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THE EFFECTS OF SOLVENT EXTRACTS OF *CITRULLUS LANATUS* SEEDS AND *CHRYSOPHYLLUM ALBIDUM* COTYLEDONS ON RAT HEPATOCYTE REGENERATION, CYTOCHROME C OXIDASE ACTIVITIES AND BACTERIOCIN-PRODUCING GRAM-NEGATIVE BACTERIA

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Abstract

Medicinal plants have been shown to exert a plethora of biological and pharmacological which has been associated with the presence of several bioactive components. In this study, the effects of crude extracts of *Citrullus lanatus* seeds (CACL) and *Chrysophyllum albidum* cotyledons (CACA) on rat hepatocyte regeneration, liver cytochrome c oxidase activities, and their antimicrobial properties on a few bacteriocin-producing Gram-negative bacteria were determined. CACL and CACA were screened for the presence of amino acids using TLC sprayed with ninhydrin, pyridine and modified Commassie brilliant blue (G250). The amino acids were elucidated by Gas Chromatography-Mass Spectrometry (GC/MS) and Fourier-transform infrared spectroscopy (FTIR). CACA enhanced tissue regeneration in rat hepatocytes, *in situ* and increased the collagen content. CACA also enhanced the activity cytochrome c oxidase in the liver mitochondrial portion, but inhibited same in the homogenate. CACA and CACL did not inhibit the bacteriocin-producing bacteria at the different concentrations tested. It is evident that CACA and CACL contain amino acids that can improve liver antioxidant functions and its mitochondrial electron transport. Both amino acid-rich fractions, combined with bacteriocin may accelerate aseptic wound healing.

Keywords: Tissue regeneration, catalase, cytochrome c oxidase, seed amino acids, *Citrullus lanatus* seeds, *Chrysophyllum albidum* cotyledons, bacteriocin-producing bacteria.

Introduction

Naturally occurring amino acids are proteinogenic and are useful as neurotransmitters, components of connective tissue, biosynthetic precursors, and lipid transport [1]. They are important in nutrition, tissue repair and growth plausible reasons they are considered useful nutritional supplements [2] and sources of ionic bio-energetic materials [3]. Seeds of *Citrullus lanatus* (CACL) and cotyledons of *Chrysophyllum albidum* (CACA) may be a good source of amino acids that can improve nutritional quality of the human diet, bioenergetic functions and tissue repair especially in combination with the bacteriocin from the tested bacteria to accelerate aseptic wound healing. Unveiling the molecular targets of these amino acids has the potential for identification of novel sources of functional foods for cellular health.

Plants have evolved a variety of mechanisms to combat pathogen attacks. These include both active and passive responses. Examples of such active responses are production of reactive oxygen species, secondary metabolites (phytoalexins), hydrolytic enzymes [4], antimicrobial proteins and peptides [5, 6]. Passive strategies can include physical barriers to pathogen invasion such as thick cuticles and cell walls. Many antimicrobial peptides (AMPs) and cyclopeptides have been identified in plants. These peptides are highly divergent at the primary sequence level and vary in their hierarchical structures. Some common biochemical features include the ability to form disulfide bonds, tandemly repeated amino acid sequences and a net charge at pH 7 [7]. Peptides are biologically occurring short chains of amino acid monomers linked by peptide (amide) bonds. All peptides except cyclic peptides have an N-terminal and C-terminal residue at the end of the peptide. Peptides are distinguished from proteins on the basis of size, and as an arbitrary benchmark can be understood to contain approximately 50 or fewer amino acids [8]. All peptides except cyclic peptides have an N-terminal and C-terminal residue at the end of the peptide.

Proteins consist of one or more polypeptides formed from amino acids arranged in a biologically functional way, often bound to ligands such as coenzymes and cofactors, or to another protein or other macromolecule (DNA, RNA, etc.), or to complex macromolecular assemblies [9]. Common features of these peptides are their small size and that they are synthesized in the ribosomes. Peptide fragments are used to identify or quantify the source protein [10]. These amino acid-rich peptides are large group of low molecular weight natural compounds that exhibit antimicrobial activity and have been isolated from animals and plants during the past few decades. Among them, cationic peptides are the most frequently studied. Interestingly, the variety and diversity of these peptides seem to be much wider than suspected. In fact, novel classes of peptides with varying chemical properties continue to be isolated from different vertebrate and invertebrate species, as well as from bacteria [11-14]. To the early characterized peptides, mostly cationic in nature, anionic peptides, aromatic dipeptides, processed forms of oxygen-binding proteins and processed forms of natural structural and functional proteins. Reactive oxygen species which cause oxidative stress if unchecked, are involved in chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease, immune dysfunction and ageing [15,16] as well as cardiovascular disease and hearing impairment via cochlear damage [17,18]. Antioxidant defense systems scavenge, and minimize the formation of reactive oxygen species but they are not 100% effective. Hence, repair systems exist to deal with molecules that have been oxidative damaged [19]. There is a growing search for functional foods that can serve as sources of various natural nutraceuticals and hormetins that can interactively and synergistically trigger endogenous antioxidant systems to neutralize free radicals [20].

Citrullus lanatus (popularly called water melon, "Elegede ajara" in Yoruba) is an annual scrambler belonging to the Cucurbitaceae family, and *Chrysophyllum albidum* (popularly called African Star Apple, "Agbalumo" in Yoruba) is a perennial shrub belonging to the Sapotaceae family. They are both rich in vitamin C and fibre, and are mostly eaten raw as snacks. While watermelon can also be eaten as salad or made into juice, only recently has the African star apple been used in juice, blended with other succulent fruits or with an alcoholic beverage. In folklore, the seeds of *Citrullus lanatus* are used for medicinal purposes including treatment of gall

stone [21] while the cotyledons of *Chrysophyllum albidum* are used for the treatment of vaginal and dermatological infections in Western Nigeria [22]. Many seeds and cotyledons, although underutilized, are edible and form part of human calories [23]. The seeds bearing the cotyledons have been identified to store several lipids, poly-unsaturated fatty acid and protein, and are used in the production of condiments [24, 25]. Plant seeds and cotyledons, like other plant parts also possess secondary metabolites such as polyphenol which are associated with high antioxidant activities [26]. Peptides are biologically occurring short chains of amino acids monomers linked by amide bonds. Some of them form a class of peptides described as antimicrobial peptides (AMPs) found in different natural sources including plants parts and animals such as amphibians that have bacteriostatic and bactericidal properties. A combination of amino acids in bacteria yield a special groups of AMPs called bacteriocins, which are known to be synthesized to prevent invasion by other related (narrow spectrum) or non-related (broad spectrum) microbiota as one of the inherent defense system weapons of bacteria [27].

The aim of this study is to identify the various amino acids extractable from the seeds of *Citrullus lanatus* (CACL) and *Chrysophyllum albidum* cotyledons (CACA) and determine their effects on rat hepatocyte regeneration, liver cytochrome c oxidase activity, and on a few bacteriocin-producing Gram-negative bacteria.

