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Evaluation OfProsopis Africana Seed Hydrolysates As Source Of Peptone For Microbial Culture Media

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Abstract: *Microbiological growth media developed from legumes as sources of protein hydrolysates was studied. This study was aimed at producing protein hydrolysates from Prosopis africana seed that could be used as a substitute to animal peptone for microbial culture. Prosopis africana seeds (2.5 kg) were obtained from a local market andselected seeds were washed with distilled water and boiled until the seed coats became soft. The softened seed coats were peeled off to obtain cotyledons which were washed, dried and powdered. The powdered cotyledon was subjected to enzymatic hydrolysis using papain, or acid hydrolysis using hydrochloric acid. Amino acid analysis was done using HPLC. Metal ion and vitamin contents of the hydrolysates were determined using standard methods. Growth patterns of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans and Aspergillusniger in the media formulated using the seed hydrolysates were monitored. Microbial growth patterns in the hydrolysates were compared with commercial samples. The analyses revealed that papain-hydrolysed Prosopis africana seed (PHP) ranked highest in terms of microbial growth support when compared with acid-hydrolysed Prosopis africana seed (AHP) and commercial peptone (CP). All the test organisms thrived well in media made with the hydrolysates, in comparison with commercially available media.*

Keywords: *Prosopis africana, hydrolysates, peptone, growth media*

I. Introduction

Studies on the use of legumes as sources of protein hydrolysate for production of microbial growth media are limited. Regrettably, such potentials of these abundant indigenous plants have been neglected, apparently due to a lack of empirical data. In West Africa, as in the other parts of the world, legumes are of great economic and nutritional value. *Prosopis africana* is a leguminous plant with such high values[1]. However, studies available on the seeds of *Prosopis africana*were limited to the microbial purity of seed hydrolysate. Bacillus species were isolated from the fermenting *Prosopis africana* cotyledons during the production of "okpeye", a food seasoning agent [2].The presence of a wide variety of bacterial species in the fermenting seeds is an indication that the seed hydrolysate could also be a source of protein (peptone) for microbial culture media. Microbial culture media are used for the purpose of isolating, studying and identification of microorganisms. In addition, culture media also provide a reliable means of estimating microbial growth as well as carrying out certain genetic manipulations in biotechnological advances. For a culture medium to be effective, it must contain all nutrients the microorganisms require for growth. The most important requirement of a culture medium is its ability to allow detectable growth from a minute inoculum within a reasonable period of incubation [3].By extension, the nutritional requirement that must be considered for microbial growth must provide sources of carbon, nitrogen and energy as well as other major mineral nutrients such as sulfur, phosphate, magnesium, calcium and trace elements [4].Peptones are protein hydrolysates that are soluble in water and are not heat coagulable [5].Most economical peptones are derived from products of bovine or porcine origin such as meat, internal organs, gelatin, and milk, as well as from certain plants and yeasts [6].Recent outbreaks of bovine spongiform encephalopathy and growing demand for raw materials that are approved and certified free of swine flu, have made peptones of non-meat origin to be increasingly important[7].Recent related researches[8] have established that peptones derived from soya beans and African locust beans supported the growth of the test organism and compared favourably well with commercial peptones. This shows that leguminous peptones contain the necessary nutrients required for microbial growth, especially the nitrogen requirement.

In view of these, it is now very pertinent that further studies and researches are carried out on more leguminous plants with the aim of exploring deeply to produce comparatively standard protein hydrolysates that can be used as alternative peptone for microbial culture in place of animal peptone. Production of alternative

plant peptone will bring about much economic benefits in the commercialization of peptones. Exploration of leguminous plants as sources of protein hydrolysates will possibly reduce the cost of peptone for research and industrial uses.This study therefore aims at evaluating the protein hydrolysates obtained from the seeds of Prosopis africana, as an alternative source of peptone for the formation of microbial culture media.

II. Materials And Methods

2.1 Materials

The materials used for this study were *Prosopis africana* seeds bought from AhoMbu, Isi-Uzo Local Government Area of Enugu State and duly identified by Miss OgechiUgwuaneke of Crop Science Department, University of Nigeria, Nsukka, Nigeria. Peptone (Guangdong Huanki, China), Papain (BDH Chemicals Ltd, Poole, England), Hydrochloric acid (BDH Chemicals Ltd, Poole, England), Sabouraud dextrose agar (Lab M Limited, United Kingdom), mannitol-salt agar (Basingstoke Hampshire, England) and MacConkey agar (Titan Biotech Ltd, India), glucose, mannitol, sucrose and lactose (Kernel Chemicals Ltd, UK), maltose (Laboratory Technology Chemicals Co. Ltd, UK). The organisms used were: *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Candida albicans*and*Aspergillusniger*. These were laboratory strains obtained from the Pharmaceutical Microbiology Department, University of Nigeria, Nsukka, Enugu State
All other reagents and materials used were of analytical grade.

2.2 Methods

2.2.1 Sample preparation

*Prosopis africana*seeds were purchased from a local market in Nigeria. The seeds were sorted to remove solid contaminants, and decaying seeds. The selected seeds were washed in de-ionized water and then boiled for 8 hours until the seed coats became soft and swollen. The seed coats were manually peeled off leaving behind the clean cotyledons. The cleaned cotyledons were dried in an oven at 40 ^{o}C for 12 hours, weighed and pulverized using a Kenwood blender (England). The resulting powder was kept in an air-tight container and stored at 4 ^{o}C prior to further procedures.

2.2.2 Acid hydrolysis

Two hundred grams of the *Prosopis africana* seed flour was soaked in 250 ml of 6 M HCl. The mixture was incubated at 80 ^{o}C for 24 hours. At the end of the incubation period, the broth was diluted with 500 ml of de-ionized water and then boiled at 100 ^{o}C for 1 hour. The solution was then cooled to room temperature, and the pH adjusted to 7.0 with 6 M NaOH. The mixture was filtered through Whatman's No.1 filter paper. The filtrate was lyophilized using a freeze-dryer (Amsco/Finn-Aqua Lyovac GT3, Germany) to yield acid-hydrolysate of Prosopis africana seed (AHP).

