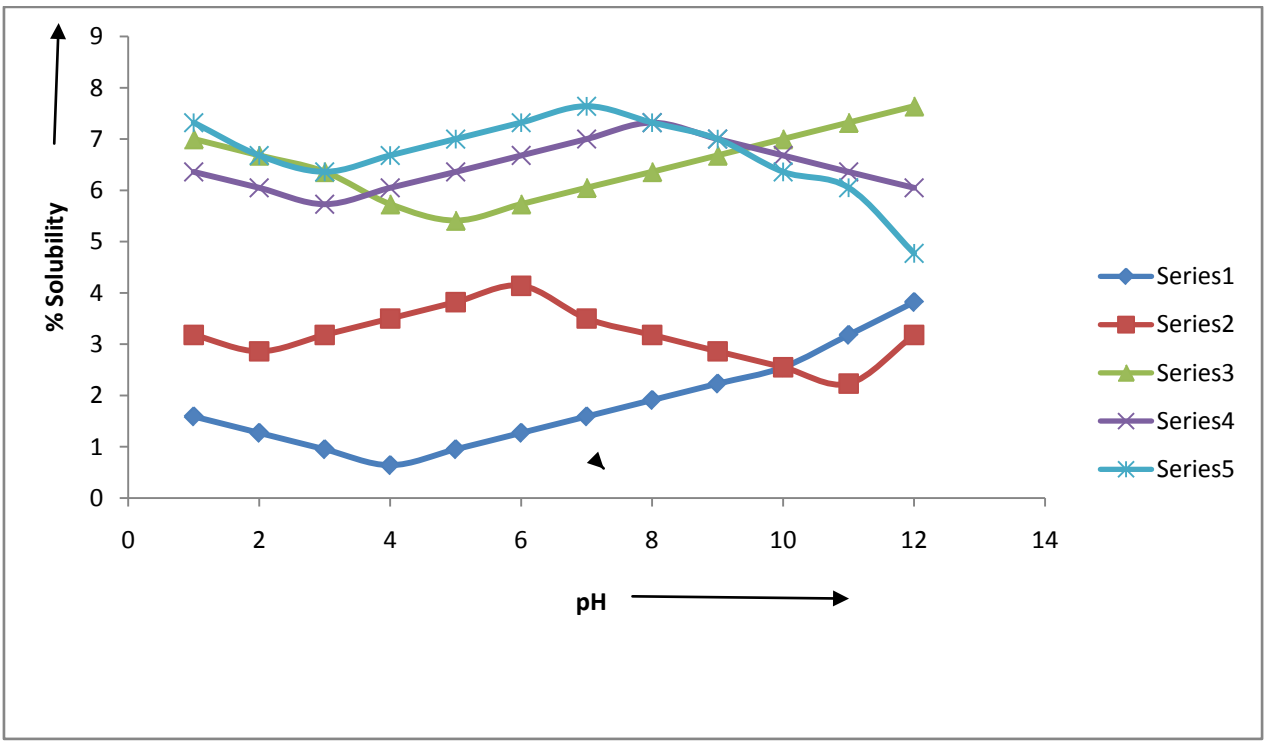

Figure 10 : Protein solubility (%) vs pH at different $NaNO_3$ concentrations in dehulled sample

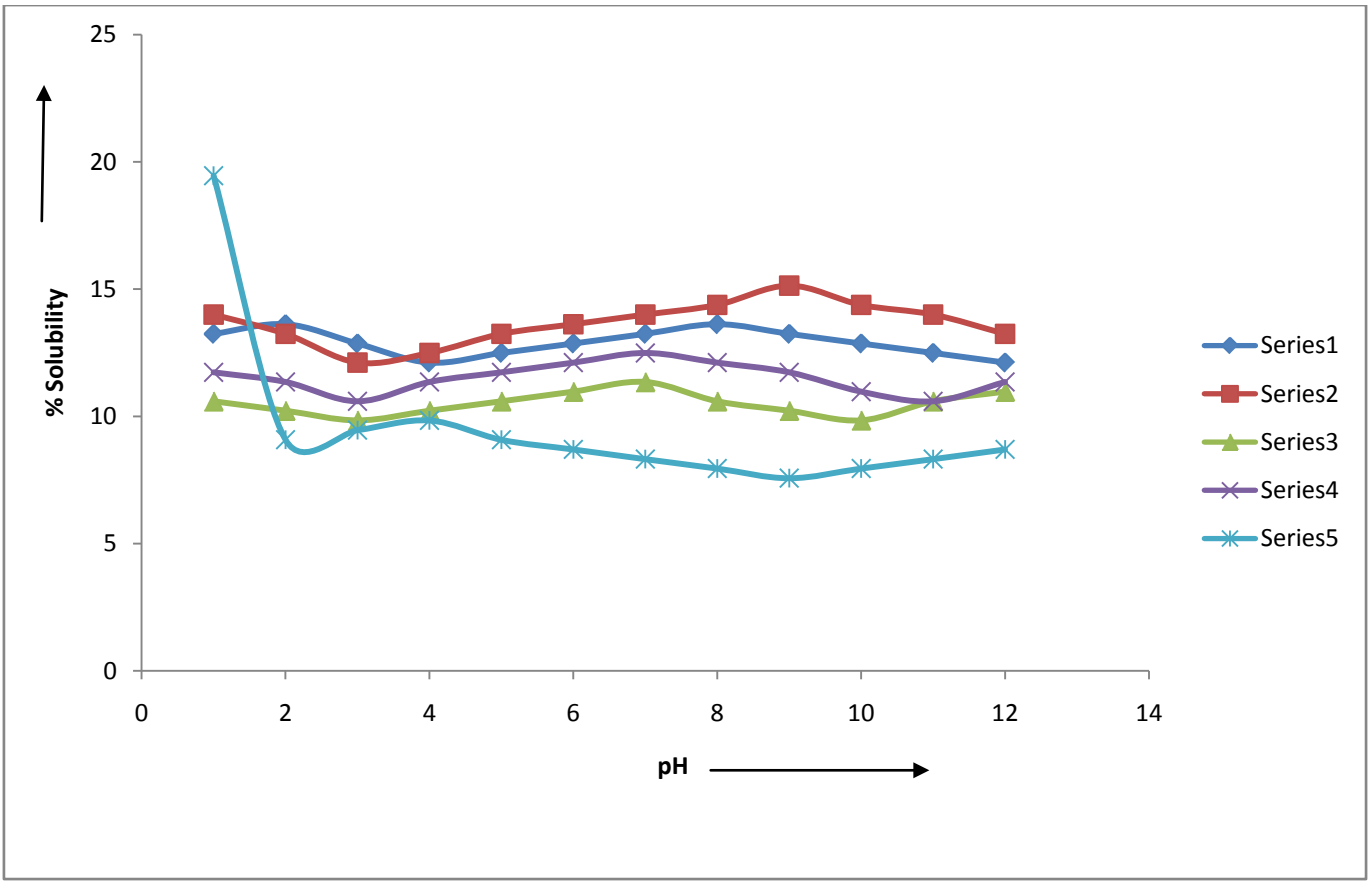

Figure 11 : Protein solubility (%) vs pH at different Na_2SO_4 concentrations in whole seed sample

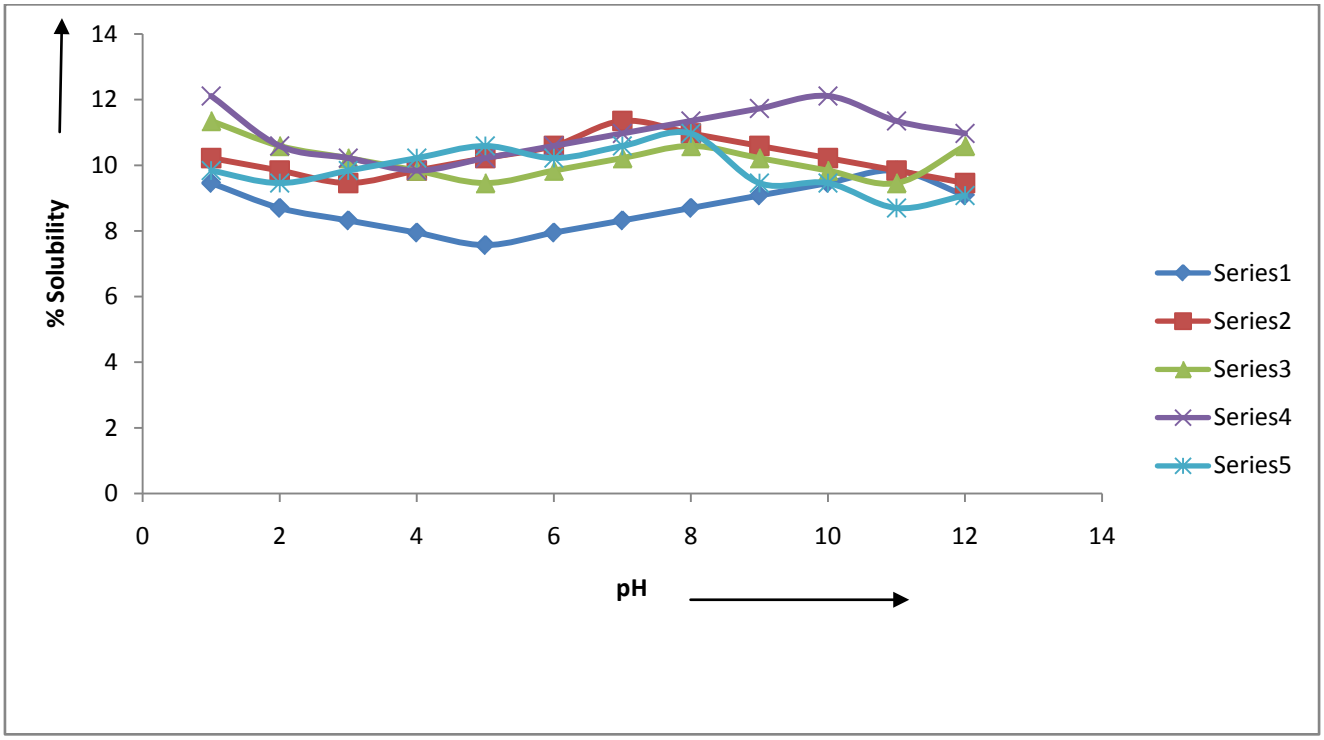

Figure 12 : Protein solubility (%) vs pH at different Na_2CO_3 concentrations in whole seed sample