Methods

Chemicals

Porcine pancreatic amylase (PPA) and α-glucosidase, dimethyl sulfoxide (DMSO), Tris-HCl buffer, and nitrophenyl glucopyranose were purchased from Sigma (St. Louis, MO, USA).. All the other chemicals used during the work were of analytical grade.

Collection and authentication

The whole fruit of watermelon (*Citrullus lanatus*) were purchased from Iworoko market in Ekiti State while *Chrysophyllum albidum* fruits were bought from Oja-Oba market in Osogbo, Osun State, Nigeria. Both plants were identified and authenticated at the Plant Biology Unit, Biological Sciences Department, Osun State University, Osogbo, Nigeria. They were deposited at the herbarium under the voucher numbers: UNIOSUN/PBH/17/002 and UNIOSUN/PBH/17/005, respectively.

Sample Preparation

Citrullus lanatus fruits were washed and cut open to obtain the seeds. The seeds obtained were washed in distilled water and air-dried for days; then pulverized using mortar and pestle under aseptic conditions and ground to powder using a blender (Waring commercial blender). Powdered seed material were then weighed and kept in air-tight containers until further usage.

Chrysophyllum albidum seeds were removed from the pulp and air-dried for five (5) days. The air-dried seeds were shelled manually to remove the cotyledon which were pulverised into fine powder using a local blending machine and then moved to Biochemistry laboratory, Osun State University, Osogbo, Nigeria for analysis.

Extraction and Isolation of Amino Acids

Peptide-rich fractions were obtained as described by Koehbach et al. [28] modified by Attah et al. [27]. 500 g of powdered seeds of *Citrullus lanatus* and *Chrysophyllum albidum* fruits were percolated in 6 L of dichloromethane-methanol mixture (1:1, v/v) in a separating funnel and left overnight at room temperature. The mixture was filtered to remove plant debris. Equal volume of water was added to the filtrate in a separating funnel, and the aqueous layer was collected and concentrated to dryness using rotary evaporator. The aqueous extract was freeze-dried in glass bottles and kept at -20°C for further use.

Determination of Presence of Amino Acids by Thin layer Chromatography using Ninhydrin, Pyridine and Coomassie brilliant blue G250

CACL and CACA were spotted and separated on pre-coated thin layer chromatographic plates in a developing chamber containing mixture of Butanol: Glacial Acetic acid: Distilled water (3:1:1). The developed plate was then dried in an oven and sprayed with ninhydrin solution, pyridine solution and modified Coomassie brilliant blue G250 in the fume hood and dried again in the oven for 15 minutes to speed up the reactions. The spots were

viewed under UV-light at 245 nm and 366 nm, respectively. The observed spots were circled using a pencil and snap shots were taken as prediction of the biologically active peptides present in CACL and CACA.

Characterization of Free Amino Acids using Gas Chromatography – Mass Spectrometry (GC-MS) and Fourier transform infrared (FTIR) Spectroscopy

CACL and CACA were subjected to GC-MS analysis (Model 7890A, Agilent Technologies) interfaced with a mass selector detector model 5975°C. The electron ionization was kept at 70 eV with an ion source temperature at 250°C. Helium gas was used as the carrier gas while HP-5MS (30 mm × 0.25 mm × 0.320 µm) was used as the stationary phase. The oven temperature was kept at 80°C held for 4 minutes and ramped to 270°C at the rate of 3.5°C/minutes holding for 6 minutes. 1 µl of the prepared extracts diluted with respective solvents was injected into the column at 300°C. The split mode was employed with a split ratio of 50:1. Relative quantity of the amino acids present in each of CACL and CACA was expressed as a percentage, based on peak area produced in the chromatogram.
FTIR spectroscopy was conducted for CACL and CACA in order to resolve the intensity of vibrations of the free amino acids. Infrared light is absorbed by molecular vibrations that oscillate with the same frequency. The frequency of the vibration and the probability of absorption are influenced by intra- and intermolecular effects. This absorption is also quantized, but vibrational spectra appear as band rather than as lines because a single vibrational energy change is accompanied by a number of rotational energy changes. Thus, information about structure and environment of amino acid side chains can be deduced from the spectral parameters band position, band width and absorption coefficient [29] .The samples resulting from column chromatography were prepared for FTIR by vortexing and sieving them through 0.4 millipore filter. They were evaporated to dryness using a rotavapour and reconstituted in 1ml of acetonitrile. Portions of the dried samples which have been reconstituted in acetonitrile were dissolved in dichloromethane in Eppendorf tubes and submitted for FTIR spectra analysis. The IR spectra were recorded on Agilent FTIR with ATR and IR microscope, and high-resolution mass spectra were measured on Bruker TOF/TOF.

Experimental Animals

Healthy adult male Wistar rats (160 – 180 g) were purchased from the Animal House, College of Health Sciences, Osun State University, Osogbo, Nigeria. Animals were kept under a natural conditions (12h light/12h dark) each day. The rats were sacrificed by cervical dislocation, the livers excised and processed for the tissue and mitochondrial isolation. The animals used received humane care in compliance with standard guidelines set up for the Care and Use of Laboratory Animals (EU Directive 2010/63/EU). Ethical approval was obtained from the Ethical Committee of the College of Health Sciences, Osun State University, Osogbo, Nigeria and given ethical approval number UNIOSUN/HREC/2017/A/005.

Preparation of Tissue and Mitochondrial Fractions of Liver Preparation of Tissue Fraction of Liver for Cytochrome C Oxidase and Catalase Activities.

Liver homogenate was prepared according to the method of Balaban et al. [30] with slight modifications. Briefly, one of the rats was sacrificed by cervical dislocation and the liver dissected and trimmed to remove excess tissue, rinsed in Buffer A (0.3M sucrose, 10 mM HEPES, 0.2 mM EDTA, pH 7.2), patted dry and weighed. A portion of the liver weighing 2g was then minced with a pair of scissors and poured into the homogenizing flask adding equal weight of Buffer B (0.1 M sodium phosphate buffer, pH 7.0) and then homogenized with Potter-Elvhenjam homogenizer for 5 seconds. The whole process was carried out on ice to preserve the integrity of the mitochondria. The homogenate was dissolved (1:5, w/v) in a volume of Buffer C (Triton X-100 prepared in Buffer B – 2%, v/v) and mixed. The homogenate dissolved in Triton X-100 was then implored into a TGL-16G high-speed cold centrifuge to sediment tissue debris at 2500 X g for 7 minutes. The supernatant was poured into a clean test-tube and stored at 4°C until further use.