2.2.3 Papain hydrolysis

Two hundred grams of powdered *Prosopis africana* seed was weighed and suspended in 800 ml of distilled water. This was stirred to form slurry. The pH of the slurry was adjusted to 6.5 with 1 M HCl. Papain (BDH Chemical Ltd, Pools, England), was added to the slurry to make a concentration of 0.25 %. Digestion was allowed to take place for 9 h at a temperature of 60 ^{o}C, and the pH was controlled using 1 M NaOH. The temperature was then raised to 80 ^{o}C for 15 minutes to inactivate the enzyme. Liquor from the hydrolysed material was separated from the protein/enzyme slurry by repeated centrifugation. The clear solution was then freeze-dried to obtain papain-hydrolysate of *Prosopis africana* seed (PHP).

2.2.4 Determination of amino acids

The analysis of the amino acids was done on Water 616 /626 LC (HPLC) instrument.

The sample preparation and determination were carried out in the following stages:

(i) Hydrolysis
(ii) Derivatisation
(iii) Separation of the derivatised amino acids
(iv) Data processing, interpretation and calculations of the final results

Step (i) Hydrolysis of the samples:

Five hundred milligram (500 mg) of the sample was weighed into a sterile furnace hydrolysis tube. Five nmolsnorleucine was added to the sample and then dried under a vacuum. The tube was then again placed in a vial containing 10.05 N HCl with a small quantity of phenol, thereby hydrolyzing the protein by the HClvapour under vacuum. This stage of hydrolysis of the sample lasted for between 20 – 23 h at 108 ^{o}C. After the hydrolysis, the sample was dissolved in ultra-pure water (HPLC) grade, containing ethylene diamine tetra

acetic acid (EDTA). The EDTA chelates the metal present in the samples. The hydrolysed samples now are stored in HPLC amino acid analyzer bottles for further analytical operations.

Step (ii): Derivatisation: The hydrolysed sample was derivatised automatically on the HPLC by reacting the amino acids, under appropriate condition, with phenylisothiocyanate (i.e. PITC) to get phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this reaction is 45 minutes per sample, as calibrated on the instrument.

A set of standard solutions of the amino acids were prepared. These standards (0.0, 0.5, 1.0, 1.5, 2.0 µmol) were used to generate a calibration file that was used to determine the amino acids contents of the samples. After the derivatisation, a methanol solution (1.5 N) containing the PTC-amino acid was transferred to a narrow bore in HPLC system for separation.

Step (iii): The HPLC separation & quantization

The separation and quantitation of the PTC-amino acids were done on a reverse phase (18 silica column and the PTC chromophores were automatically and digitally detected at the wavelength of 254 nm. The elution of the whole amino acids in the samples took 30 min. The buffer system used for separation was 140 M sodium acetate, pH 5.50, as buffer A and 80 % acetonitrile as buffer B. The program was run using a gradient of buffer A and buffer B concentrations and ending with a 55 % buffer B concentration at the end of the gradient.

Step (iv): Data interpretation and calculations

The intensity of the chromatographic peaks areas were automatically and digitally identified and quantified using a Dionexchromeleon data analysis system which is attached to the HPLC system.

The calibration curve or file prepared from the average values of the retention times (in minutes) and areas (in mm^2) at the amino acids in 5 standards runs was used. For a known amount of each amino acid in the standard loaded into the HPLC, a response factor (mm^2/pmol) was calculated by the software that was inter-phased with the HPLC. This response factor was used to calculate the amount of each of the amino acid (in pmols) in the sample and displayed on the system digitally. The amount of each amino acid in the sample is finally calculated by the software by dividing the intensity of the peak area of each (corrected for the differing molar absorptivity of the various amino acids) by the internal standard in the chromatogram and multiplying this by the total amount of internal standard added to the original sample. After the picomole of each amino acid has been ascertained by the software, the digital chromatographic software extrapolates back to the internal standard (Norleucine), and displays for the total amount that was pipetted into the hydrolysis tube at the beginning of the analysis.

Calculation

mg/ml (in extract) = Dilution factor x Peak height intensity

$$\text{mg/ml (in sample)} = \frac{\text{µg/ml in extract} \times \text{sample volume}}{\text{Weight of sample}}$$

2.2.5 Determination of vitamins

Determination of vitamin B_1 (Thiamine)

Five gram of the sample was homogenized in 50 ml ethanolic sodium hydroxide and filtered. Its 10 ml filtrate was added to 10 ml potassium dichromate and the absorbance was recorded at 360 nm in a spectrophotometer after the development of colour. The absorbance obtained from the sample extract was converted to thiamin concentration by means of calibration curve generated using standard concentrations [9].

$$\text{Vitamin B1 content} = \frac{\text{Standard vitamin B1}}{10\ \text{mg/100 g}} - \frac{\text{Absorbance}}{0.025}$$

Determination of vitamin B_2 (Riboflavin)

Five grams of sample was extracted with 100 ml of ethanol for 1 h. To 10 ml of the filtered extract was added 10 ml of 5 % potassium permanganate and 10 ml of 3 % hydrogen peroxide and it was allowed to stand on hot water bath for 30 minutes. To this was added 2 ml of 40 % sodium sulphate. The volume was made up to 50 ml with distilled water. The sample was centrifuged at 1500 rpm and the supernatant taken for spectrophotometric reading at 510 nm. The absorbance obtained from the sample extract was converted to riboflavin concentration by means of calibration curve generated using different standard concentrations [9].