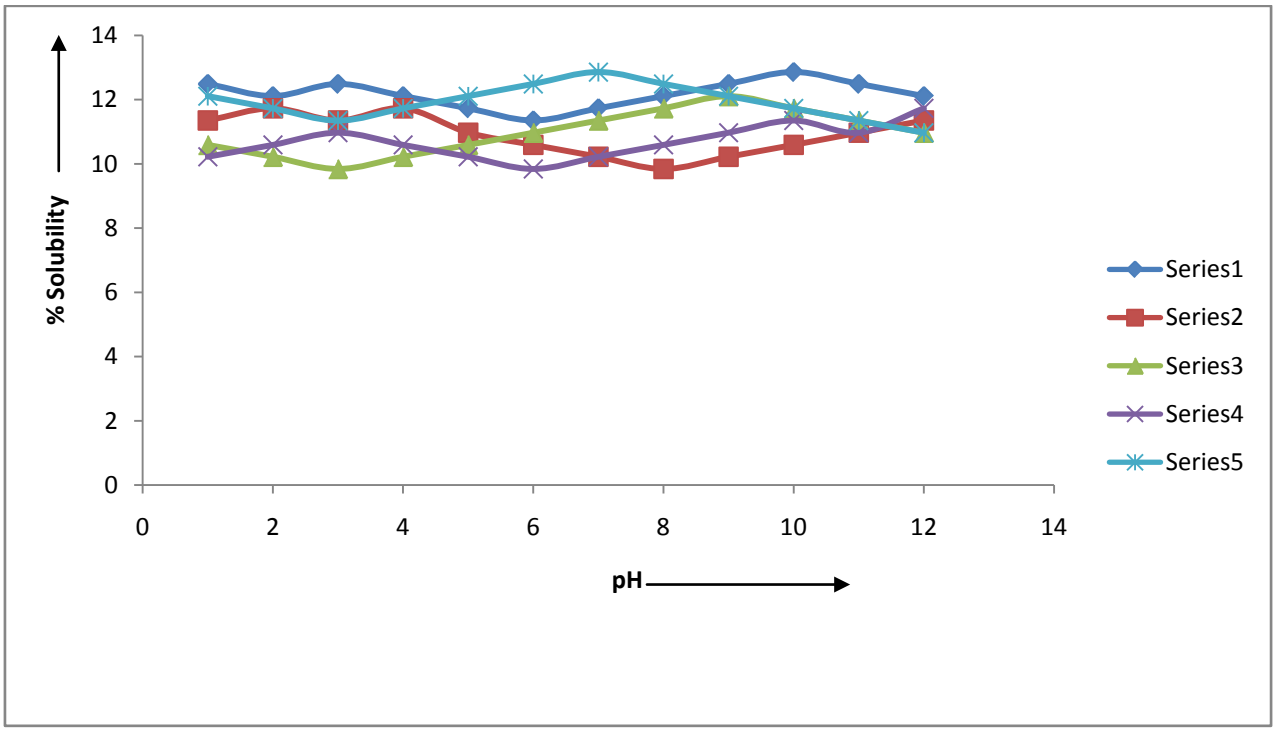

Figure 13 : Protein solubility (%) vs pH at different Na_2SO_3 concentrations in whole seed sample

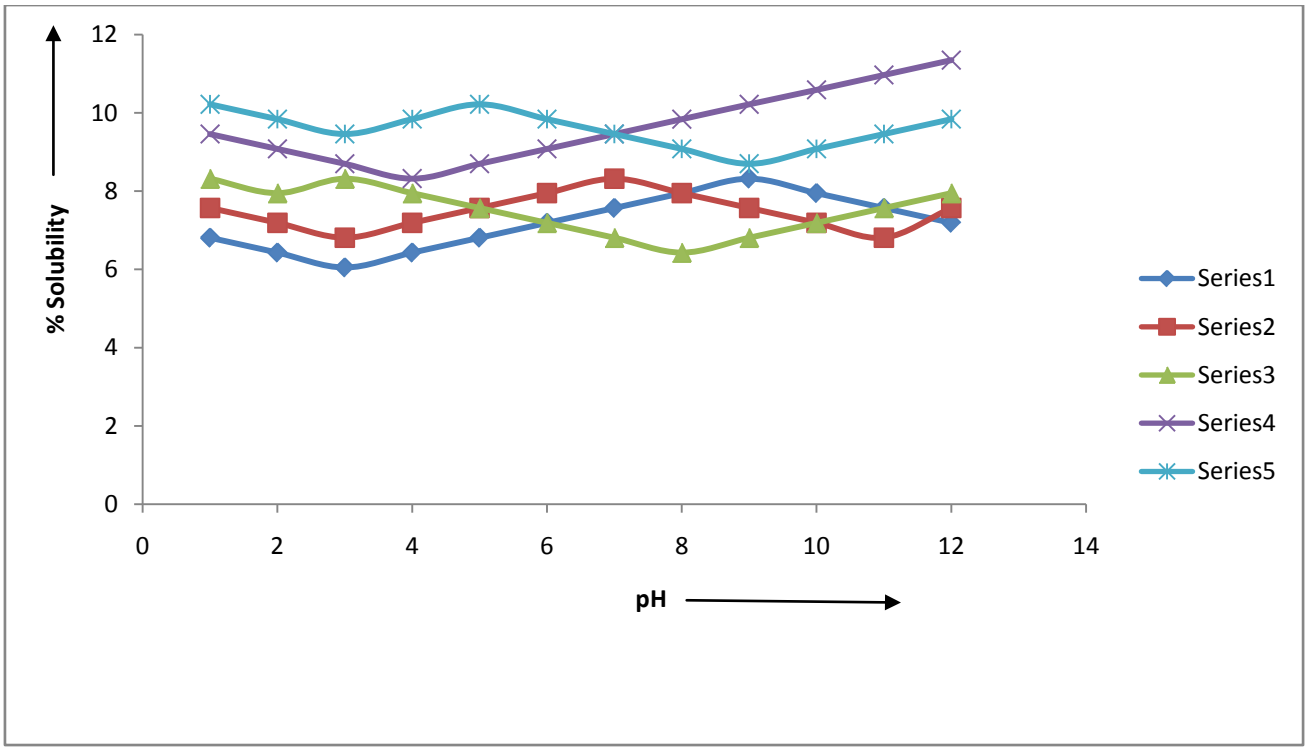

Figure 14 : Protein solubility (%) vs pH at different CH_3COONa concentrations in whole seed sample

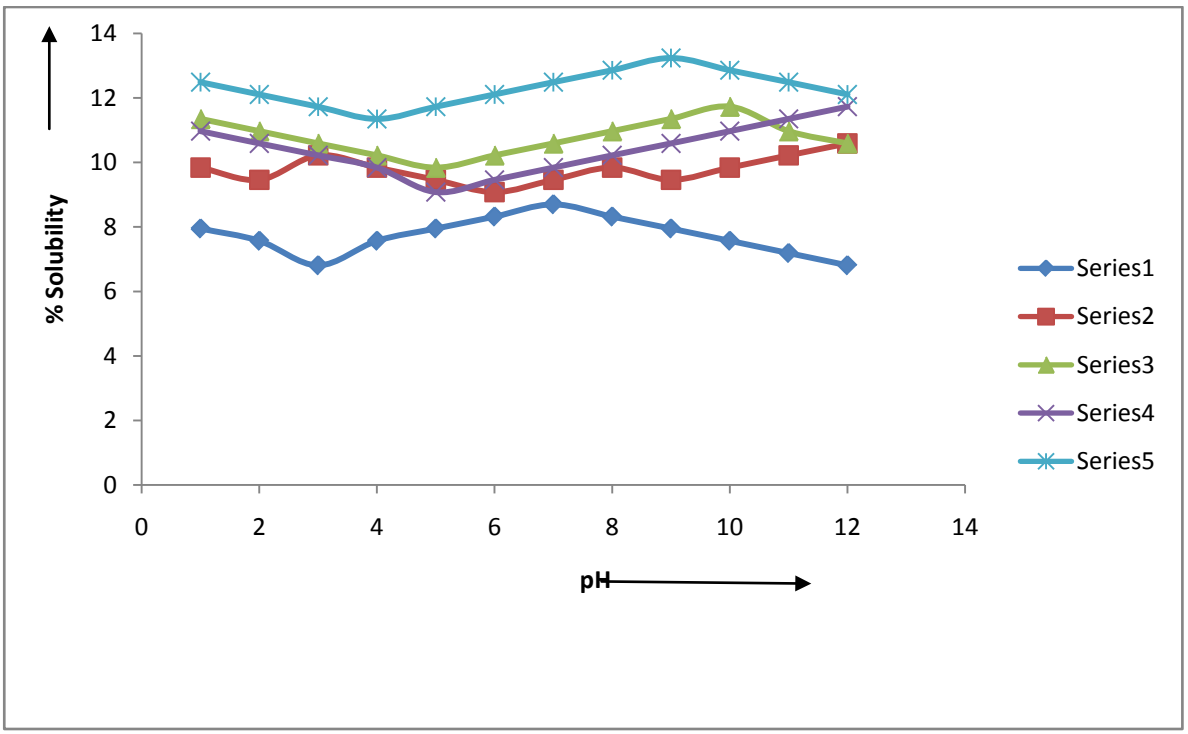

Figure 15 : Protein solubility (%) vs pH at different $NaNO_3$ concentrations in whole seed sample

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Synthesis, Structural Characterization, Spectral Analysis and Antimicrobial Activities of Schiff Base Ligands and Their Metal Complexes Derived from 3-Aldehydosalicylic Acid

By B. M. Kalshetty, R. S. Gani, S.S.Karabasannavar & M.B.Kalashetti
Bharathier University, Coimbatore

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Keywords : 3-aldehydosalicylic acid, schiff bases, metal complexes, characterization and antimicrobial activity.

GJSFR-B Classification : FOR Code: 039999

Strictly as per the compliance and regulations of :

Synthesis, Structural Characterization, Spectral Analysis and Antimicrobial Activities of Schiff Base Ligands and Their Metal Complexes Derived from 3-Aldehydosalicylic Acid

B. M. Kalshetty,[α] R. S. Gani,[σ] S.S.Karabasannavar[ρ] & M.B.Kalashetti [ω]

Abstract - The synthesis of new Schiff base ligands formed by the condensation of 3-Aldehydosalicylic acid with equimolar quantities of 2-Amino-5-methyl pyridine, 4-Amino-5-phenyl-3-mercapto-[1,2,4] -Triazole, 4-Amino-3-methyl-5-mercapto-Triazole, 7-Amino-4-ethyl Coumarin forming 2-(5-methyl pyridine-2-yl amino)-methyl-2-hydroxy-Benzoic acid (Scheme 1), 3-[(3-phenyl-5-mercapto-[1,2,4] triazole-4-yl amino)-methyl] -2-hydroxy-Benzoic acid (Scheme 4), 3-[(3-methyl-5-mercapto-[1,2.4] triazoile-4-yl amino)-methyl]-2-hydroxy-Benzoic acid (Scheme 7), 3-[(4-Ethyl-Coumarin-7-yl amino)-methyl] -2-hydroxy-Benzoic acid (Scheme 11).

The coordination complexes of metal ions like Cu(II), Zn(II), Ni(II), Co(II), Cd(II) with the Schiff base ligands (LH2) having N- and S- donor system have been synthesized at various pH ranges. All the synthesized ligands and metal complexes were characterized by elemental analysis, IR, 1HNMR and Mass spectral data. Hence, the study reveals that the Schiff base ligands involved the donor groups – COOH, -OH, -SH and Nitrogen atom. The ligand LH_2 in metal complexes Scheme 2, 5, 8 behaves as a monobasic bidantate OO-donor at pH- 4 suggesting the non-involvement of Nitrogen and Sulphur atoms present in the Schiff base ligands. At the same pH-4 the (Scheme 12) also monobasic bidantate ON- donor.

At pH-7 the ligands behave as dibasic tridentate OON-donor (Scheme 4), OOS-donor (Scheme 9), OON- donor (Scheme 13) suggesting the non-involvement of sulphur atom with metal ion in (Scheme 4) and in (Scheme 13) and non-involvement of Nitrogen atom with metal ion in (Scheme 9). In case of (Scheme 9) sulphur makes coordination with metal ion first rather than Nitrogen atom because of its electronic configuration.

At pH-9 the ligand behaves as dibasic tetra dentate OOSN- donor with metal ions, suggesting the coordination of Nitrogen and Sulphur atom to the metal ions in the coordination complex (Scheme 6). And (Scheme 10). At the same pH-9 the ligand behaves dibasic tetra dentate of OONO-donor, suggesting the coordination of Nitrogen atom and Oxygen atom of Coumarine unit to the metal ions in the complex compounds.