Preparation of Mitochondrial Fraction of Liver for Cytochrome C Oxidase Activity

The liver mitochondria fraction was prepared according to San et al.[31] The remaining 2g portion of liver was homogenized in Buffer D (210 mM

mannitol, 70 mM sucrose, 5 mM HEPES, 1 mM EDTA, 0.2% BSA, pH 7.4 – 1:5, w/v). The homogenate was centrifuged in a TGL-16G high-speed cold centrifuge at 1000 rpm for 10 minutes to pellet the nuclear fraction, and the supernatant was centrifuged at 10,000 rpm for 10 minutes to pellet the mitochondria, which was suspended in 10 ml of Buffer E (130 mM KCl, 20 mM HEPES, 2.5 mM $MgCl_2$, 0.5 mM EDTA, pH 7.2) and stored at 4°C until further use.

Spectroscopic Determination of Cytochrome C Oxidase Activity

One milliliter each of the tissue or mitochondrial fraction was dissolved in 1.5 ml of Buffer C in the presence or absence of 50 µl each of 4 M KCN, 3 M ascorbate and 50 mg/ml CACL or CACA, respectively. The absorbance was read at 605 nm and 630 nm in Camp Spec M105 UV-Vis spectrophotometer[30]. Briefly, homogenized tissue was dissolved in 2% Triton X-100 solution to eliminate light scattering effects. The differential absorbance between 605 and 630 nm during chemically induced maximum oxidation and reduction of the sample was used to determine the absolute activity of cytochrome oxidase. Strong oxidizing agent potassium cyanide and reducing agent ascorbic acid were utilized[32] to ascertain the effects of CACL and CACA on the activity of cytochrome c oxidase. Triton X-100 (2.5 mL) solubilized tissue or mitochondria was placed in a cuvette and placed in a spectrophotometer. All measurements were made as the optical absorbance were read over 605 – 630 nm. Typically, 50 µL of peptide extract, potassium cyanide and ascorbate was added to 2.45ml of triton X 100 solubilized tissue or mitochondria. The absorbance was read (605, 610, 615, 620, 625 and 630 nm) and recorded for tissue and mitochondria.

Bacteria Sensitivity Test

Four isolate were revived in a nutrient agar for 24 hours and inoculated in 5 ml Ringer solution using 0.5 % McFarland standard as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. Three various concentrations of CACL and CACA (50 mg/ml, 5.0 x 10^{-3} mg/ml and 5.0 x 10^{-6} mg/ml) were prepared. Small discs of sterile filter paper were soaked in each of the extract concentrations. A sterile swab stick was used to spread the pure isolate of bacteria on a Mueller-Hinton agar evenly and 2 discs of small soaked filter paper of each extract was placed on the Petri dish containing each of the microorganisms in duplicates and incubated in an inverted position for 48 hours at 37°C. The zones of inhibition were measured in millimeters after 48 hours. The above studies were done in an aseptic condition in order to avoid contamination[33].

Tissue Regeneration Studies

Adult bovine blood was aseptically collected from slaughter house in clean containers. It was allowed to stay for 30 minutes on the laboratory work bench after which it was placed in the freezer for 3 hours at 4°C. Thereafter, the serum which had exuded to the surface was collected into clean centrifuge tubes and spun at 2300 rpm for 10 minutes. The supernatant was collected and dispensed into clean Eppendorf tubes and spun at 1000 rpm for 10 minutes at 4°C after which the clear serum was dispensed into clean sera bottles and placed in the water bath to inactivate at 56°C for 30 minutes. The serum was stored at 4°C until used.

The various constituents of the modified Eagle's medium was weighed accurately and poured into a 250 ml beaker. 200 ml of double distilled water was added and placed on the magnetic stirrer to solubilize. After solubility has been achieved, the solution was poured into a flat bottom flask and made up to 1000 ml. Five grams of agar-agar was weighed into a conical flask. 500 ml of the modified Eagle's medium was added and autoclaved at 120 °C for 15 minutes. The medium was allowed to cool at room temperature after which 10% serum was added to the medium and mixed. The medium was poured into Petri dishes in a sterile environment and allowed to set. The plates were kept in the refrigerator until used [34].

Preparation of Hepatocytes for Cell Culture and Wound Healing Studies

The rat was sacrificed by cervical dislocation and the liver dissected and trimmed to remove excess tissue and weighed. The liver was rinsed in phosphate-buffered saline (PBS) until cleaned of blood stains. It was then minced with a pair of scissor and the tissue was then poured into the

homogenizing flask adding equal weight of PBS. The tissue was homogenized for 5 seconds. The homogenate was sieved through a millipore membrane to remove larger tissues. The filtrate was then dispensed into clean Eppendorf tubes and centrifuged at 1000rpm for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 5 ml of the modified Eagle's media (without agar-agar). The cells were inoculated on the Petri dishes by dispensing 200 µl of cell solution on each plate and evenly distributed across the surface area of the plate using a sterile spreader. The cells were incubated in an inverted position at 37 ^{o}C over 24 to 48 hours.

After the cells have grown to confluence over 24 – 48 hours, a 3 mm^2 wound area was cut into the mid-portion of the plates and re-filling the area with extract-contained media. They were allowed to set and then incubated in inverted position over 3 days and observed for regeneration. The tissue regeneration studies were was carried out by growing rat hepatocytes on Eagle's medium and subsequently making a scar on a pre-determined area of the Petri dish. The scar area was refilled with fresh media containing extract and the cells were allowed to grow. The extent of wound closure was photographed and measured using Image J.

Collagen Quantification in Scar Tissue

Collagen content was quantified according to the method of Yura and Vogel [35]. To 1 ml of test sample (supernatant recovered from wound area reconstituted in DMSO, gently homogenized and centrifuged) or standard (1 mg proline was added 1 ml of freshly prepared 0.05 M $CuSO_4$ and 1 ml of 2.5 N NaOH and mixed by gentle swirling. The tubes were placed in a water bath at 40°C for 3-5 minutes. 1 ml of freshly prepared 6 % H_2O_2 was added and the solution mixed gently and kept for another 10 minutes in the water bath with continuous shaking. Upon cooling, 4 ml of concentrated H_2SO_4 and 2 ml of 5 % p-DMBA solution were added and content mixed and further kept in water bath at 70 °C or 16 minutes. Upon cooling to room temperature, the absorbance was read at 555 nm.

Statistical analysis

Data were presented as Mean ± Standard Deviation and analyzed using one-way ANOVA ($p< 0.005$) on IBM SPSS Statistics 19.0.

Results

Amino acid contents of extracts of Citrullus lanatus seeds and Chrysophyllum albidum cotyledons

The development of purple color upon reaction with ninhydrin and pyridine, and the development of bright blue color upon reaction with modified Coomassie brilliant blue G250 indicated the presence of amino acids, which can occur in both linear and circular configurations.

CACL tested positive for various amino acids (purple colour development with ninhydrin and pyridine) and arginine, histidine, lysine, phenylalanine, tyrosine, and tryptophan, which may occur in circular configuration (blue colour development with Commassie brilliant blue, G250 [36].