$$\text{Vitamin B2 content (mg/100 g)} = \frac{\text{Standard Vitamin B2}}{10\ \text{mg/100 g}} - \frac{\text{Absorbance}}{0.626}$$

Determination of vitamin B_3 (Niacin)

Five grams of the sample was treated with 50 ml of 1 M sulphuric acid for 30 minutes. Then 0.5 ml ammonia solution was added to it and then filtered. To 10 ml of the filtrate was added 5 ml of 0.5 % potassium cyanide. This was further acidified with 5 ml of 0.02 M sulphuric acid. The absorbance of the resultant solution was recorded at 420 nm. The absorbance obtained from the sample extract was converted to niacin concentration by means of a calibration curve generated using different standard concentrations [9].

$$\text{Vitamin B3 content (mg/100 g)} = \frac{\text{Standard Vitamin B3}}{3.23\ \text{mg/100 g}} - \frac{\text{Absorbance}}{0.090}$$

Determination of vitamin B_6 (Pyridoxine)

One gram of the sample was weighed separately into a 100 ml conical flask and extracted with 10 ml of 0.1 M HCl with vigorous shaking for 10 minutes. The sample was filtered through Whatman's No. 1 filter paper. The filtrate was then made up to 10 ml with distilled water. Five milliliters of the slightly acidic filtrate is treated with 1 ml of 0.04 % ferric chloride. The optical density of the resultant brown solution was measured in a spectrophotometer at 450 nm. The absorbance obtained from the sample extract was converted to pyridoxine concentration by means of a calibration curve generated using different standard concentrations [9].

$$\text{Vitamin B6 content (mg/100 g)} = \frac{\text{Standard Vitamin B6}}{0.25\ \text{mg/100 g}} - \frac{\text{Absorbance}}{0.05}$$

Determination of vitamin B_{12} (Cyanocobalamin)

One gram of the sample was weighed into a flat-bottom flask and 50 ml of 0.1 M HCl was added to it. The flask was thoroughly swirled and allowed to stand for 2 h. The mixture was then filtered and 10 ml aliquot taken in a test tube for spectrophotometric reading. A volume 0f 0.5 ml of 0.2 % ferric chloride in ethanol and 0.5 ml of 0.5 % alpha-alpha dipyridyl were added to the sample in the presence of acetate buffer (5 ml) which was read within 10 minutes in a spectrophotometer at 550 nm [10].

$$\text{Vitamin B12 content (mg/100 g)} = \frac{\text{Standard Vitamin B6}}{50\ \text{mg/100 g}} - \frac{\text{Absorbance}}{0.108}$$

Determination of vitamin C (Ascorbic acid)

Five grams of the sample was weighed into a flat-bottom flask and 60 ml TCA/acetic acid solution was added. The mixture was left for about an hour before it was filtered. The filtrate was made up to 200 ml with distilled water. A volume of 10 ml was taken for titration with 0.005 % of 2, 6-dichlorophenols [11]. The vitamin C content was calculated as follows:

$$\text{K} = \frac{\text{Y} \times \text{Z} \times \text{DF}}{\text{Weight of sample (g)}}$$

where Y = Titre value; Z is the figure obtained when 50 g of the standard vitamin C is divided by its titre value, and DF: Dilution factor.

Determination of vitamin A

One gram of the sample was weighed. Then the proteins in the weighed sample were first precipitated with 3 ml of absolute ethanol before the extraction of vitamin A with 5 ml of heptane. The test tube containing this was shaken vigorously for 5 minutes. On standing, 3 ml from the heptane layer was taken up in a cuvette and read at 450 nm against a blank of heptane. The standard was prepared following same procedure, and read at 450 nm [12].

Determination of Vitamin E (Tocophenrol)

One gram (1 g) of the sample was extracted with 50 ml of petroleum ether and evaporated to dryness. The residue was saponified with 5 ml of 0.1 M potassium hydroxide under reflux. Twenty milliliters (20 ml) of petroleum ether was used to extract the unsaponifiable matter and evaporated to dryness. Twenty milliliters(20 ml) of ethanol was added to dissolve the residue. One milliliter (1 ml) was transferred into 3 separate test tubes to which was added 1 ml of 0.2 % ferric chloride in ethanol and 1ml of 0.5 % α-dipyridyl and was made up to 5 ml with ethanol. The absorbance was taken at 520 nm wavelength [9].

Calculation:$\%\ \text{Vitamin E} = \frac{\text{Absorbance of test} \times \text{concentration of standard} \times \text{DF}}{\text{Absorbance of standard} \times \text{Weight of sample (g)}} \times\times \frac{100}{1}$

2.2.6 Determination of metals

Calcium

Five grams of sample was dissolved in 100 ml of distilled water. Ten milliliters (10 ml) of the sample solution was pipetted into a 250 ml of 0.5 M KOH in a conical flask and 25 ml of water was added and finally a pinch of calcine indicator was added. The mixture was titrated against 0.1 M EDTA solution to an end point. The volume of the EDTA is equivalent to volume of calcium in the solution [13].
Calculation:

$$\% \text{ Calcium } = \frac{\text{Vol. EDTA} \times \text{ Mol. EDTA} \times \text{Atomic weight of Ca } \times 100 \times \text{DF}}{1000 \times \text{ Weight of sample}}$$