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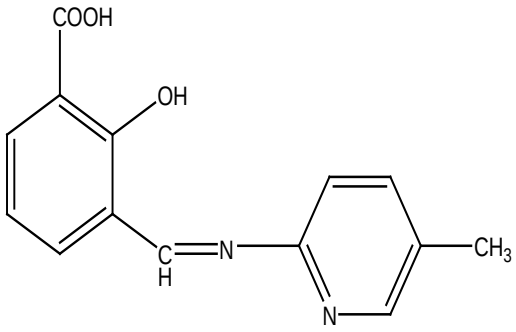
Keywords : 3-aldehydosalicylic acid, schiff bases, metal complexes, characterization and antimicrobial activity.

I. Introduction

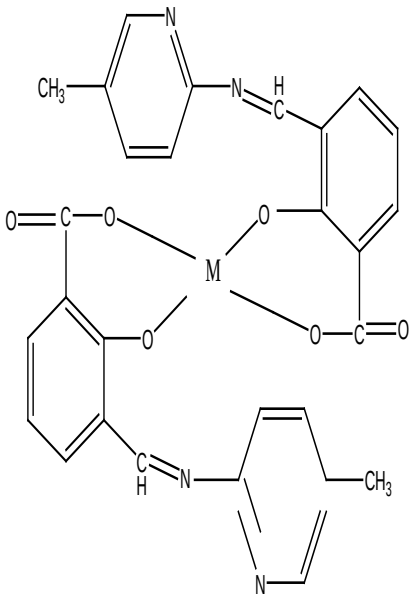
Schiff bases have been widely used as ligands in the formation of transition metal complexes. Schiff base ligands containing various donor atoms like O,N,S show broad biological activity and are special interest because of the variety of ways in which they are bonded to the transition metal ions [1,2]. The Schiff bases of salicylaldehyde and amino pyridines have been characterized in solid state and in ligand solution [3,4] and proposed as highly sensitive spectrometric and spectrofluorimetric reagents for Cu(II) [5,6].

Metal complexes of Schiff bases show biological activities including antibacterial, antifungal, anticancer and herbicidal [7, 8]. The Schiff base complexes and derivatives of Coumarin, thiazolidinone and triazoles so far reported as anti-inflammatory[9], anti-oxidant [10] , vascorelant [11] , Cytotoxic [12] , anti HIV [13] , anti-tubercular [14] and anti-microbial [15] and develop effective therapies [16] . The medical application in the treatment of wounds and tumors, where the metal complex compounds of triazole is to impart strength, elasticity and impermeability of water [17]. The metal complexes are still a major line of approach to develop new drugs. Thiazolidine ring present in a large number of biologically active compounds, the development of penicillin which shows the thiozolidine ring. However, analogous heteroaromatic Schiff bases derived from 3-aldehydosalic acid (3ASA) have not been investigated so thoroughly the synthesized Schiff bases and metal complexes have been found to possess biological activity. The present developments of above Schiff bases with metal ions are most fascinating. The continuation of work on the synthesis of Schiff base derived from 3-aldehydosalicylic acid and its complex compounds with metal ions at different pH. All the Schiff bases were synthesized followed by standard procedures[18, 19] contains the acidic groups such as – COOH and -OH (Phenolic) groups. It is predicted that the coordination behavior of ligand would be developed

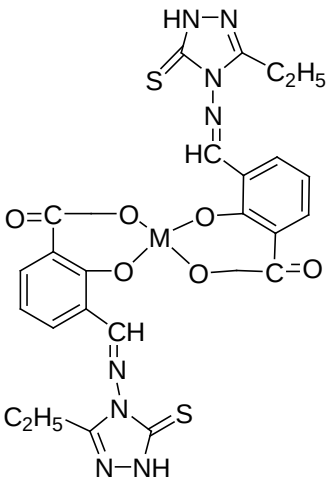
on pH using different reaction conditions and are markedly different from these reported by Nag et al [20].



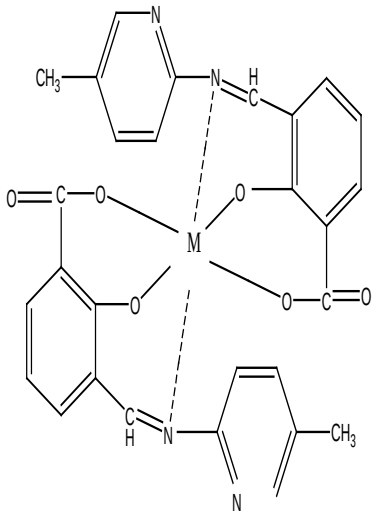
2-(5-methyl pyridine-2-yl amino)-methyl-2-hydroxy-Benzoic acid (Scheme 1)



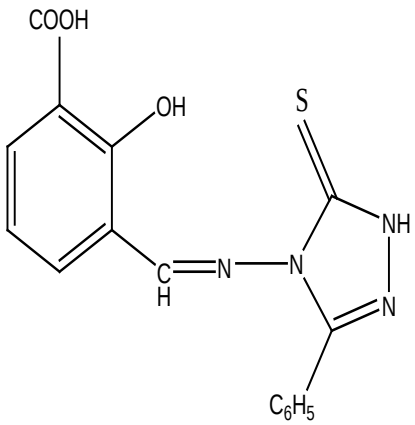
[M (LH) 2]: M=Cu, Zn, Cd. OO-Donor At pH=4 (Scheme 2).



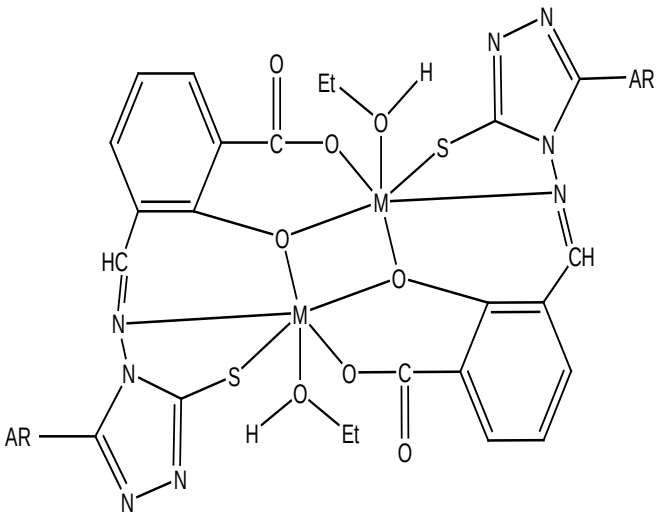
[M (LH) $_2$]: M= Cu, Zn Cd. OO- donor At pH= 4 (Scheme 5).



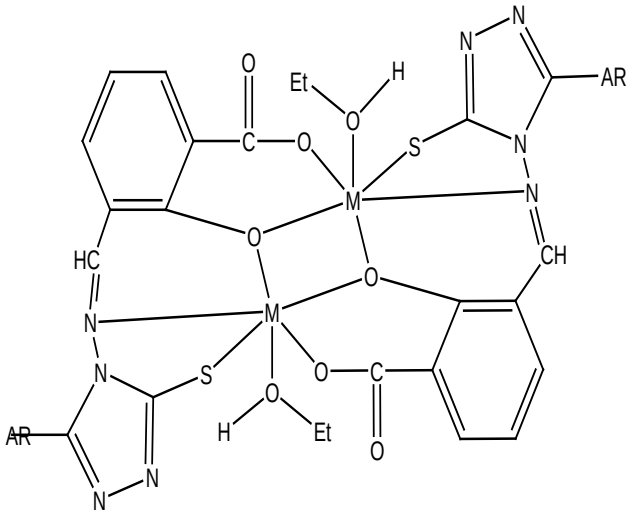
[M L] : M= Zn, Cd.
OON- donor at=7 (Scheme 3)



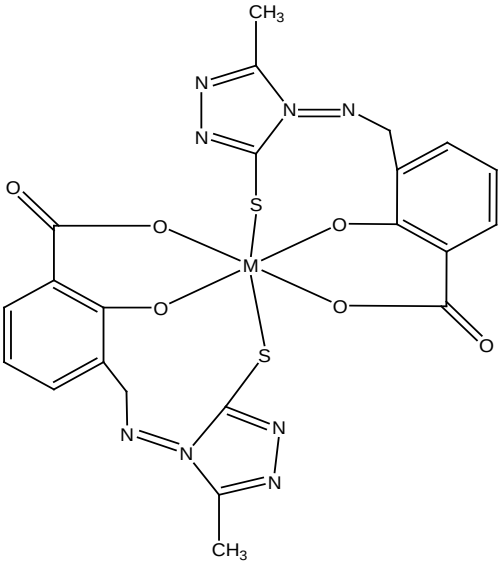
3-[(3-phenyl-5-mercapto-[1,2,4] triazole-4-yl amino)-methyl] -2-hydroxy-Benzoic acid (Scheme 4)



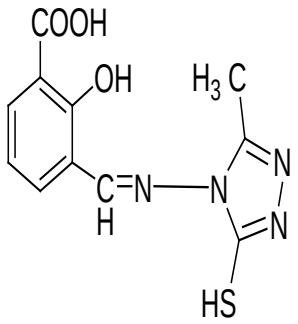
[ML(EtOH)$_2$]: M+ Zn, Cd, Cu, Ni, Co. OONS-donor At pH=9 (Scheme 6)



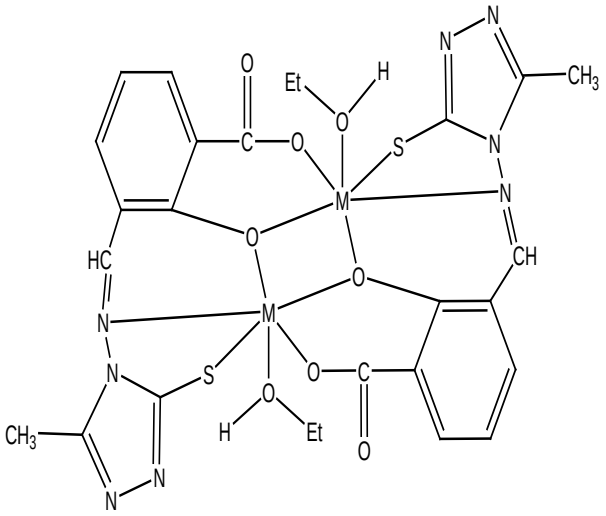
[$ML(EtOH)_2$]: M+ Zn, Cd, Cu, Ni, Co. OONS-donor At pH=9 (Scheme 6)



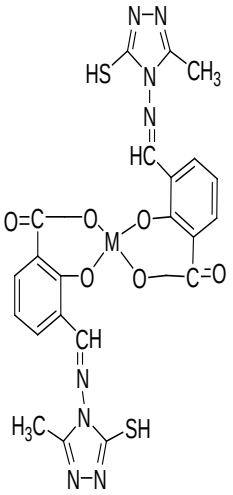
[M (LH)2] M: Zn, Cd. OOS-donor at pH= 9 (Scheme9).