Ex vivo tissue regeneration by extracts of Citrullus lanatus seeds and Chrysophyllum albidum cotyledons

The Image J densitiometric measurements revealed that the hepatocyte cultures progressively regenerated (0, 24, 36 and 72 hours) in response to the treatment of the amino acid-rich fraction of CACA (Figure 6). CACA appeared to significantly sustain increasing cell proliferation and wound closure as there was steady increased cell population over 72 hours, though the cells apparently experienced a slight initial shock upon exposure to CACA unlike in CACL and Control which initially increased cell division towards quick wound healing, but later declined progressively over 72 hours.

Antimicrobial effects of extracts of Citrullus lanatus seeds and Chrysophyllum albidum cotyledons

The effects of the factions screened against bacteriocin-producing bacteria; *Serantia marcenscense 1*, *Serantia marcenscense 2*, *Escherichia coli* and *Proteus mirabilis* using agar well diffusion in Mueller-Hinton's agar showed a stronger inhibitory effect on the activity of *Escherichia coli* maximally at 50 mg/ml, 5.0 x 10^{-3} mg/ml and 5.0 x 10^{-6} mg/ml. Its zone of inhibition was observed to be 9 mm (±0.10), although the inhibition was higher with penicillin 23 mm at 50 mg/ml and 27 mm at 5.0 x 10^{-3} mg/ml but

at a low concentration of 5.0 x 10^{-6} mg/ml the zone of inhibition was 11 mm which is close to the inhibition with the fraction from *Chrysophyllum albidum* cotyledons. There was no detectable activity at 50 mg/ml and 5.0 x 10^{-3} mg/ml on *Serantia marcenscense* 2, and 50 mg/ml and 5.0 x 10^{-6} mg/ml on *Proteus mirabilis*. The fraction had no effect on the activity of *Serantia marenscense* 1.

Effects of extracts of Citrullus lanatus seeds and Chrysophyllum albidum cotyledons on cytochrome c oxidase activity

Table 3 showed that the activity of cytochrome c oxidase in the mitochondria fraction of the rat liver treated with amino acid-rich fraction of *Citrullus lanatus* seeds was not significantly different ($p>0.05$) when compared to the control. Treatment with ascorbic acid showed significant increase ($p<0.05$) in cytochrome c oxidase activity in the mitochondria fraction of the rat liver while treatment with potassium cyanide showed significant decrease ($p>0.05$) in mitochondria fraction of the rat liver in comparison to control. This could indicate that the fraction from *Citrullus lanatus* seeds had little or no effect on the activity of cytochrome c oxidase in the mitochondria fraction of the rat liver while potassium cyanide inhibited the activity of cytochrome c oxidase and ascorbic acid increased the activity of cytochrome c oxidase in the mitochondria fraction of the rat liver.

The activity of cytochrome c oxidase showed significant increase ($p<0.05$) in the cytochrome c oxidase activity of the tissue fraction when compared to the control. In contrast, treatment with KCN showed no significant difference in cytochrome c oxidase activity of the tissue fraction of rat liver while treatment with ascorbic acid showed significant increase in the cytochrome c oxidase activity, whereas KCN inhibited the activity of cytochrome c oxidase in the mitochondria fraction.

Discussion

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings [37]. The use of natural products for the prevention and treatment of different pathologies is continuously expanding throughout the world [38]. *Citrullus lanatus* is an excellent source of antioxidants flavonoids like lycopene, beta-carotene, lutein, zeaxanthin and cryptoxanthin. These antioxidants have been found to offer protection against colon, prostate, breast, endometrial, lung, and pancreatic cancers. Phytochemicals present in *Citrullus lanatus* like lycopene and carotenoids have the ability to help protect cells and other structures in the body from oxygen-free radicals. Antioxidation is an extremely significant activity which can be used as a preventive agent against diseases. Phenolic compounds are the most active natural antioxidants in plants. They are very important plant constituents because their hydroxyl groups which confer scavenging ability and because of the reactivity of the phenolic moiety [39]. Cui-ping [40] in his work stated that the antioxidant activity of watermelon was higher in the epicarp (rind, outer layer) than the flesh (mesocarp). Also, the free radical scavenging and antioxidant activities of plant extract may be attributed to the presence of phenolic compounds.

Amino acids in the form of peptides have been described to be involved in the daily metabolic activities of plants which could help such plants to adapt to extreme environmental conditions, particularly cysteine-rich peptides. A host of these peptides are reported to produce anti-oxidative property [31, 41]. These peptides are currently described as host defence peptides with broad antimicrobial function [42, 43] and immunomodulatory property [44]. Host Defence Peptides (HDPs) also known as Antimicrobial Peptides (AMPs) are disulphide stabilized peptides rich in cysteine residues. In addition to their innate defense roles, they are reported to possess antioxidant property [45]. The redox-active cysteine residues abundantly expressed in these class peptides make them interesting antioxidant molecules. In addition, disulphide linkages within these classes of peptides may equally contribute to the level of hardness of the seeds as well as their resistance to pest attack. The phosphoric acid modified G250 TLC spray increases the detection sensitivity of cysteine-containing amino acids. It has been used to detect sulphur-rich cyclotides in some plant extracts [27, 46]. The G250 stain can serve the phytochemist a useful tool for the preliminary detection of cysteine-rich peptides in botanical samples especially seeds where these host defense

peptides are commonly stored. The observed bioactivity of amino acids-rich *C. lanatus* seed extract may be associated with the presence of the detected cysteine-containing amino acids considering their reported antioxidant, immunomodulatory and antimicrobial properties. There is therefore need to investigate the configuration, molecular targets and mechanism of action of these interesting amino acids to understand their occurrence and significance in intrinsically disordered proteins and antimicrobial peptides.

Conclusion

The results confirmed the presence of amino acids in *C. albidum* cotyledons and *C. lanatus* seeds if embraced a novel sources of nutritionally useful amino acids and proteins. The amino acid-rich extract of *C. lanatus* seeds improved hepatocyte regeneration, *in situ* in a time-dependent manner. Thus, the amino acids from *Citrullus lanatus* seeds seem to possess wound healing properties and may hold great promise in health and disease conditions, including diabetic foot ulcer.

The results revealed that the seed extract of *Citrullus lanatus* may possess a promising anti-oxidative property that, if harnessed, may be useful to attenuate the production of free radicals and deter the deleterious effects of oxidative stress in health and disease. Thus, the seeds should also be consumed while eating the fruit rather than discarding them which is very common practice among its consumers. Lastly, with the findings of this study in primary rat liver culture, clinical works are required to further validate the current findings.

The inability of the amino acid-rich extract to inhibit the activity of the bacteria might be as a result of possible cooperative interaction between the amino acids and the bacteriocin produced by the bacteria. Therefore the naturally occurring amino acids are considered harmless to the activity of the bacteria. *Escherichia coli* is known as a wound contaminant but the inhibitory effect of the amino acid-rich extract of *Citrullus lanatus* seeds on its activity accelerated wound healing process.