Magnesium

The sample solution was prepared by dissolving 5 g of samples each (PAH and CP) in 100 ml of distilled water. Ten milliliters (10 ml) of the sample solution was pipetted into a 250 ml conical flask. About 25 ml of NH_3-NH_4 buffer solution at the pH of 7.2 was added to it. Twenty–five millilitres (25 ml) of water was added to the mixture followed by 2-3 drops of Eriochrome Black T indicator and titrated against 0.1 M EDTA solutions. The volume of EDTA used was the volume equivalent of magnesium in the mixture [14].
The volume of magnesium = (Volume of Calcium and Magnesium – Volume of Calcium)

$$\% \text{ Mg} = \frac{\text{Vol. EDTA} \times \text{ Mol. EDTA} \times \text{Atomic weight of Mg} \times \text{DF}}{1000 \times \text{Weight of sample (g)}} \times \frac{100}{1}$$

where DF = Dilution factor

Iron and Zinc

Two milligrams of the sample was weighed into a crucible and ashed in a muffle furnace at 555 ^{o}C for 6 hours. The ash was cooled and 6 ml of 1 M HCl added and boiled for 10 min, while covering the crucible with a watch glass. After boiling, the sample was cooled and filtered into 100 ml volumetric flask. The crucible was washed with distilled water and ash filtrate was added. The ash filtrate was made up to 100 ml with distilled water. An aliquot of the filtrate was aspirated into atomic absorption spectrophotometer (PYE UNICAM, England) and the absorbance values of the different minerals read. Standard solutions of iron and zinc were prepared and aspirated into the atomic absorption spectrophotometer and their absorbance values were recorded. The percentage of element present was calculated from the absorbance values of the samples and standard solutions [14].

Copper

This was determined using [14] method. One gram of the sample was weighed into a digestion flask and 20 ml of acid mixture (650 ml conc. HNO_3; 80 ml of perchloric acid (PCA); 20 ml conc. H_2SO_4) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to 500 ml mark. A stock solution of 100 mg/ml of Cu^{2+} ions was prepared by dissolving 2.682 g of $CuCl_2$. $2H_2O$ in de-ionized water and finally diluted to 1000 ml. Standard concentration of 0.5 ppm was prepared from this stock solution and absorbance was taken at 360 nm.

Sodium and potassium

Two grams (2 g) of the sample was weighed into a digestion flask and 20 ml of acid mixture (650 ml of conc. HNO_3, 80 ml PCA, and 20 ml conc. H_2SO_4) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to 500 ml. An aliquot of the sample digest was taken for photometry using Flame analyzer. Absorbance for sodium was read at 767 nm while that of potassium was read at 589 nm. Sodium and potassium concentrations are obtained from the calibration curves obtained from the standard [14].
Calculation:

$$\text{Ppm} = \frac{\text{Gf} \times \text{Total volume of extract} \times \text{Ab} \times \text{DF}}{\text{Weight of sample (g)}}$$

where DF = Dilution factor; Gf = Gram factor, and Ab = Absorbance.

Manganese

A volume of 2 ml of phosphoric acid was added to 2 ml of the sample (prepared by dissolving 2 g of sample in 50 ml of distilled water). Appropriate amount of 0.3 g potassium periodate was added and the beaker was covered with a watch glass. The solution was boiled on a hot plate and simmered gently for 5 minutes. The beaker was allowed to stand on a boiling water bath for 30 minutes. The solution was allowed to cool and transferred to a 50 ml graduated flask and diluted to 50 ml. The reading of the absorbance was taken at 525 nm [9].

Mercury

This was determined using titrimetric method. Two grams of the sample was digested by wet-ashing with perchloric acid and nitric acid in the volume of 6:3 and made up to 100 ml with distilled water. Twenty five milliliters aliquot was measured into a conical flask and 2 ml of dithizone was added. This was acidified by adding 10 ml of acidified sodium nitrite. The solution was titrated with 0.001 M dithizone solution. Titration continued until colour change occurred.

Calculation

$$\text{Mercury} = \frac{\text{T} \times \text{concentration} \times \text{TV} \times 96}{\text{Weight of sample} \times \text{aliquot}} \times \frac{100}{1}$$

where T: Titre value; TV: Total volume obtained after wet-ashing, and Concentration: Concentration of dithizone solution used for titration.

2.2.9 Microbial growth evaluation

Microbial growth media were formulated using either commercially available peptone (1 %) or peptone hydrolysates from *Prosopis africana* seed (1 %). The pH range of the peptone hydrolysate media was 6.8 to 7.2. The solutions were dispensed as 50 ml aliquots into a 250 ml Erlenmayer flask and autoclaved at 121 ^{o}C for 15 minutes. A volume of 9.9 ml of each formulated medium was distributed into five test tubes in which the different test organisms were placed. An inoculum of 0.1 ml of 10^5 colony forming units (0.5 McFarland's standard) of each test organism was introduced into the test tubes and the culture was incubated at 30 ^{o}C on a rotary shaker (150 rpm) for 24 hours. The absorbance of the biomass was recorded at 650 nm using a spectrophotometer (Model 752, China). These procedures were repeated for varied PAH and CP concentration of 2, 3, 4 and 5 mg % respectively. For solid media, the molten agar media containing the seed hydrolysate or commercial peptone were also added to five separate petri dishes. The contents were thoroughly mixed and allowed to solidify. An inoculum of 0.1 ml of 10^5 colony forming units of each test organism was streaked on the media, and incubated at 37 ^{o}C for 24 hours for bacteria and 25 ^{0}C for 72 hours for the fungi.

2.3 Statistical analyses

Mean and standard deviations were calculated for the result generated using Microsoft excel 2013.

III. Results

3.1 Yield of the hydrolysate

For AHP, the weight of the freeze-dried sample was 104 g and the percentage yield was 52 %. For PHP, the weight of the lyophilized hydrolysate was 132 g and its percentage yield was 66 %.