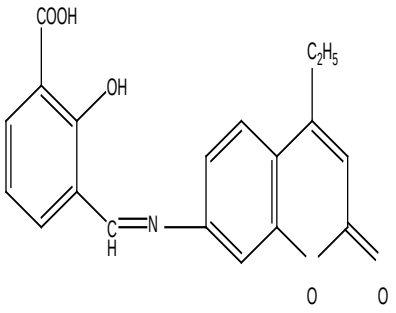
3-[(3-methyl-5-mercapto-[1,2.4] triazoile-4-yl amino)_ methyl]_2_hydroxy_Benzoic acid (Scheme 7)



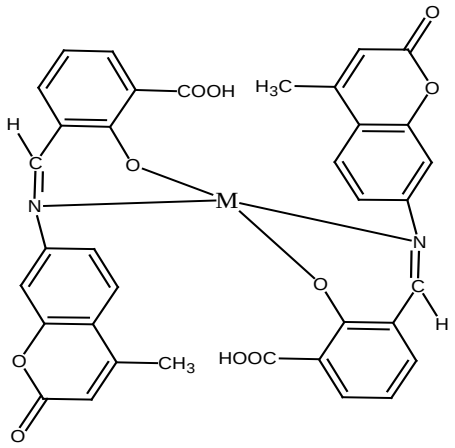
[$ML(EtOH)_2$] M: Cu, Zn, Ni, Co, Cd. OOONS- donor at pH=9



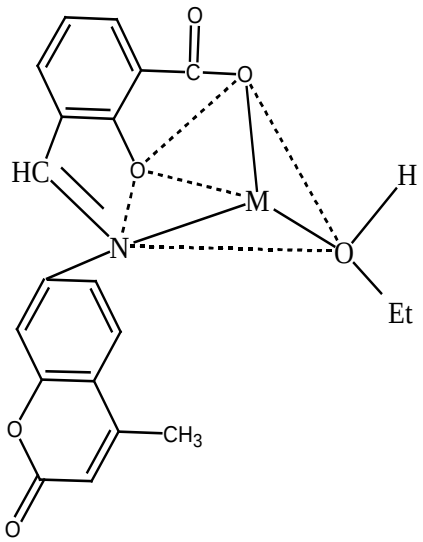
[ML2] M: Cu, Zn, Cd. OO-donor at pH= 4 (Scheme 8).



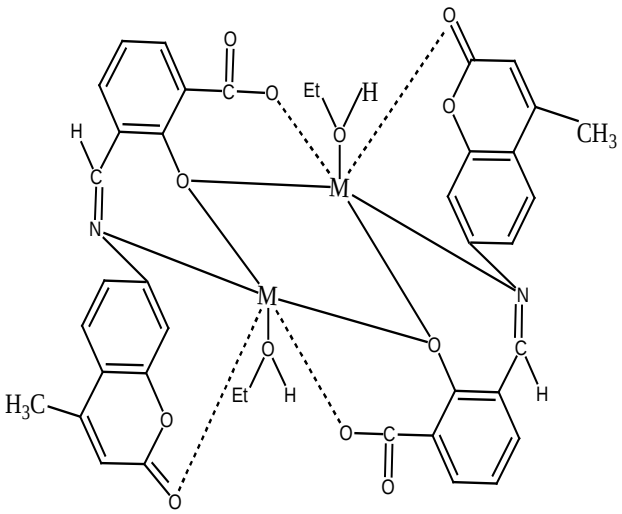
3-[(4-Ethyl-Coumarin-7-yl amino)-methyl] -2-hydroxy-Benzoic acid (Scheme 11)



[M(LH)2] M: Cu, Zn, Cd. ON- donor at pH=4 (Scheme 12).



Scheme 13[ML(EtOH)] M: Zn, Cd. OON-donor at pH= 7 (Scheme 13.



[ML (EtOH) 2] M: Cu, Zn, Ni, Co, Cd. OONO- donor at pH=9 (Scheme 14)

II. Results and Discussion

All the Schiff base ligands were dark yellow in colour; they were air and moisture free crystalline solids. They were sparingly soluble in water, in aqueous sodium hydroxide solution. But, easily soluble in alcohol, owing to the possession of the –OH (Phenolic) and –COOH (Carboxylic) groups. The synthesis of coordination complexes with selected metal ions at various reaction conditions reported as follows:

An aqueous or 50% water-ethanol solution of the Schiff base ligands LH_2 Scheme 1,4,7,11 react with metal ions in (2:1) molar ratio forming metal complexes of the type $[M(LH)_2]$ where M= Cu(II),Zn(II) and Cd(II). Hence, the formed metal complexes Scheme 2,5,8,12 indicating monomeric nature of metal complex with OO-donor at pH-4.

$$MCl_2 + 2LH_2\ (aq) \longrightarrow [M(LH)_2] + 2HCl.$$

Whereas M = Cu (II), Zn (II), and Cd (II).

50% water- alcohol solution of Schiff base ligands (Scheme 1,4,7,11) refluxed separately with alcoholic solution of metal ions in an equimolar quantities, forming the metal complexes of the type [M(LH)2]: M= Zn, Cd, Scheme 3 suggesting the monomeric nature of metal complexes with OON- donor at pH-7. Scheme 9 also indicating the monomeric nature of metal complex with OOS- donor at pH-7.

$$MCl_2\ (alc) + 2LH_2\ (alc) \xrightarrow[\text{Reflux}]{\text{EtOH}} [M(LH)_2] + 2HCl.$$

In the same pH condition Scheme 13 were formed suggesting the monomeric nature of the metal complex with OON- donor.

The alcohol solution of metal ions react with an alcoholic solution of LH2 (Scheme 4) in 1:1 ratio in the presence of sodium- ethoxide and forming the bimetallic complexes of the type $[ML(EtOH)_2]$ (Scheme 6), (Scheme 10) and (Scheme 14) where M= Cu , Zn, Ni, Co and Cd. Suggesting dimeric nature of metal complexes with OONS-donor, OONS- donor and OONO- donor respectively at pH-9.

$$2MCl_2 + 2LH_2 \xrightarrow[\text{Reflux}]{\text{EtOH}} [ML(EtOH)]_2 + 4HCl$$

M= Cu (II), Zn (II), Ni (II), Co (II), Cd (II).

All the synthesized metal complexes were sparingly soluble in water but were fairly soluble in absolute alcohol.

The physical characteristics and analytical data of the Schiff base ligands and metal complexes formed at various pH conditions reported in Table 1. The

molecular measurements of the complex compounds indicated the monomeric nature of complex compounds Scheme 2,5,8,12 at pH-4. The metal complex compounds Scheme 3, 9, 13, formed at pH-7 showed monomeric nature and the complexes Scheme 4, 6, 10, 14 at pH-9 showed diametric in nature. The synthesized complex compounds remained stable up to certain temperature and pH as recorded in the Table 2.

Table 1 : The Physical and Analytical data of ligands and their Metal Complexes

Scheme	Mol.Formula	Mol.Wt. (Obs)	Metal Ions	C (Obs)	H (Obs)	N (Obs)	S (Obs)
1.	$C_{14}H_{12}O_3N_2$	256	----	65.63	4.69	10.94	----
2.	Cu $C_{28}H_{20}O_6N_4$	573.5	11.07	58.59	3.49	9.77	----
	Zn $C_{28}H_{20}O_6N_4$	575.7	11.41	58.36	3.47	9.73	----
	Cd $C_{28}H_{20}O_6N_4$	622.4	18.06	53.99	3.21	8.99	----
3.	Zn $C_{28}H_{20}O_6N_4$	573.7	11.45	58.57	3.49	9.76	----
	Cd $C_{28}H_{20}O_6N_4$	620.4	18.12	54.16	3.22	9.03	----
4.	$C_{16}H_{12}O_3N_4S$	340.0	----	56.47	3.53	16.47	9.41
5.	$CuC_{32}H_{20}O_6N_8S_2$	739.5	8.59	51.93	2.70	15.15	8.65
	$ZnC_{32}H_{20}O_6N_8S_2$	741.7	8.86	51.77	2.69	15.10	8.63
	$CdC_{32}H_{20}O_6N_8S_2$	788.4	14.26	48.70	2.54	14.21	8.12
6.	$Cu_2C_{36}H_{30}O_8N_8S_2$	893.0	14.22	48.38	3.36	12.54	7.17
	$Zn_2C_{36}H_{30}O_8N_8S_2$	897.4	14.64	48.14	3.34	12.48	7.13
	$Ni_2C_{36}H_{30}O_8N_8S_2$	883.4	13.29	48.90	3.40	12.68	7.25
	$Co_2C_{36}H_{30}O_8N_8S_2$	882.2	13.17	48.97	3.40	12.70	7.26
	$Cd_2C_{36}H_{30}O_8N_8S_2$	990.8	22.69	43.60	3.03	11.30	6.46
7.	$C_{11}H_{10}O_3N_4S$	278.0	----	47.48	3.60	20.14	11.51
8.	$CuC_{22}H_{16}O_6N_8S_2$	615.5	10.32	42.89	2.60	18.20	10.40
	$ZnC_{22}H_{16}O_6N_8S_2$	617.7	10.64	42.74	2.59	18.13	10.36
	$CdC_{22}H_{16}O_6N_8S_2$	664.4	16.92	39.74	2.41	16.85	9.63
9.	$ZnC_{22}H_{14}O_6N_8S_2$	615.7	10.67	42.88	2.27	18.20	10.40
	$CdC_{22}H_{14}O_6N_8S_2$	662.4	16.97	39.86	2.11	16.91	9.66
10.	$Cu_2C_{26}H_{26}O_8N_8S_2$	769.0	16.52	40.57	3.38	14.56	8.32
	$Zn_2C_{26}H_{26}O_8N_8S_2$	773.4	16.99	40.34	3.36	12.41	8.28
	$Ni_2C_{26}H_{26}O_8N_8S_2$	759.4	15.46	41.09	3.42	14.75	8.43
	$Co_2C_{26}H_{26}O_8N_8S_2$	758.2	15.33	41.15	3.43	14.77	8.44
	$Cd_2C_{26}H_{26}O_8N_8S_2$	866.8	25.94	35.99	2.99	12.92	7.39

Scheme	Mol.Formula	Mol.Wt. (Obs)	Metal Ions	C (Obs)	H (Obs)	N (Obs)	S (Obs)
11.	$C_{19}H_{15}O_5N$	337.0	----	67.66	1.34	4.15	----
12.	Cu $C_{38}H_{28}O_{10}N_2$	735.5	8.63	61.99	3.81	8.70	----
	Zn $C_{38}H_{28}O_{10}N_2$	737.7	8.91	61.81	3.80	3.80	----
	Cd $C_{38}H_{28}O_{10}N_2$	784.4	14.33	58.13	3.57	3.57	----
13.	Zn $C_{21}H_{19}O_6N$	446.7	14.71	56.41	4.25	3.13	----
	Cd $C_{21}H_{19}O_6N$	493.4	22.78	51.07	3.85	2.84	----
14.	$Cu_2C_{42}H_{38}O_{12}N_2$	889	14.29	56.69	4.28	3.15	----
	$Zn_2C_{42}H_{38}O_{12}N_2$	893.4	14.71	56.41	4.25	3.13	----
	$Ni_2C_{42}H_{38}O_{12}N_2$	879.4	13.35	57.31	4.32	3.18	----
	$Co_2C_{42}H_{38}O_{12}N_2$	878.2	13.23	57.39	4.33	3.19	----
	Cd C_2 H_{42} O_{38} N_{12} $_2$	986.8	22.78	51.07	3.85	2.84	----

Table 2 : The synthesized organometallic complex compounds and the nature of coordination

scheme	pH	Metal-complex	Nature of Complex	Stable up to Temp.in °C	Donor-Atoms
2	4	$[M(LH)_2]$	Monomer	236°C	OO-donor
3	7	$[ML_2]$	Monomer	228°C	OON-donor
5	4		Monomer	232°C	OO-donor

6	9	$[ML(EtOH)_2]$	Dimeric	101°C	OONS-donor
8	4	$[ML_2]$	Monomer	218°C	OO-donor
9	9	$[M(LH)_2]$	Monomer	230°C	OOS-donor
10	9	$[ML(EtOH)_2]$	Dimeric	98°C	OONS-donor
12	4	$[M(LH)_2]$	Monomer	212°C	ON-donor
13	7	[ML(EtOH)]	Monomer	89°C	OON-donor
14	9	$[ML(EtOH)_2]$	Dimeric	94°C	OONO-donor

a) Characterization of the Schiff Base Ligands

The IR Spectra of Schiff base ligands (Scheme 1,4,7 and 11) and metal complex compounds (Scheme 2,5,8,12) at pH-4, pH-7 (Scheme 3,9 and 13) and the metal complexes at pH-9 (Scheme 6,10 14) were recorded in KBr pellets. The IR spectra of the 3ASA ligands exhibit intra molecular H-bonded (carboxylic – √(COOH) and Phenolic –√(OH) groups) stretches at 2910 Cm^{-1} and 2710 Cm^{-1}, √(C=O) carboxylic stretches at 1695 Cm^{-1}, √(C-O) phenolic stretches at 1575 Cm^{1} The IR spectra of Aniline compounds show a characteristic band due to√(N-H) at 3260 Cm^{-1} another band at 1100 Cm^{-1} is due to √(C=S). After the deprotonation of N-H moiety of Triazole indicated by the absence of the bands in the metal complexes at 3260 Cm^{-1} and 1100 Cm^{-1} respectively due to√(N -H) and √(C=S).