Conflict of interest

The authors report no conflict of interest whatsoever during the compilation of the manuscript.

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Table 1: The results for the amino acid-rich seed extract of *Citrullus lanatus* on cytochrome c oxidase activity in the mitochondria fraction of the rat liver.

PARAMETER (nm)	MEAN±SD
BASAL	0.136±0.13[b]
KCN	0.048±0.01[a]
EXTRACT	0.117±0.051[b]
ASC	0.202±0.02[c]

Data are expressed as Mean± Standard deviation for 6 determinations, n=6. Values with different superscript are significantly different at P<0.05.
BASAL- Homogenate with no treatment added
EXTRACT- Homogenate treated with extract
KCN- Homogenate treated with KCN
ASC- Homogenate treated with ASC

Table 2: The result for the crude amino acid-rich seed extract of *Citrullus lanatus* on cytochrome c oxidase activity in the tissue fraction of the rat liver.

PARAMETER (nm)	MEAN±SD
BASAL	0.62±0.31[a]
KCN	0.68±0.60[a]
EXTRACT	0.76±0.38[b]
ASC	2.54±0.96[c]

Data are expressed as Mean± Standard deviation for 6 determinations, n=6. Values with different superscript are significantly different at P<0.05.
BASAL- Homogenate with no treatment added
EXTRACT- Homogenate treated with extract
KCN- Homogenate treated with KCN
ASC- Homogenate treated with ASC

Table 3: The results for the crude amino acid-rich seed extract of *Citrullus lanatus* on catalase activity in the mitochondria fraction of the rat liver.

PARAMETER (mm)	MEAN±SD
BASAL	0.20±0.00[b]
KCN	0.00±0.00[a]
EXTRACT	0.70±0.00[c]
ASC	0.25±0.71[b]

Data are expressed as Mean± Standard deviation for duplicate determinations, n=3. Values with different superscript superscript are significantly different at P<0.05.
BASAL- Homogenate with no treatment added
EXTRACT- Homogenate treated with extract
KCN- Homogenate treated with KCN
ASC- Homogenate treated with ASC

Table 4: The results for the crude amino acid-rich seed extract of *Citrullus lanatus* on catalase activity in the tissue fraction of the rat liver.

PARAMETER	MEAN±SD
CONTROL	1.50±0.00[b]
KCN	0.00±0.00[a]
EXTRACT	1.67±0.35[b]
ASC	0.53±0.33[a]

Data are expressed as Mean± Standard deviation for duplicate determinations, n=2. Values with different superscript are significantly different at P<0.05.

BASAL- Homogenate with no treatment added
EXTRACT- Homogenate treated with extract
KCN- Homogenate treated with KCN
ASC- Homogenate treated with ASC

Table 5a: Result of peptide characterization using Fourier transform infared (FTIR) in the seed peptides of *Citrullus lanatus* seeds

Band position (cm^{-1})	Intensity of vibration	Type of vibration	Assignment
734.88	30.68	δ, γ_r	Threonine
896.90	95.72	δ, γ_r	Threonine
1022.27	41.96	*v (c-o)*	Serine
1112.93	93.18	*v (c-o)*	Threonine
1267.23	80.96	γ_t (CH_2)	Tryptophan
1454.33	90.88	δ (CH_2)	Proline
1647.21	98.01	*v (c=o)*	Glutamine
1741.72	98.62	*v (c=o)*	Glutamate

δ - Plane bending vibration, γ r- Rocking vibration, *v*- Stretching vibration, γ t- Twisting vibration

Table 5b: Result of peptide characterization using Fourier transform infared (FTIR) in the cotyledon peptides of *Chrysophyllum albidum* cotyledons

Band position (cm^{-1})	Intensity of vibration	Type of vibration	Assignment
414.70	87.80	δ, γ_r	Threonine
734.90	28.69	δ, γ_r	Threonine
896.90	95.70	*v (C-O)*	Serine
1022.27	46.26	*v (C-O)*	Threonine
1111.00	93.75	γ_t (CH_2)	Tryptophan
1267.23	79.40	δ (CH_2)	Proline
1454.33	91.72	*v (C=O)*	Glutamine
1647.21	97.82	*v (C=O)*	Glutamate
2034.90	98.13	ND	ND
2160.27	98.50	*v (C=C)*	ND
2833.43	92.35	*v (O=C-H)*	ND
2945.30	81.77	*v (C-H)*	ND
3331.07	87.77	ND	ND

δ - Plane bending vibration, γ r- Rocking vibration, *v*- Stretching vibration, γ t- Twisting vibration, ND – Not Determined