Table 1: Amino acid composition of PHP, AHP and CP

	Media		
	PHP (%)	AHP (%)	CP (%)
Essential amino acids			
Arginine	0.229±0.02	0.236±0.01	0.903±0.01
Histidine	0.393±0.03	0.406±0.03	1.551±0.002
Leucine	0.354±0.001	0.262±0.01	0.735±0.02
Isoleucine	1.607±0.002	0.450±0.03	1.262±0.01
Lysine	2.632±0.02	1.948±0.01	5.470±0.01
Methionine	0.912±0.01	0.075±0.03	1.210±0.03
Phenylalanine	0.914±0.03	0.158±0.01	0.744±0.004
Threonine	3.354±0.004	0.262±0.01	7.735±0.03
Tryptophan	0.165±0.01	0.218±0.03	0.647±0.01
Valine	0.529±0.01	0.236±0.001	0.903±0.01
Non-essential amino acids			
Alanine	1.703±0.02	1.758±0.01	6.719±0.002
Aspartic acid	0.066±0.01	0.068±0.02	2.258±0.02
Asparagine	2.138±0.001	0.143±0.04	1.545±0.01
Glutamic acid	0.102±0.002	0.106±0.003	0.430±0.03
Glutamine	0.576±0.003	0.763±0.001	0.514±0.02
Glycine	0.576±0.01	0.763±0.01	5.514±0.003
Proline	0.990±0.03	1.311±0.005	0.883±0.02
Serine	4.289±0.01	5.679±0.02	3.827±0.05
Tyrosine	0.158±0.05	0.177±0.03	1.328±0.01

Cystine	0.348±0.01	0.461±0.05	0.311±0.001

PHP: Papainhydrolysed*Prosopis africana* seed, AHP: Acidhydrolysed*Prosopis africana* seed, CP: Commercial peptone

Table 2: Vitamin compositions of PHP, AHP and CP

Vitamins	PHP	AHP	CP
Vitamin B1 (mg/100 g)	8.00±0.02	0.182±0.02	4.44±0.01
Vitamin B2 (mg/100 g)	0.684±0.01	0.023±0.02	1.04±0.01
Vitamin B3 (mg/100 g)	9.556±0.01	0.405±0.03	7.46±0.02
Vitamin B6 (mg/100 g	1.367±0.02	0.088±0.01	1.06±0.01
Vitamin B12 (mg/100 g)	0.616±0.03	0.094±0.01	0.146±0.02
Vitamin C (%)	2.20±0.01	0.802±0.01	=
Vitamin A (IU/100 g)	70.922±0.02	7.751±0.01	0.08±0.03
Vitamin E (mg/100 g)	0.86±0.05	0.37±0.03	0.11±0.01

PHP: Papainhydrolysed*Prosopis africana* seed, AHP: Acidhydrolysed*Prosopis africana* seed, CP: Commercial peptone

Table 3: Metal ions composition of PHP, AHP and CP

	Media		
Minerals (mg/100g)	**PHP**	**AHP**	**CP**
Calcium	10.15±0.003	4.239±0.02	68.80±0.01
Magnesium	26.05±0.001	3.186±0.03	32.64±0.004
Zinc	2.31±0.04	1.783±0.01	1.70±0.006
Iron	4.38±0.01	5.140±0.02	5.25±0.007
Copper	5.01±0.02	2.416±0.003	0.11±0.10
Potassium	48.17±0.01	36.423±0.002	16.00±0.05
Sodium	31.64±0.04	28.598±0.01	18.42±0.03
Manganese	2.03±0.02	1.371±0.003	2.11±.0.03
Mercury	0.42±0.03	0.607±0.002	0.12±0.01

PHP: Papainhydrolysed*Prosopis africana* seed
AHP: Acidhydrolysed*Prosopis africana* seed
CP: Commercial peptone

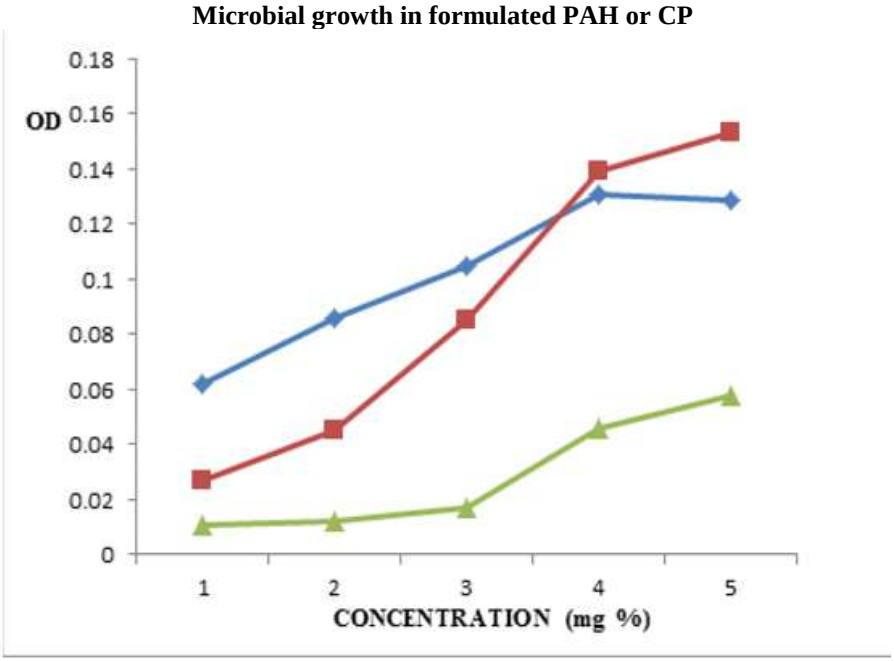

Fig. 1: Growth of *Staphylococcus aureus* in PAH and CP at 24 hours of incubation

Key:

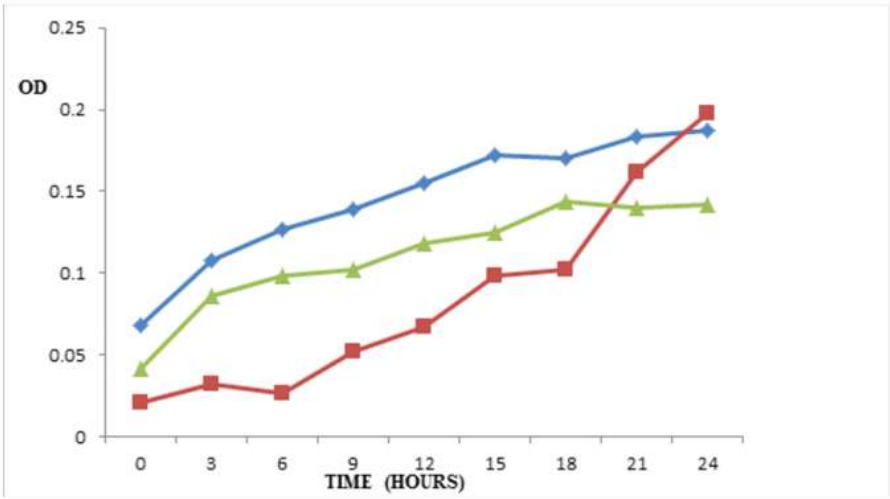

Fig. 2: Growth curve of Staphylococcus aureus in 4 mg % concentration of PHP, AHP or CP

Key: PHP AHP CP

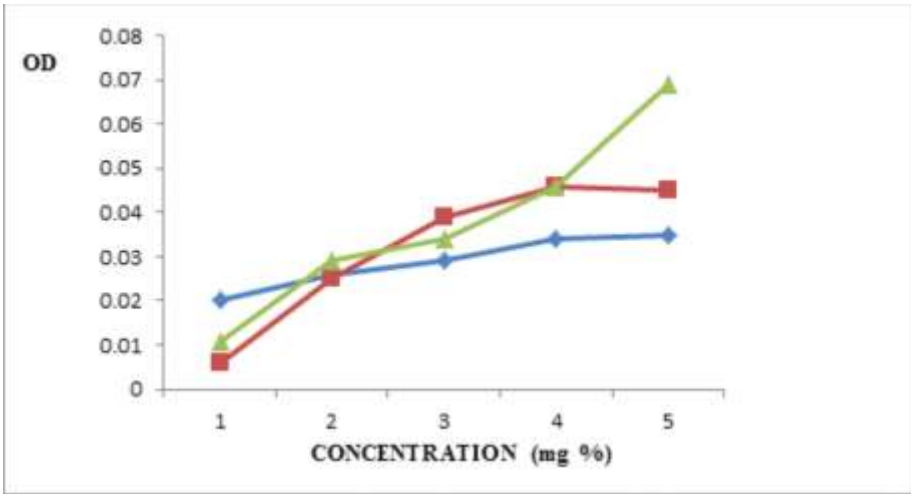

Fig. 3: Growth of *Escherichia coli* in PAH and CP at 24 hours of incubation

Key: PHP AHP CP

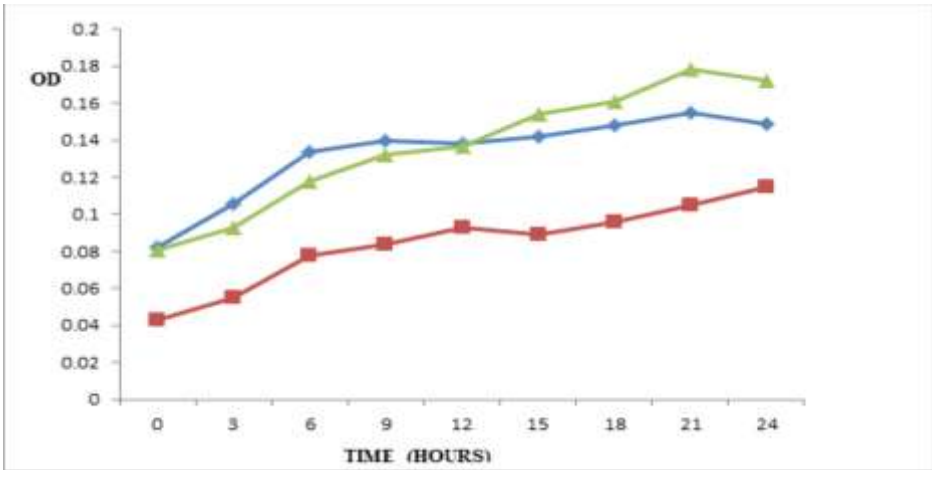

Fig. 4: Growth curve of *Escherichia coli*in 4 mg % concentration of PHP, AHP or CP

Key: PHP AHP CP

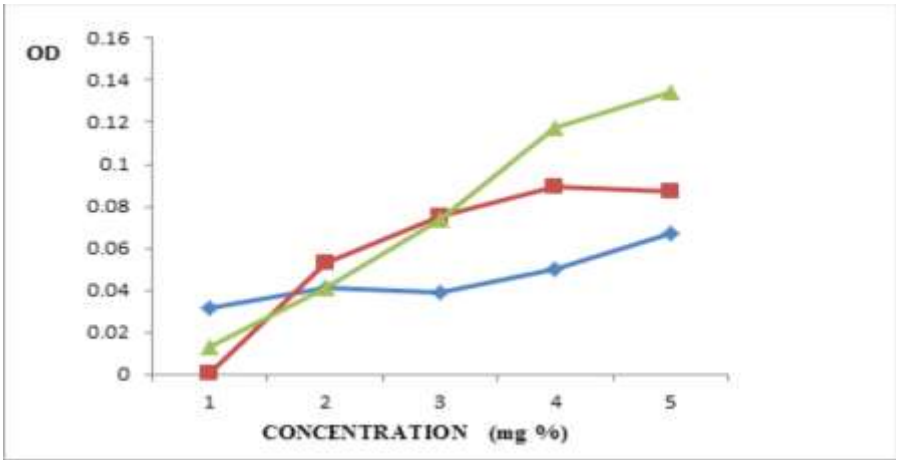

Fig. 5: Growth curve of *Pseudomonas aeruginosa* in PAH and CP at 24 hours of incubation