A new band seen at 745 Cm^{-1} assigned to √(C-S), this type of band indicates the complexation of the metal ion through sulphur atom. A band 458 Cm-1 to 421 Cm^{-1}, supported to know, √(M-S) band formation in the metal complex formation.

A sharp band in the region 1620 Cm^{-1} -1601 Cm^{-1} for √(N=C)[21] in case of ligand is shifted to higher region 1628 Cm^{-1} to 1612 Cm^{-1} in the spectra of metal-complexes [22] indicating coordination through the ligands nitrogen atom to the metal ion. The formation of a metal Nitrogen bond is further supported by the presence of a band at 578 Cm^{-1} for √(M -N)[23] indicating the coordination of ligand to the central metal atom through the Nitrogen atom of the ligands. The IR-spectral data of ligands and the metal complexes listed in Table 3.

b) ^{1}HNMR – SPECTRA

^{1}HNMR spectra of ligands and metal complexes were recorded in recorded in DMSO- d6 using tetra methyl silence (TMS) as internal standard. The ^{1}HNMR-spectra of Schiff bases show the –SH proton at δ13.2 – δ13.7 ppm. Disappearance of this signal to the –SH proton in the metal complexes. In the metal complexes the deprotonation of the thio-group and this supported the coordination of ligand through sulphur atom to the central metal ion.

^{1}HNMR-Spectra of triazole ligands, the signals of NH2 protons appear at δ10.3 ppm and δ 9.2 ppm. These signals shifted to high field in the spectra of the metal complexes. In Scheme 6 , Scheme 10 in both cases the signals shifted and appear at δ9.5 ppm to δ9.7 ppm for organ metallic complexes with the ligand molecules. This indicates the bonding through the Nitrogen atom of the Schiff base ligands to the central metal ion. The aromatic protons gave signals at δ1.2 ppm – δ 1.3 ppm. The 1HNMR spectroscopic data of the ligands and their metal complexes are given in the Table 4.

Table 3 : IR Spectroscopic data (Cm-1) of the ligands and their metal complexes

Scheme	Functional group	IR-Values in Cm-1		Scheme	Functional group	IR-Values in Cm-1
Scheme1	√(-COOH)	3300cm^{-1}		Scheme8	√(-COO-)	1748 cm^{-1}
"	√(-OH)	3150 cm^{-1}		"	√(-C=N)	1628 cm^{-1}
"	√(-C=N)	1675 cm^{-1}		"	√(-C-H)	3000 cm^{-1}
"	√(-C-H)	3000 cm^{-1}		"	√(-C=O)	1690 cm^{-1}
"	√(-C=O)	1690 cm^{-1}				
Scheme2	√(-COO-)	1748 cm^{-1}		Scheme9	√(-COO-)	1748 cm^{-1}
"	√(-C=N)	1675 cm^{-1}		"	√(-C=N)	1628 cm^{-1}
"	√(-C-H)	3000 cm^{-1}		"	√(-C-H)	3000 cm^{-1}
"	√(-C=O)	1690 cm^{-1}		"	√(-C=O)	1690 cm^{-1}
				Scheme10	√(-COO-)	1748 cm^{-1}
Scheme3	√(-COO-)	1749 cm^{-1}		"	√(-C=N)	1628 cm^{-1}
"	√(-C=N)	1620 cm^{-1}		"	√(-M-S)	458 cm^{-1}
"	√(-C=O)	1690 cm^{-1}		"	√(-C=O)	1690 cm^{-1}
"	-M-	578 cm^{-1}		"	-M-	577 cm^{-1}

Scheme4	√(M N) √(-COOH)	3300 cm^{-1}	Scheme11	√(M √(-COOH)	3300 cm^{-1}
''	√(-OH)	3149 cm^{-1}	''	√(-OH)	3150 cm^{-1}
''	√(-C=N)	1628 cm^{-1}	''	√(-C=N)	1628 cm^{-1}
''	√(-C-H)	3000 cm^{-1}	''	√(-C-H)	3000 cm^{-1}
				√(-C=O)	1690 cm^{-1}
Scheme5	√(-COO-)	1748 cm^{-1}	Scheme12	√(-COOH)	3300 cm^{-1}
''	√(-C=N)	1628 cm^{-1}	''	√(-M-N)	578 cm^{-1}
''	√(-C=O)	1690 cm^{-1}	''	√(-C=N)	1628 cm^{-1}
''	√(-C-H)	3000 cm^{-1}	''	√(-C-H)	3000 cm^{-1}
			''	√(-C=O)	1690 cm^{-1}
Scheme6	√(-COO-)	1748 cm^{-1}	Scheme13	√(-COO-)	1748 cm^{-1}
''	√(-C=N)	1628 cm^{-1}	''	√(-C=N)	1628 cm^{-1}
''	√(-C=O)	1690 cm^{-1}	''	√(-C=O)	1690 cm^{-1}
''	√(-M-S)	458 cm^{-1}	''	√(-M-N)	578 cm^{-1}
			''	√(-C-H)	3000 cm^{-1}
Scheme7	√(-COOH)	3300 cm^{-1}	Scheme14	√(-COO-)	1748 cm^{-1}
''	√(-OH)	3150 cm^{-1}	''	√(-C=N)	1628 cm^{-1}
''	√(-C=N)	1675 cm^{-1}	''	√(-C=O)	1690 cm^{-1}
''	√(-C-H)	3000 cm^{-1}	''	√(-M-N)	578 cm^{-1}
''	√(-C=O)	1690 cm^{-1}	''	√(-C-H)	3000 cm^{-1}

Table 4 : 1HNMR Chemical shifts of the ligand and their metal complexes

Compound	Aromatic H	-CH3	-CH2	-CH=N	-SH
Scheme 1	7.1 – 8.0 (m)	δ 1.2(t)	----	δ 10.3(s)	----
Scheme 2	7.1 – 8.0 (m)	δ 1.2(t)	----	δ 10.3(s)	----
Scheme 3	7.1 – 8.0 (m)	----	----	δ 10.3(s)	----
Scheme 4	7.1 – 8.0 (m)	----	----	δ 10.3(s)	δ 13.7(s)
Scheme 5	7.1 – 7.9 (m)	----	----	δ 9.7(s)	δ 13.7(s)
Scheme 6	6.9 – 8.0 (m)	δ 1.3(t)	δ 2.7(q)	δ 9.5(s)	δ 13.6(s)
Scheme 7	6.4 – 7.2 (m)	δ 1.3(t)	----	δ 9.2(s)	δ 13.7(s)
Scheme 8	6.7 – 7.8 (m)	δ 1.2(t)	----	δ 9.4(s)	δ 13.6(s)
Scheme 9	6.9 – 8.0 (m)	δ 1.3(t)	----	δ 9.3(s)	δ 13.6(s)
Scheme 10	6.9 – 7.2 (m)	δ 1.2(t)	δ 2.8(q)	δ 9.4(s)	δ 13.2(s)
Scheme 11	7.1 – 8.0 (m)	δ 1.2(t)	δ 2.9(q)	δ 9.2(s)	----
Scheme 12	7.1 – 8.0 (m)	δ 1.2(t)	δ 2.8(q)	δ 9.2(s)	----
Scheme 13	7.1 – 8.0 (m)	δ 1.3(t)	δ 2.9(q)	δ 9.3(s)	----
Scheme 14	7.1 – 8.0 (m)	δ 1.3(t)	δ 2.8(q)	δ 9.7(s)	----

III. Experimental

3-Aldehydosalicylic acid24, 7-Amino-4-ethyl Coumarin25, 2-amino-5-methyl Pyridine26, 4-Amino-5-Phenyl-3-Mercapto-1, 2, 4-Trizole27, 4-Amono-5-methyl-5-mercapto-Triazole28, and Schiff base compounds were prepared by adopting the published standard procedures29, 30. The chemicals such as metal chlorides, sodium acetate and other solvents were obtained from Merck.

a) Synthesis of Schiff Base ligands

i. *Synthesis of 2-(5-methyl pyridine-2-yl imino)-methyl-2-hydroxy-Benzoic acid*

Equimolar ethanol solution (50 ml) of 3-Aldehydosalicylic acid and 2-amino-5-methyl pyridine was refluxed for 2 hours in round bottom flask. During the reflux a crystalline orange compound was separated (Scheme 1) filtered and dried and purified by crystallizing in appropriate solvent. Followed by the same experimental methods the other Schiff base ligands Scheme 4, 7 and 11 were synthesized, the compounds were confirmed by physical and analytical data, IR and 1HNMR- spectra were evidences for the Schiff base formation by the selected compounds.

ii. *Preparation of Metal Complexes*

All the metal complexes of Schiff bases were prepared by the standard reported method The metal salts were dissolved in minimum quantity of water and the solutions were added to hot ethanolic solution of the corresponding Schiff bases. After complete addition, little amount of sodium acetate was added and the mixture was refluxed for 4 hours. Crystalline solid was obtained, which was isolated by filtration, washed with hot water and dried in air.

b) Antibacterial Activity

Antibacterial activity of the all the Schiff base ligands and metal-complexes were evaluated against Staphylococcus aureus, Bacillus subtitles', Escherichia Coli and pseudomonas aeruginosa. Bacterial culture was prepared; each culture was added to the sterilized medium before solidification. The media with bacteria was poured into sterilized Petri dishes under aseptic condition. Different weights of Schiff base ligands and their metal complexes (1 mg, 5 mg and 10 mg) were placed on the surface of the culture and incubated at 30oc for one day. After incubation zone of inhibitions (mm) were recorded.

IV. Conclusions

The different types of complex compounds of same ligand with metal ions have been obtained by varying pH and solvent during their synthesis. The basicity and denticity of the Schiff base scheme 1, 4,7,11 derived from 3-Aldehydosalicylic acid and different amines changes considerably with the role of pH from 3 to 9. At pH-3 ligands act as monobasic bidentate OO-donor ligand forms monometallic complex compounds of the type [M(LH)2] where M= Cu,Zn, Cd (Scheme 2,5,8,12). At pH-7 the ligand acts as a bibasic tridentate OON-donor ligand forming the monometallic complex compound of the type [ML_2]: M= Zn, Cd (Scheme 3). At the same pH-7 the ligand formed OOS-donor monometallic complex compound of the type [M $(LH)_2$]: M= Zn,Cd (Scheme 9). The Scheme 13 indicates the ligand acts as a dibasic tridentate OON-donor ligand forming the monometallic complex compound of the type [ML (EtOH)]: M= Zn, Cd.