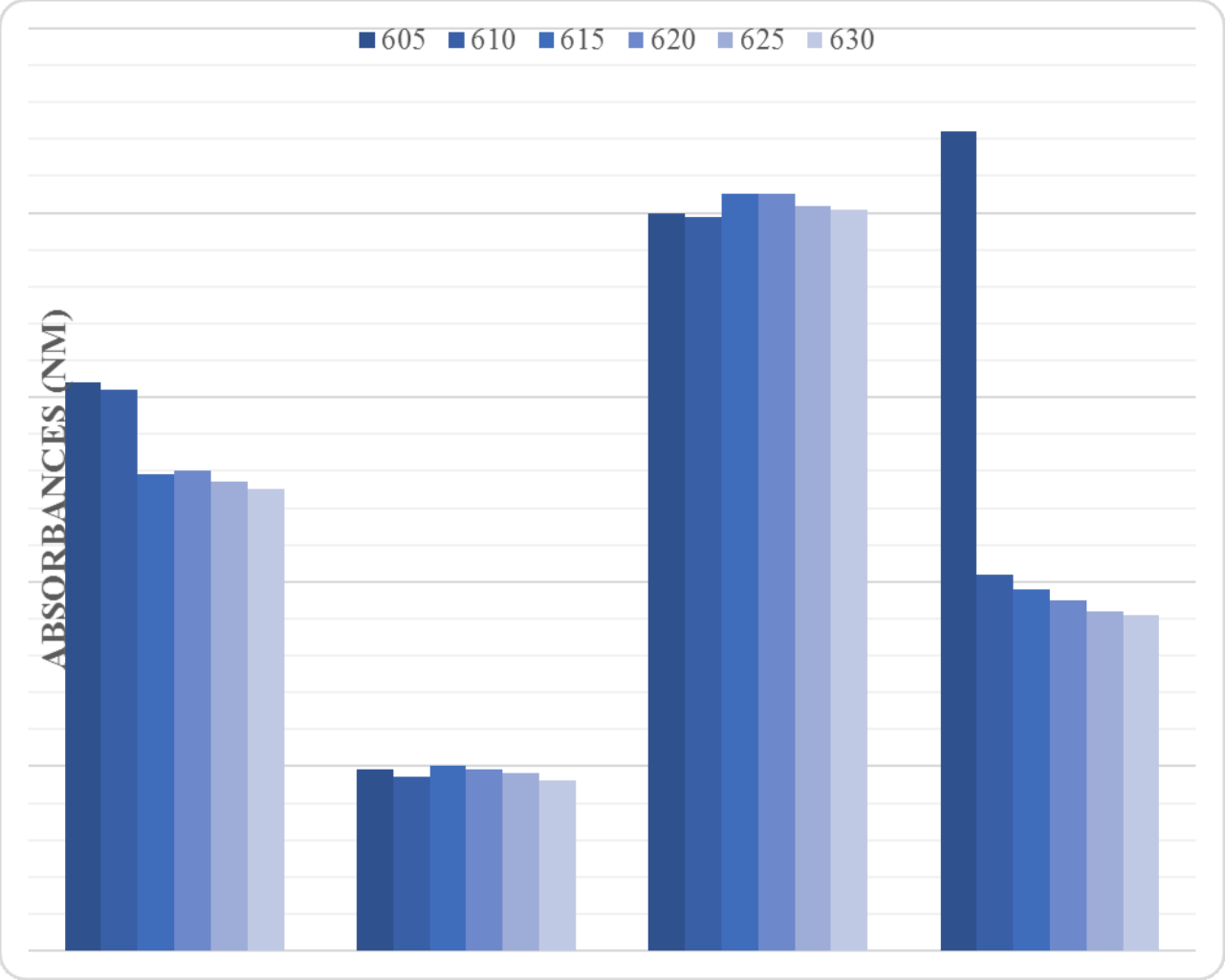


Figure 1: Up-regulation of cytochrome c oxidase activity by amino acid-rich seed extract of *Citrullus lanatus* in the mitochondria fraction of the rat liver

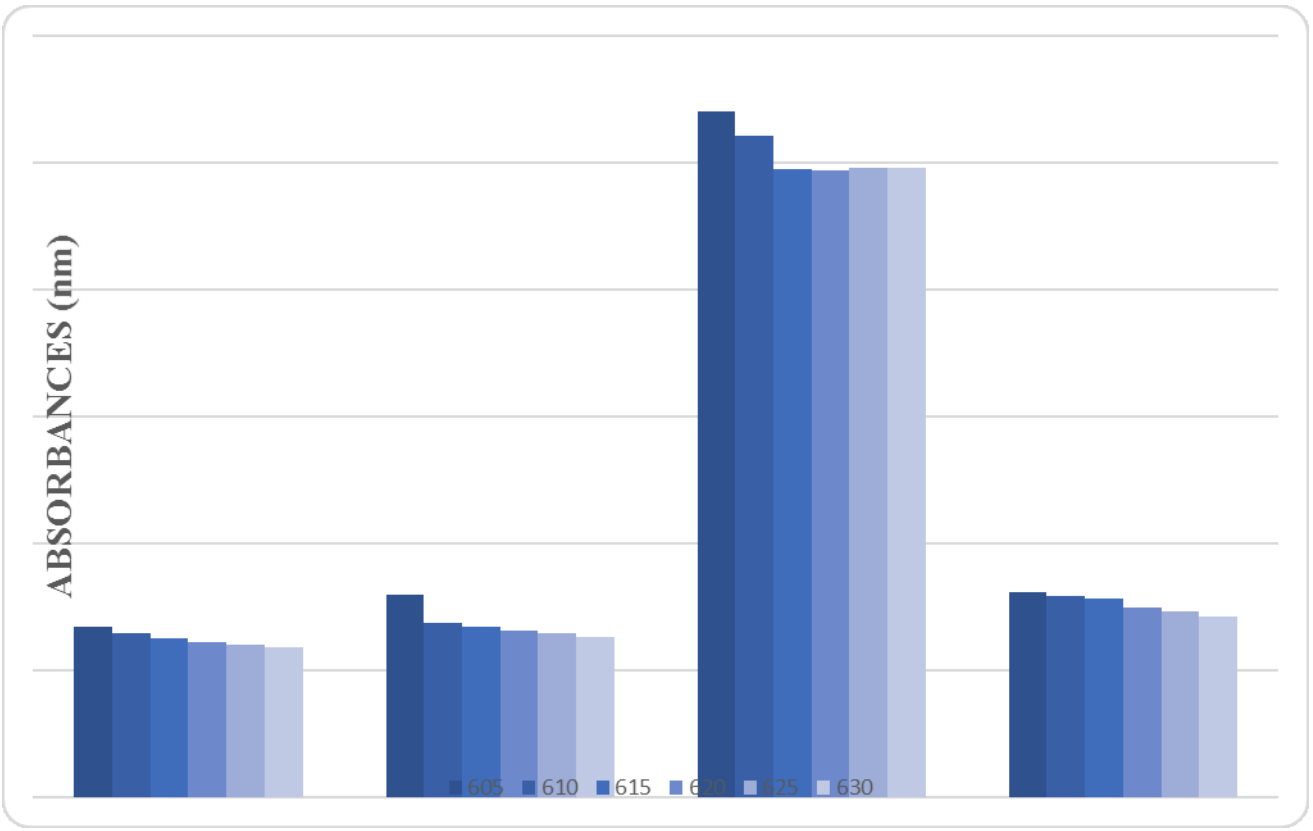


Figure 2: Up-regulation of cytochrome c oxidase activity by amino acid-rich seed extract of *Citrullus lanatus* in the tissue fraction of the rat liver