Key: PHP AHP CP

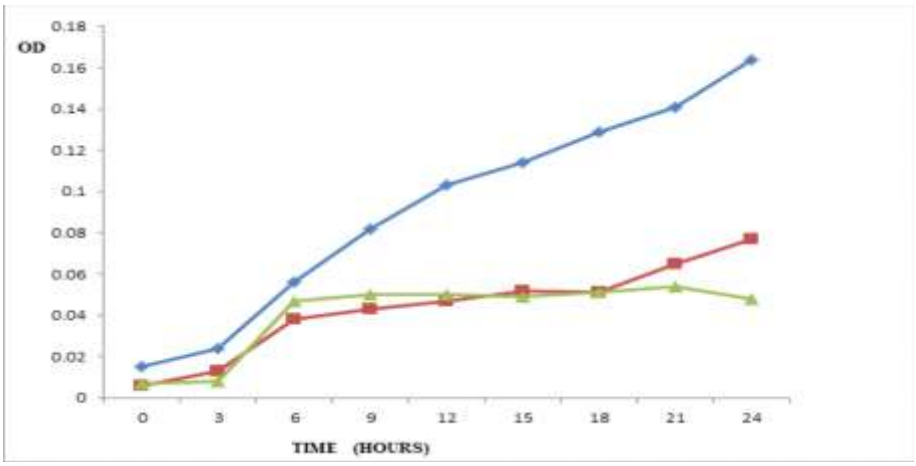

Fig. 6: Growth curve of *Pseudomonas aeruginosa*in 4 mg % concentration of PHP, AHP or CP

Key: PHP AHP CP

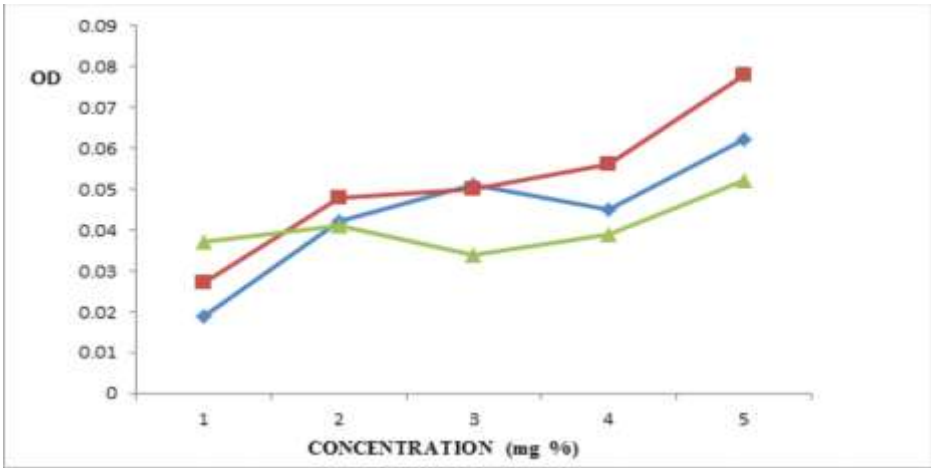

Fig. 7: Growth of *Candida albicans* in varied concentration of PAH and CP at 24 hours of incubation

Key: PHP AHP CP

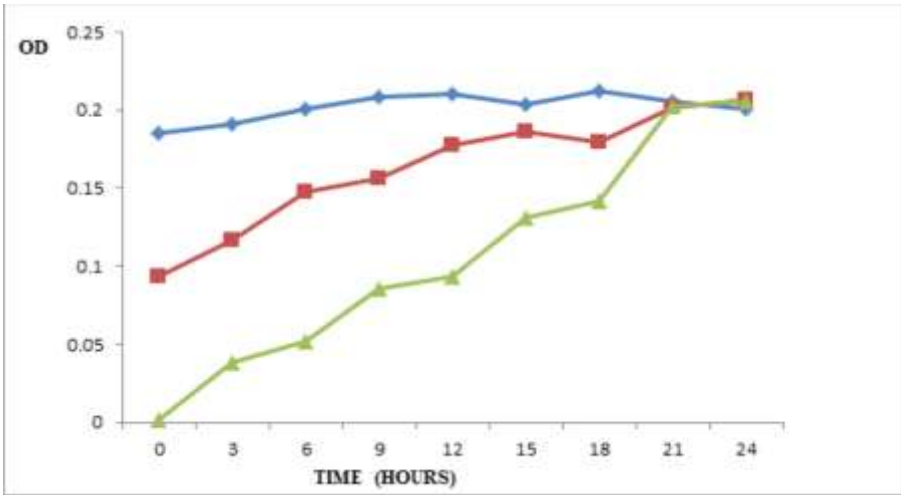

Fig. 8: Growth curve of Candida albicans in 4 mg % concentration of PHP, AHP or CP