Metal complexes at Ph-9 the ligands act as a dimeric tetra dentate OONS-donor (Scheme 10) and OONO-donor (Scheme 14). The basicity and denticity confirmed by various methods. The IR Spectra of the Schiff bases (Scheme 1,4,7,11) reveals that the non-involvement of carboxylic atom in coordination at pH-4, and metal complexes Scheme 2,5,8,12 reveals that the non-involvement of Nitrogen atom in the coordination. Here in these metal complexes the carboxylic O-atom and O- atom (Phenolic) involved in the coordination by exhibiting the δ_{as}(COO) and δ_s(COO) stretches of carboxylate group in the ranges 1560-1650 Cm^{-1} (i.e., 1600 Cm^{-1}) and 1340 – 1430 Cm^{-1} (ie., 1400 Cm^{-1}) respectively. The energy difference between δ_{as}(COO) and δ_s(COO) is more than 200 Cm^{-1} indicates the monodentate nature of the carboxylate moiety[31].

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Chemical Analysis and Nutritional Assessment of Defatted Garcinia Mangostana Seeds Used as an Additive on the Feed of Fish *(Clarias Gariepinus)*

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Keywords : *clarias gariepinus, DGMS, growth performance, histology, post juveniles.*

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Ibironke A. Ajayi [α], Flora E. Olaifa,[σ] & Muhammed M. Omoniyi [ρ]

Abstract - Chemical analysis and nutritional assessment of defatted *Garcinia mangostana* seeds (DGMS) were undertaken to determine its suitability as an additive at 0.00g, 18.00g, 36.00g, 54.00g and 72.00g inclusion levels in diets and performance of *Clarias gariepinus* post juveniles. Proximate analysis of DGMS showed that the defatted seeds were high in carbohydrate (71.00 ± 0.79%) but low in protein content (8.10 ± 0.22%). The mineral element analysis detected different minerals with potassium as the highest (270.00ppm). All the fish increased in weight and length significantly ($p<0.05$) above the initial values though no significant differences were observed among treatments at 49days in all growth indices showing that the diets were similar in nutritional qualities and adequate for growth of fish. Hematological analysis showed increments in the final blood parameters at day 49 except MCH, MCV and heterophils and very high levels of leucocytes and platelets ($p<0.05$). No significant differences were observed in the haemoglobin content, AST, ALT, globulins and albumin in fish on DGMS-containing diets. Histology showed sub mucosal congestion in gill (36.00g DGMS), severe interstitial congestion in kidney (72.00g DGM) and vacuolations in liver in all groups except diet 4 (54.00g DGMS) and control (0.00g DGMS). However higher survival rates were observed in all DGMS- containing diets than the control.

Keywords : clarias gariepinus, DGMS, growth performance, histology, post juveniles.

I. Introduction

Uncountable plant resources with important benefits abound around us unexploited. Many have high nutritive values like proteins, carbohydrates, lipids and minerals while others have industrial potentials as sources of dyes, starches and vitamins. Plants hitherto considered of little or no value are being investigated, evaluated and developed into drugs with little or no side effects (Adedeji et al., 2006). Several biological and synthetic compounds have been shown to enhance non specific immune system of cultivated fish (Cao & Lin, 2003; Lin & Zhang, 2004; Sakai, 1999; Shan et al, 1999; Soosean et al. 2010). Lack of competition for and high cost of readily available nutritive fish feed ingredients have continued to plague aquaculture in the competitive global food production system (Munguti et a*l.*, 2012; Tacon, 1993). In view of these, researchers have been studying several plant and animal sources as supplement or likely substitutes for the conventional ingredients currently in use. Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (Nwanna et al.., 2008). Protein is the most expensive component of fish diets and is usually supplied as fish meal due to its balanced amino acid profile (Munguti et al, 2012). As alternatives to fishmeal, plant products tend to be less balanced in amino acid profiles than fish meal. Omoregie and Ogbemudia (1993) advised that it would be more economical to use plant protein and have reduced growth rates of aquatic animals than using fish meal at high cost.

Feed additives are substances which are usually included in feeds in trace amounts to preserve its nutritional characteristics prior to feeding (antioxidants and mould inhibitors), facilitate ingredient dispersion or feed pelleting (emulsifiers, binders or stabilizers), promote growth (growth promoters, antibiotics and hormones), facilitate feed intake and acceptance (feeding stimulants and colourants), or supply some essential nutrients such as minerals and vitamins (Food and Agricultural Organization, 1987).Some workers have also suggested the addition of immunostimulants of biological origin to reduce disease outbreaks in fish culture by enhancing the non-specific immune system (Anderson,1992).

The Genus *Garcinia* belongs to the family *Clusiaceae* (syn. *Guttiferae*) which consists of about 35 genera and up to 800 species (Osman & Milan, 2006). The family is pan tropical and comprises mostly of large evergreen trees or erect shrubs with smooth, thin bark and white or yellow latex. *Garcinia* is the biggest Genus in the family with about 400 species (Jantan et al., 2011). The Genus *Garcinia* has been used in ayurvedic preparations to treat disorders and contain bioactive

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molecules like hydroxycitric acid, flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garinol, xanthochymol and guttiferone (Hemshekhar et al., 2011; Lim, 2012). Flavonoids present in G. *kola* are assumed to have anti-asthmatic activities (Okojie et al., 2009), inhibit platelet activating factor, phospholipidaseA2 and phosphodiesterase (Dorsch & Wagner, 1991; Miller, 2001), prevent allergies, inflammation, free radicals, platelet aggregation and inhibit histamine release (Ferguson, 2002; Farquar, 1996; Hodek et al., 2002; Okwu, 2004). *Garcinia mangostana* L., (mangosteen) is a slow-growing tree and well known for its fruits particularly in Southeast Asia. The fruits possess a sweet pulp which is eaten fresh, but is also used in processed form. It has a long history of use as a medicinal plant majorly in Southeast Asia for its anti inflammatory properties (Obolskiy et al., 2009) and for treatment of skin infections and wounds (Pedraza-Chaverri et al., 2008). Other applications include treatment of dysentery, urinary disorders, cystitis and gonorrhea.

The biologically active ingredients in G mangostana L. responsible for these medicinal properties have been identified as xanthones (Obolskiy et al., 2009) which are polyphenols found in the pericarp from the mangosteen fruit .The juice also contains α-mangostin, garcinones C, D, and E, γ-mangostin, gartanins and other xanthones (Chitchumroonchokchai et al., 2012; Pedraza-Chaverri et al., 2008;). α-mangostin has been shown to enhance betulinic acid's cytotoxicity to colon cancer cells, showed cytoprotective effect against cisplastin-induced cytotoxicity (Aisha et al., 2012) and is a multi-target inhibitor of mutans streptococci and useful as an anti-caries agent (Phuong et al., 2011). Poly isoprenylated benzophenone and xanthone are known for having antioxidant (Joseph et al., 2005; Okoko, 2009), antibacterial, anti viral, anti fungal, anti ulcer, anti protozoan activities (Hemshekhar et al., 2011), anti platelet (Jantan et al., 2009) and anti-cancer (Han et al., 2008). Ajayi et al., (2007) observed that rats were able to utilize the oil of *Garcinia mangostana* seeds and suggested that the seeds could be utilized as sources of dietary fibre and roughage in livestock feeds because of its high crude fibre and carbohydrate contents. The low protein content can be supplemented with high protein residues, such as groundnut or soy cakes. A lot of studies using G. *mangostana* for treatment of human disorders abound including heart problems and asthma (Buelna-Chontal et al, 2011; Okojie, et al., 2009). This study examined the utilization and growth performance, haematology and tissue pathology of *Clarias gariepinus* post juveniles on feeds containing defatted G. *mangostana* seed meal included as additives of 0.00g, 18.00g, 36.00g, 54.00g and 72.00g.

II. Materials and Methods

a) Sample Preparation

Garcinia mangostana fruits were obtained from the Botanical Garden of the University of Ibadan. The seeds were removed from the fruits, washed with water and left to air-dry. Oil from the seeds was obtained by soxhlet extraction using n-hexane (bpt 60 °C) as described by Ajayi et al. (2006) and left to air dry for a week to remove the solvent from the defatted seeds.

b) Proximate Analysis

Proximate analysis of the defatted seeds, formulated feeds and fish samples were carried out following the procedure described by AOAC (2000).

c) Mineral element analysis

The mineral element analysis was carried out using the wet-ashing method for the digestion of the defatted seed sample: 1.00g of defatted G. *mangostana* seed was digested with 20 ml of concentrated HNO^3 and perchloric acid (1:1 v/v) and thereafter transferred to a 50 ml volumetric flask. It was diluted to volume with de-ionized water and stored in a clean polyethylene bottle. The mineral element content was determined using an atomic absorption spectrophotometer (Perkin–Elmer model 703, USA) as described by Onyeike and Acheru (2002).

d) Fish housing and treatment

Clarias gariepinus post juveniles (One hundred and fifty, initial mean length and weight 16.20 ± 0.01cm and 47.92 ± 0.00g respectively) were purchased and transported from the Department of Wildlife and Fisheries Management's fish farm, University of Ibadan, Oyo State to the laboratory in plastic bowls between 5pm-6pm to reduce stress and mortality due to high temperature. The fish were acclimatized for five days during which vitamin C was administered in water. The water in the bowls was well aerated using air stone and pump (Lawson, 1995) and fish were fed at 3% of their body weight twice daily. At the end of acclimatization period, the fish were starved for 12 hours to empty their guts and prepare them for the experimental feed.

The fish were assigned randomly to five treatments in 40- litre plastic bowls with each treatment having three replicates and 10 fish per replicate. De-chlorinated water (tap water exposed to air for more than 24 hours) was used. Water quality parameters monitored were temperature using mercury- in- glass thermometer (Paragon Scientific Ltd, Birkenhead, and Wirral, UK). The dissolved oxygen content and pH of the water were measured at 0.01 accuracy using dissolved oxygen metre, (Jenway 3015 pH metre, Genway, Staffordshire, UK) after standardizing the metre. These water quality characteristics were monitored throughout the feeding trials and water in each bowl was changed every three days. Fish were fed experimental diets twice daily for 49 days at 3 % of

their body weight. Their weights and lengths were recorded weekly. The plastic bowls were covered with nets to prevent the fish jumping out and avoid intrusion by other animals and insects.

e) Feed Formulation

The feedstuffs purchased from the feed mill were soybean meal (44% crude protein), fish meal (72 % crude protein), wheat offal (18 % CP), starch, di-calcium phosphate (DCP), salt, maize, vegetable oil and vitamin/mineral premix. The ingredients were milled into powder and used to formulate a 40 % crude protein diet and turned into dough. Each diet mixture was treated separately and extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine (Hobart GmbH, Rben-Bosch, Offenburg, Germany) to form noodle- like strands which were broken into suitable sizes for the fish. The pellets were sun- drieds, packed in labeled polythene bags and stored in a cool dry place to prevent fungal growth. The defatted *Garcinia mangostana* (DGMS) was incorporated in the formulated diet as an additive at 0.00g, 18.00g, 36.00g, 54.00g, 72.00g inclusion levels before the extrusion process representing diets 1(control) to 5 respectively as shown in table 1.

f) Food utilization parameters

Specific growth rate (SGR): This was calculated from data on changes of the body weight over the given time intervals according to the method of Brown (1957) as follows:

$$\text{SGR\%} = \frac{\text{Ln W2} - \text{Ln W1}}{\text{T} - \text{t}} \text{ X } 100$$

Where W1 is the initial weight (gram at time t), W2 is the final weight gain (gram at time t) (Brown, 1957)

i. *Food conversion ratio*

$$\text{FCR} = \frac{\text{weight of food consumed fortnightly (g)}}{\text{weight gain by fish fortnightly (g)}}$$

Weight gain (g) was calculated as the difference between the initial and final mean weights of the fish in the plastic bowl.

weight gain(g)=final weight-initial weight

Survival rate (SR): The survival rate, SR was calculated as total fish number harvested / total fish number stocked expressed in percentage.

$$\text{survival (\%)} = \frac{\text{number of fish harvested}}{\text{number of fish stocked}} \text{ X } 100$$

Percentage weight gain (%WG): This is expressed by the equation:

$$\text{\%WG} = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} \text{ X } 100$$

Where: Wo = Initial weight, and Wt = Weight at time t.