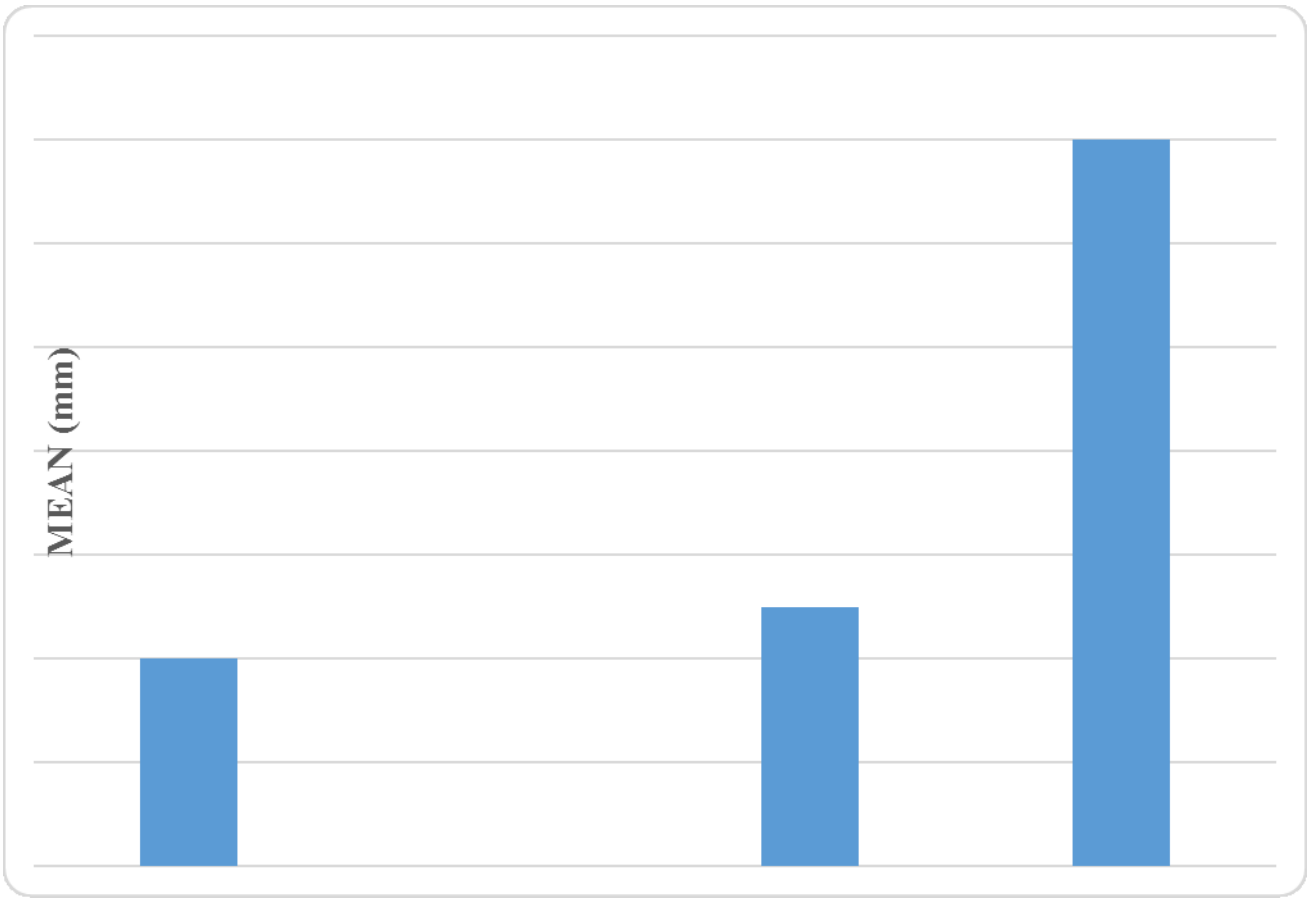


Figure 3: Up-regulation of catalase activity by amino acid-rich seed extract of *Citrullus lanatus* in the mitochondrial fraction of the rat liver

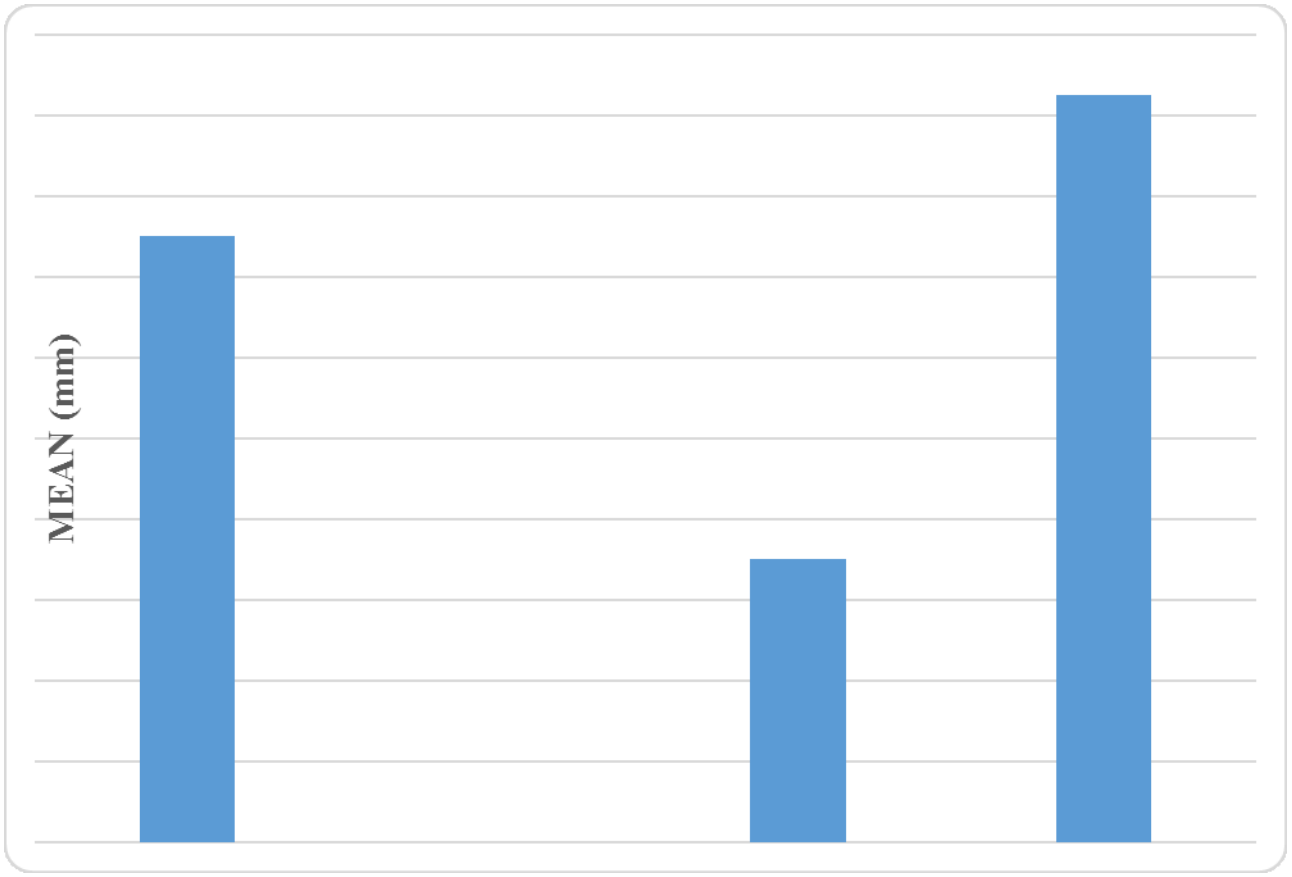


Figure 4: Up-regulation of catalase activity by amino acid-rich seed extract of *Citrullus lanatus* in the tissue fraction of the rat liver