Key: PHP AHP CP

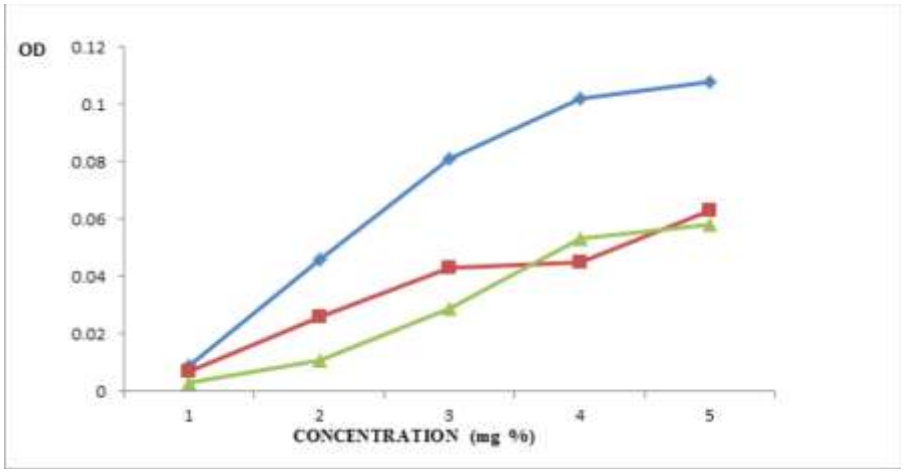

Fig. 9: Growth of Aspergillusniger in PAH and CP at 72 hours of incubation

Key: PHP AHP CP

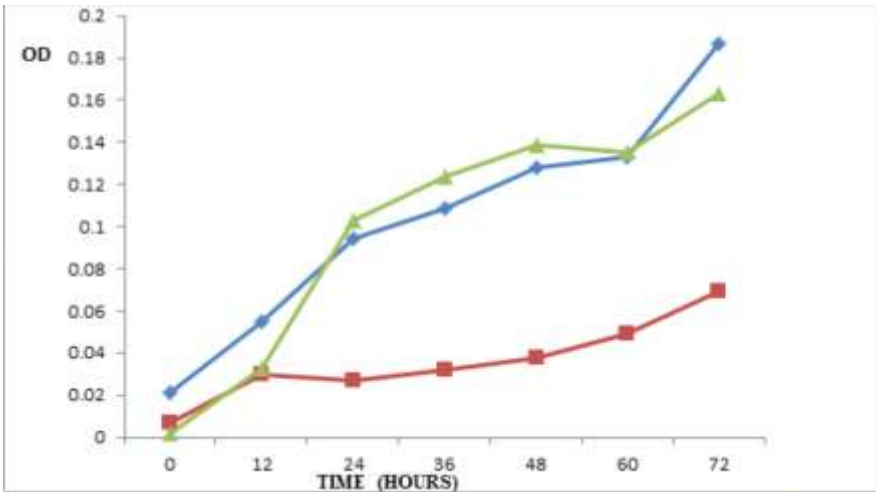

Fig. 10: Growth curve of *Aspergillusniger*in 4 mg % concentration of PHP, AHP or CP

Key: PHP AHP CP

IV. Discussion

Fig. 1 shows the growth of *S. aureus* in the various media tested. It is apparent that microbial growths in the media were concentration dependent. However, media prepared with PHP or AHP were found to produce better growth of the organisms than media made with CP after a cumulative incubation period of 24 hours. In addition, when the growth of *S. aureus* was monitored at regular time intervals, there was no significant difference in the growth rate pattern due to the various media (Fig. 2). Similar results were obtained for other bacteria strains (Figs. 3-6), with only slight variations. In specifically comparing the effectiveness of the two test hydrolysates (PHP and AHP), it was noticed that in most situations, the degree of growth support produced by AHP was less than that caused by PHP. Comparable findings have been recorded in other related studies. In a study for the development of fish peptone as nitrogen source for *Staphylococcus aureus*,it was observed that the mode of hydrolysis has considerable effects on the performance of the resulting peptone[15]. Enhanced activity associated with peptones produced by enzymatic hydrolysis can be explained by the fact that enzymatic hydrolysis is largely complementary to bacterial peptide uptake [16]. The relatively lower level performance of the AHP in supporting bacterial growth may also be due to extensive breakdown of proteins and peptides into component amino acids in the process of acid hydrolysis.

The nutritional requirements of microbial species may also affect their growth response in various media. It appears that concentrate of a hydrolysate in the medium influences bacterial growth rate. Concentration of ram horn hydrolysate beyond an optimum level produced an inhibitory effect on microbial growth [17]. It is known that certain procedures, such as overheating of media during autoclaving, can yield toxic oxygen species [18]. Additionally, storage condition and age of media constituents are known to influence medium performance.

The amino acid composition of microbial growth media is a clearly dominant factor in the performance of the medium [19]. Table 1 represents the amino acids of *P. africana* seeds as determined by either acid or enzyme hydrolysis. The presence of branched-chain amino acids such as leucine, valine and isoleucine, are essential for the growth of the test organisms [20]. Such other micronutrients-vitamins and minerals (Tables 2 and 3) present in the hydrolysates have also contributed to the observed growth pattern of the organisms.

It is noteworthy that the *P. africana* hydrolysates also supported the growth of *Candida albicans* (yeast) and *Aspergillusniger* (fungus) in equally concentration-dependent manners (Figs. 7-9). In all cases the effects were comparable with the commercial peptone. Similar reports have been recorded elsewhere, using a variety of other materials as sources of peptone for microbial growth [21- 23]. There is therefore, overwhelming evidence that alternative sources of peptone, from non-animal sources, are abundantly available, and such great potentials lie also in the seed hydrolysate of *P. africana*.

V. Conclusion

Results from this study have shown that peptone derived from *Prosopis africana* seed hydrolysates, can compare favourably with commercially available peptone for supporting the growth of test organisms. It is our submission that proper manipulations of in vitro enzyme hydrolysis will result in high grade peptones rich in peptides and polypeptides, which can further be hydrolysed by microbial enzymes for better growth effect. Acid hydrolysis breaks down proteins into their component amino acids and, being a harsh process, some amino acids are lost and salt formation is also inevitable. Prosopis africana seed hydrolysates have, therefore, added to the number of leguminous peptones available for use in microbial media. This product can be used in enrichment media or as a basic nutrient medium. In consideration of the impressive way in which it has supported the growth of the test organisms, it is believed that the production of this peptone will serve as alternative source of peptone, for growth of a wide variety of microorganisms, including bacteria, yeast and fungi.

VI. Declaration of interest

The authors report no conflicts of interest in the work.

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