Protein efficiency ratio (PER): This was calculated as:

$$\text{PER} = \frac{\text{wet body weight gain}}{\text{crude protein feed}}$$

Condition factor (K): This was expressed by the equation

$$K = \frac{100\ W}{\text{L}^3}$$

Where W is weight of the fish
L is standard length
Nitrogen metabolism (NM) was calculated as:

$$\text{NM} = \frac{(0.549)(a+b)h}{2}$$

Where a = initial weight of fish
b = final weight of fish
h = experimental periods in days (Nwanna, 2003).

g) Heamatological Analysis

Hematological analyses were carried out both at the beginning and end of the experiment. Initial and final fish blood samples were collected before the feeding trial (that is day 10) and on day 49 for all treatments with the aid of needles and syringes into heparinised bottles with disodium EDTA as anticoagulant. The samples collected were analyzed on each occasion as described by Schalm et al., (1975). The blood parameters determined for each sample were packed cell volume (PCV), haemoglobin concentration, white blood cell (leucocyte) count, red blood cells (erythrocyte) count, protein, albumin, and globulin. Others were mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

h) Histopathological Analysis

For tissue histopathology, the internal organs were exposed by dissection and the liver, kidney, heart, brain were observed for gross lesion and stored in formalin. Small portions of each organ were fixed and put through series of dehydration in graded concentrations of xylene. They were embedded in wax, sectioned at 5µ and transferred onto glass slides. The thin sections were stained with heamotoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes (MAFF, 1984).

i) Statistical Analysis

Each experiment was performed in triplicate. Results were expressed as the mean ± standard deviation. The one-way analysis of variance (ANOVA) was used to determine significant differences of the treatments. P-values<0.05 were considered significant. Duncan multiple range test was applied while all data

were analyzed with SPSS (IBM statistic Computer Program 2010).

III. Results and Discussion

The proximate composition of *G. mangostana* defatted seed was presented in able 2 and showed the protein content of the defatted seed as low and similar to quantities obtained from some cereal grains like maize (8-9%). The crude protein for *G. mangostana* obtained during this study was higher than 6.57 g/100g reported by Ajayi et al. (2007) but lower than for *Gnetum africanum* 17.50% (Ekop, 2007). The crude fibre content (6.50 ± 0.02 %) was higher than the range for legumes (Prakash et al. 2001). Carbohydrate content was high at 71%.This implies that the seeds can complement energy sources or supply energy in livestock rations. The ash content was 1.80 ± 0.02% and similar to Ajayi et al. (2007) but lower than 4.5% reported for *Caesalpinia pulcherrima* (Yusuf et al. (2007). The crude fat content (2.13 ± 0.04) is quite low when compared with that of Ajayi et al. (2007). The low fat content of the defatted seed reported was because of the removal of the oil by solvent extraction during the defatting process.

The defatted seeds of *G.mangostana* had a good supply of mineral elements (Table 6) with the highest being potassium (270 ppm), followed by magnesium (110 ppm), iron (68.62 ppm) and calcium (30 ppm). A diet that contains *Garcinia mangostana* will help to prevent deficiencies of potassium, iron and calcium since they are rich in these elements. Potassium helps to regulate blood pressure; calcium is needed for good bones, muscle contraction and blood clotting while magnesium works along with calcium to maintain healthy bones. The minerals obtained in the defatted seeds of G. mangostana were lower than those obtained in the seed oil (Ajayi et al., 2007).This may imply that most of the mineral elements were lost to the oil fraction during the defatting process.

The gross and proximate compositions of the diets were presented in tables 1 and 2 respectively. All diets were formulated with similar gross compositions except for the addition of 0.00, 18.00, 36.00, 54.00 and 72.00g of the defatted *G.mangostana* seed to the experimental diets for diets 1-5 respectively. Though the moisture contents of all the diets were significantly different ($p<0.05$), the crude protein of the experimental diets were not. Crude fat (ether extract), crude fibre, ash and Nitrogen free extractives varied significantly among treatments ($P<0.05$).These could have been due to the presence of DGM at different levels in the diets.

Growth and nutrient utilization of *Clarias gariepinus* on the experimental diets were presented (Table 3). Fish on all treatments significantly ($p<0.05$) increased in weights and lengths over the experimental period above the initial observations though no significant differences ($p<0.05$) were observed in weights and lengths among treatments. Feed conversion ratio, specific growth rate, percentage weight gain, and nitrogen metabolism in fish did not differ significantly among the diets. This reveals that the addition of defatted seeds of *G. mangostana* as an additive to the feed of *C. gariepinus* had a positive effect on the growth performance of the fish throughout the experimental period. These observations were similar to those observed by (Dada & Oviawe, 2011, Prasad & Priyanka, 2011; Dada & Ikuerewo 2009) but differed from Soosean et al. (2010) .Higher survival rates were recorded in DGM-containing diets above the control with the highest in diet 5(72.00g DGM). The condition factors of the fish varied non- significantly in all treatments. The feed conversion ratios for diets 2, 3 and 4 were lower than for the control and treatment 5 showing that diets containing between 18.00g DGM and54.00g DGM were better utilized by the fish for growth than the control and highest inclusion level of DGM though the observed differences were not significantly different ($p>0.05$).

The proximate analyses of *C.gariepinus* post juveniles after the feeding trial were presented in table 2. Moisture contents, crude protein and crude fibre of the fish differed significantly ($P<0.05$) among treatments. The crude protein was highest in 18.00g DGM-containing diet while the crude fibre was highest in 36.00g DGM-containing diet. The crude fat (ether extract and ash were not significantly different among treatments and varied from 20.30±0.27 to 23.70±1.67. All the DGM-containing diets had higher ash contents than the control fish with diet 3(36.00g DGM) having the highest value (10.60 ± 1.96) and the lowest in the control and 18.00g DGM diet. This observation tends to support the view that DGM-containing rations could improve the mineral content of the fish. The mineral composition of *Clarias gariepinus* (Table 6) after the feeding trial showed significant differences ($p<0.05$) among treatments in potassium, calcium, magnesium and sodium which are macro elements with diet 4(54.00g DGM) producing the highest concentrations of the elements and diets 1 and 2(18.00g DGM) the lowest of all four in the fish. Manganese, iron, copper and zinc were present in very low quantities and were not significantly different among diets.

All the blood parameters studied were higher after 49 days than at the beginning of the study except heterophils, MCH and MCV which decreased from initial values (Table 4).These observations were similar to the reports of other workers (Prasad & Priyanka, 2011; Soosean et al, 2010) who reported increased haematological parameters in fish after diets containing different extracts of Garcinia species. Packed cell volume varied from 30.00 ± 2.83 to 34.00 ± 1.41% for all the treatments though not significantly different ($p>0.05$). Haemoglobin concentration (mg/l) of the fish in all treatments and control varied but not significantly. The hemoglobin content in the blood and oxygen

consumption increases when fishes are stressed and allows an increase in release of immature RBCs from the haemopoietic organs, which in turn elevate hemoglobin concentration in blood (Choudhury et al, 2005; Soosean et al, 2010).

White blood cell counts in all the treatments were significantly higher than the initial values and differed significantly among the treatments. This supports the immune- system boosting effect of Garcinia species reported by some workers (Dada & Ikuerewo, 2009; Prasad & Priyanka, 2011). The platelets also showed similar trend. Percentage lymphocytes and monocytes varied non-significantly among diets from 71.00±1.41 to 72.00 ± 2.83% and 2.00 ± 1.41 to 4.00 ± 1.41respectively. The survival rate of the fish was high generally during the experiment though the least was reported for the contro

The blood biochemistry -plasma proteins (albumin and globulin) and blood serum enzymes (aspartate amino transferase and alanine amino transferase) were presented in table 5. All the final mean values were higher than the initial observations except for aspartate amino transferase (AST) in which the mean value for fish on control diet was lower than the initial observations .However all DGM-containing diets produced higher AST at 49 days than at day 0 of the experiment. Total protein ranged from 3.50±1.4 to 3.80 ± 0.14 and albumin 1.10 ± 0.14 to 1.40 ± 0.14 for all the diets. The highest value of globulin was 2.8 ± 1.4 in diet 3(36.00g DGM) and the lowest 2.20 ± 0.14 in diet 2(18.00g DGM). The values of AGR (albumin –globulin ratio) were not significantly different, ranging from 0.30 ± 0.14 to 0.60 ± 0.14 for all diets. The blood biochemistry showed variations that were not significant ($p>0.05$). Fish plasma proteins are important in regulating water balance in fish (Wedemeyer & Yasutake, 1977)

The tissue examination showed no lesions in the organs of the control fish. Other diets produced no lesions in the organs except the gills in diet 2(36.00g DGM) fish which showed mild submucosal congestion. Diet 5(72.00g DGM) produced severe interstitial congestion in the kidney. The kidney is involved with excretion of wastes from the body of animals and that could explain why there was severe interstitial congestion at the highest inclusion level of DGM inclusion treatment 5 (72.00g DGM). Similar reports of kidney malformations were reported on rats (Ajayi et al, 2007). The organ majorly impacted in *C.gariepinus* post juveniles was the liver which presented different degrees of hepatocyte vacuolation in diets 2, 3 and 5(18.00, 36.00 and 72.00g DGM). Diet 4 (54.00gDGM) was the only group where the fish showed no visible lesions in all organs in fish on DGM-containing diets. This observation differed from Ajayi et al. (2007) who reported no lesions in the liver of rats. The liver is the organ involved in the metabolism, detoxification and excretion of chemicals and xenobiotics in the body (Pathan et al, 2010) and that could explain why it was so affected. Vacuolation has been observed to be a common response to the presence of chemicals in fish (Meyer & Henderick, 1985; Clearwater et al, 2002; Shaw & Handy, 2006). No lesions were observed in all the heart tissues of fish during this study.

IV. Conclusion

The proximate analysis of the defatted *Garcinia mangostana* seed (DGM) depicted it as high in carbohydrate and good in mineral elements though the protein content was low. The mineral analysis of defatted *Garcinia mangostana* seeds showed high values of potassium, magnesium and iron which indicated that DGM could be used as an additive in fish feed. However, further studies are recommended due to the problems identified during the tissue examination of the liver. High survival rate and good performance of the fish would be an incentive to further look at methods of processing the DGM to be compatible with the requirements of *C.gariepinus*.

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Table 1 : Gross composition of experimental diets (g) containing defatted G. mangostana seeds

Ingredients(g)	Control	18gDGM	36g DGM	54gDGM	72gDGM
Fish meal	9.69	9.69	9.69	9.69	9.69
Soy bean	19.39	19.39	19.39	19.39	19.39
Maize	31.71	31.71	31.71	31.71	31.71
Wheat offal	31.71	31.71	31.71	31.71	31.71
Vit-min	2.00	2.00	2.00	2.00	2.00
Starch	1.00	1.00	1.00	1.00	1.00
DCP	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
Vegetable oil	2.00	2.00	2.00	2.00	2.00
DGM(g)	-	18.00	36.00	54.00	72.00

Table 2 : Proximate composition of formulated diets at different inclusion levels of defatted *G. mangostana* seeds

Constituents(%)	Control	18g DGM	36g DGM	54g DGM	72g DGM
Moisture	6.64 ± 0.045^{d}	6.07 ± 0.061^{b}	5.68 ± 0.03^{a}	6.31 ± 0.01^{c}	7.60 ± 0.02^{e}
Crude protein	40.39 ± 0.40^{a}	40.49 ± 0.22^{a}	40.36 ± 0.25^{a}	40.21 ± 0.01^{a}	40.96 ± 0.01^{a}
Crude fibre	4.82 ± 0.15^{b}	3.53 ± 0.01^{a}	3.49 ± 0.02^{a}	5.53 ± 0.01^{c}	5.77 ± 0.08^{d}
Crude fat	7.70 ± 0.01^{c}	7.07 ± 0.05^{a}	7.14 ± 0.01^{b}	15.60 ± 0.32^{e}	15.11 ± 0.04^{d}
Ash	8.45 ± 0.27^{b}	8.31 ± 0.16^{b}	8.2 ± 0.01^{ab}	8.00 ± 0.03^{a}	7.95 ± 0.14^{a}
NFE	32.00 ± 0.80^{c}	34.52 ± 0.47^{d}	35.33 ± 0.26^{e}	24.35 ± 0.01^{b}	22.62 ± 0.13^{a}

Means followed by different letters in the same rows are significantly different ($P<0.05$)

Table 3 : Result of proximate analysis of fish after feeding trial using experimental feeds

Constituent (%)		Mean ± SD				
	Garcinia mangostana defatted seeds	Control	18.00 g DGM	36.00g DGM	54.00g DGM	72.00g DGM
Moisture	10.47 ± 1.12	9.48 ± 0.55^{a}	9.08 ± 0.99^{b}	9.92 ± 0.09^{a}	10.46 ± 0.58^{c}	9.48 ± 0.55^{b}
Crude protein	8.10 ± 0.22	63.00 ± 2.00^{cd}	63.60 ± 0.26^{d}	55.40 ± 0.27^{a}	60.70 ± 0.34^{b}	61.47 ± 1.27^{bc}
Crude fibre	6.50 ± 0.02	0.27 ± 0.06^{a}	0.23 ± 0.06^{a}	0.200 ± 0.00^{a}	0.40 ± 0.10^{b}	0.70 ± 0.00^{c}
Crude fat	1.80 ± 0.02	20.60 ± 1.85^{a}	20.30 ± 0.27^{a}	23.70 ± 1.67^{b}	21.07 ± 0.78^{a}	20.50 ± 0.46^{a}
Ash	2.13 ± 0.04	6.65 ± 0.55^{a}	6.79 ± 1.83^{c}	10.60 ± 1.98^{b}	7.37 ± 0.22^{b}	7.85 ± 0.55^{b}
Carbohydrate	71.00 ± 0.79					

Means followed by different letters in the same rows are significantly different ($P<0.05$).
The treatments produced very negligible nitrogen free extracts in fish

Table 4 : Mineral composition of Clarias gariepinus post juveniles after feeding trial (ppm of dry matter) in 49 days and Defatted *Garcina mangostana*

Mineral element	Defatted *Garcinia mangostana*	Control	18.00g DGM	36.00g DGM	54.00g DGM	72.00g DGM
Potassium	270	73.30^{b}	32.80^{a}	249.50^{d}	364.50^{e}	147.00^{c}

Calcium	30	160.00^{b}	120.00^{a}	258.00^{d}	268.50^{e}	242.00^{c}
Magnesium	110	12.50^{b}	6.87^{a}	35.65^{d}	49.65^{e}	23.75^{c}
Sodium	7.95	50.41^{b}	20.11^{a}	147.05^{d}	134.05^{e}	58.31^{c}
Manganese	9.76	0.12^{a}	0.12^{a}	0.30^{a}	0.38^{a}	0.37^{a}
Iron	68.62	1.85^{a}	1.88^{a}	4.42^{b}	4.27^{b}	1.95^{a}
Copper	6.58	0.10^{a}	0.02^{a}	0.09^{a}	0.16^{a}	0.13^{a}
Zinc	25.63	0.50^{a}	0.14^{a}	0.87^{a}	1.50^{a}	0.69^{a}

Table 5 : Growth and nutrient utilization of Clarias gariepinus fed with different defatted G. mangostana-based diets

Parameter	Control	18.00g DGM	36.00g DGM	54.00g DGM	72.00gDGM
IMW	47.89±0.11^{a}	47.92±0.06^{a}	47.89±0.11^{a}	47.93±0.17^{a}	48.00±0.19^{a}
FMW	56.16±4.23^{a}	59.06±1.22^{a}	58.31±2.19^{a}	56.75±3.84^{a}	55.44±1.42^{a}
PER	0.25±0.12^{a}	0.33±0.04^{a}	0.33±0.07^{a}	0.29±0.12^{a}	0.30±0.06^{a}
CF	0.58±0.01^{a}	0.67±0.04^{ab}	0.70±0.03^{b}	0.65±0.10^{ab}	0.59±0.07^{ab}
FCR	2.15±0.92^{a}	1.46±0.14^{a}	1.55±0.28^{a}	1.94±0.78^{a}	2.05±0.33^{a}
NM	1399.58±55.55^{a}	1439.07±17.12^{a}	1428.44±30.86^{a}	1407.95±53.90^{a}	1391.37±20.85^{a}
WG	8.27±4.33^{a}	11.14±1.16^{a}	10.42±2.09^{a}	8.82±3.67^{a}	7.44±1.31^{a}
SGR	0.32±0.16^{a}	0.43±0.04^{a}	0.40±0.07^{a}	0.34±0.13^{a}	0.29±0.05^{a}
PWG	17.29±9.10^{a}	23.24±2.40^{a}	21.75±4.31^{a}	18.39±7.59^{a}	15.50±2.68^{a}
IL	16.20±0.00	16.20±0.00	16.20±0.00	16.20±0.00	16.20±0.00
FL	21.33±0.58^{a}	20.67±0.29^{ab}	20.33±0.29^{a}	20.67±0.73^{ab}	20.83±0.29^{ab}
LG	5.13±0.58^{b}	4.47±0.29^{ab}	4.13±0.29^{a}	4.47±0.76^{ab}	4.63±0.29^{ab}
SR	80.00	83.33	83.33	96.67	93.33

Means followed by different letters in the same rows are significantly different (P<0.05).
Please note that INW= initial mean weight, FMW= final mean weight, PER =protein efficiency ratio, CF=condition factor, FCR= feed conversion ratio, NM = nitrogen metabolism, WG =weight gain, SGR =specific growth rate, PWG =percentage weight gain, IL=initial length, FL= final length, LG= length gain, SR= survival rate

Table 6 : Haematology of Clarias gariepinus fed defatted G.mangostana- based diets at 0 and after 49 days

Parameters	Initial Value	Final values at Different DGM inclusion rates, %				
		Control	18gDGM	36g DGM	54g DGM	72gDGM
PCV (%)	26.00	32.00±1.41^{a}	32.00±1.41^{a}	30.00±2.83^{a}	30.00±1.41^{a}	34.00±1.41^{a}
Haemoglobin (g%)	8.00	10.00±1.41^{ab}	11.40±0.28^{b}	9.80±0.28^{ab}	9.6±0.14^{a}	11.30±0.14^{ab}
RBC x10^{12}/L	1.59	3.54±0.06^{b}	3.61±0.00^{b}	3.29±0.01^{a}	3.34±0.06^{a}	3.63±0.01^{b}
WBC x10^{9}/L	19425	19700±141.42^{d}	18100±141.42^{b}	25750±70.71^{e}	15900±70.71^{a}	18950±70.71^{c}
Platelet	107500	144000±5656.85^{c}	216000±2828.43^{d}	128000±1414.21^{a}	135900±212.13^{b}	128000±1414.21^{a}
Lymphocyte (%)	63.00	66.00±1.41^{a}	72.00±2.83^{b}	69.00±1.41^{ab}	71.00±1.41^{b}	71.00±1.41^{b}
Heterophil	34.00	29.00±1.41^{b}	22.00±2.83^{a}	23.00±1.41^{a}	19.00±1.41^{a}	22.00±2.83^{a}
Monocytes (%)	2.00	2.00±1.41^{a}	2.00±1.41^{a}	3.00±1.41^{a}	4.00±1.41^{a}	4.00±1.41^{a}
Eosinophils	1.00	3.00±1.41^{a}	4.00±1.41^{a}	5.00±1.41^{a}	6.00±1.41^{a}	3.00±0.00^{a}
Basophil	0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
MCHC (%)	30.00	31.38±5.81^{a}	31.58±0.79^{a}	32.77±2.15^{a}	32.03±1.04^{a}	33.15±0.82^{a}
MCH(Pg)	50.29	28.29±4.45^{a}	31.49±0.78^{a}	29.79±0.73^{a}	28.49±0.30^{a}	31.13±0.27^{a}
MCV(Fl)	163.28	90.38±2.56^{a}	94.18±3.92^{a}	91.17±8.19^{a}	86.06±0.36^{a}	93.66±3.53^{a}

*Means followed by different letters in the same rows are significantly different (p<0.05).*PCV=packed cell volume, RBC=Red blood cells, WBC=white blood cells, MCV = mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH = mean corpuscular haemoglobin

Table 7 : Blood biochemistry of Clarias gariepinus fed Defatted G.mangostana- based diets at 0 and 49 days

Parameter	Initial value	Final values at Different DGM inclusion rates				
		Control	18.00g DGM	36.00g DGM	54.00g DGM	72.00g DGM
Protein	3.3	3.90±0.14^{a}	3.50±0.14^{a}	3.90±0.14^{a}	3.80±0.14^{ab}	3.70±0.14^{ab}
Albumin	1.3	1.40±0.14^{a}	1.30±0.14^{a}	1.10±0.14^{a}	1.40±0.14^{a}	1.40±0.14^{a}

Globulin	2.0	2.50 ± 0.14^{ab}	2.20 ± 0.14^{a}	2.80 ± 0.14^{b}	2.40 ± 0.14^{a}	2.30 ± 0.14^{a}
AGR	0.65	0.60 ± 0.14^{a}	0.50 ± 0.14^{a}	0.30 ± 0.14^{a}	0.60 ± 0.14^{a}	0.60 ± 0.14^{a}
AST(IU/L)	35.5	33.00 ± 0.14^{a}	42.00 ± 0.14^{b}	36.00 ± 0.14^{a}	34.00 ± 0.14^{a}	40.00 ± 0.14^{b}
ALT(IU/IL)	20.5	26.00 ± 0.14^{b}	34.00 ± 0.14^{c}	29.00 ± 0.14^{b}	21.00 ± 0.14^{a}	32.00 ± 0.14^{c}

Means followed by different letters in the same rows are significantly different ($P<0.05$)

Table 8 : Tissue pathology of C. gariepinus post juveniles at 49 days after feeding on defatted G. mangostana-containing diets

Organs	Control	18g DGM	36g DGM	54g DGM	72gDGM
Gills	No visible lesions seen	No visible lesions seen	There is mild submucosal congestion	No visible lesions seen	No visible lesions seen
Kidney	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen	There is severe interstitial congestion
Liver	No visible lesions seen	There are severe vacuolations of the hepatocytes, with mild central venous congestion	There is diffuse hepatic vacuolation.	No visible lesions seen.	There is diffuse vacuolation of the hepatocytes, marked
Heart	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen

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· Present your points in sound order

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- Fundamental goal
- To the point depiction of the research
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Approach:

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- Center on shortening results - bound background information to a verdict or two, if completely necessary
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- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
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Approach:

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- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
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Approach:

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- Leave out information that is immaterial to a third party.

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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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- Present a background, such as by describing the question that was addressed by creation an exacting study.
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Approach

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- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
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	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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