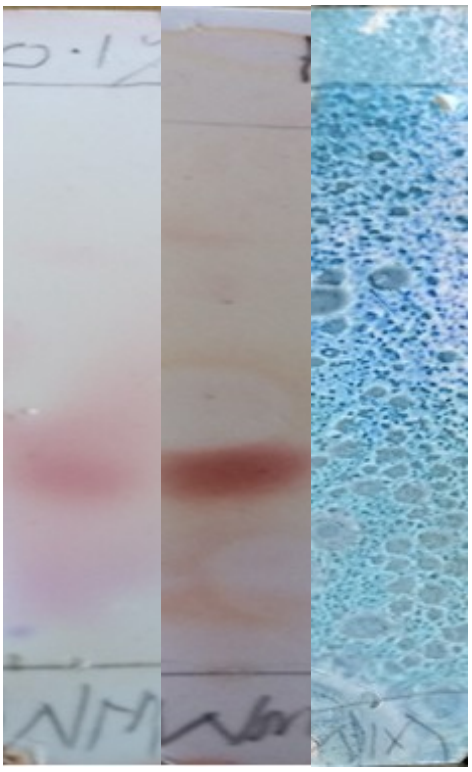


Figure 5a: Detection of the free amino acids, peptides and proteins present in the crude seed extract of *Citrullus lanatus* seeds

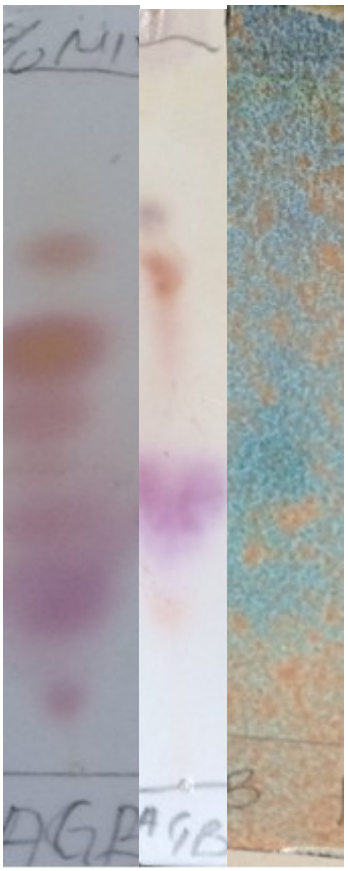


Figure 5b: Detection of the free amino acids, peptides and proteins present in the crude seed extract of *Chrysophyllum albidum* cotyledons

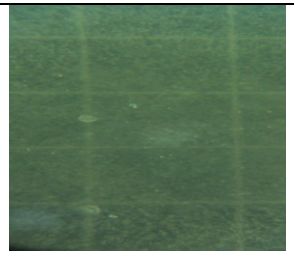
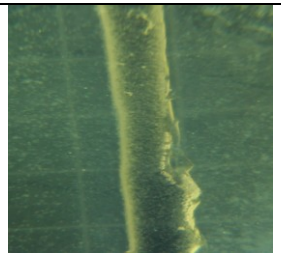
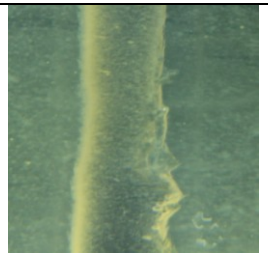
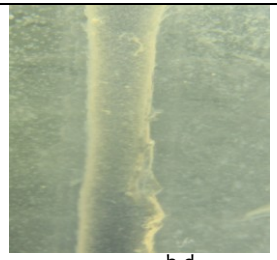
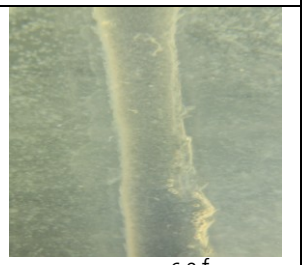
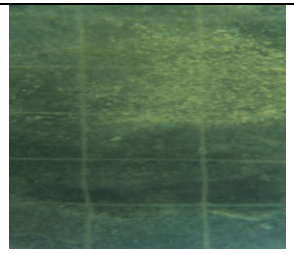
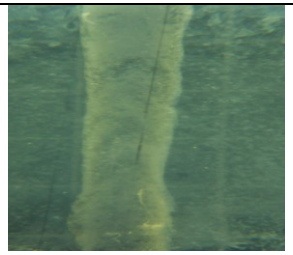
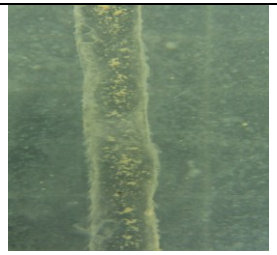
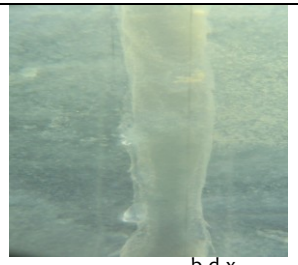
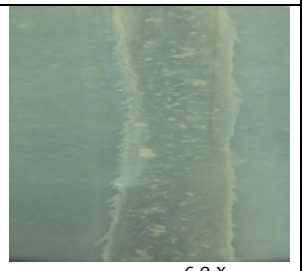
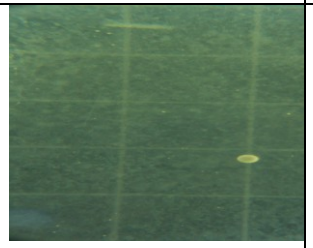
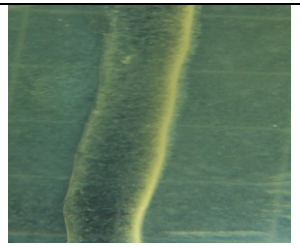
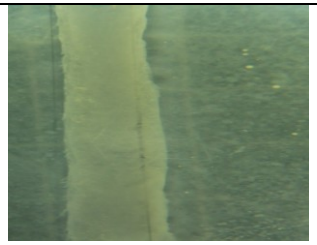
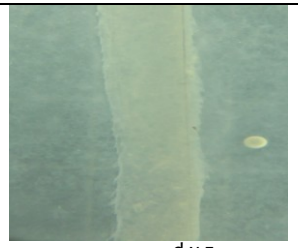
Crude Peptide	Confluence Growth (Pre-wound)	Day 0	Day 1	Day 2	Day 3
Control		 90.05 ± 11.11	 140.06 ± 7.47^{a}	 96.73 ± 5.18^{b,d}	 73.99 ± 4.42^{c,e,f}
Chrysophyllum albidum cotyledons (CACL)		 98.74 ± 8.45^{x}	 96.23 ± 12.39^{x}	 102.59 ± 14.25^{b,d,x}	 104.39 ± 15.11^{c,e,x}
Citrullus lanatus seeds (CACA)		 91.43 ± 4.85^{z}	 128.08 ± 11.68^{a,y,z}	 90.30 ± 7.03^{d,y,z}	 83.76 ± 5.49^{c,e,f,y,z}

Figure 6: Wound closure assessment in rat liver hepatocytes treated with amino acid-rich extracts of *Citrullus lanatus* seeds and *Chrysophyllum albidum* cotyledons

Day 0 vs Day 1 – a; Day 0 vs Day 2 – b; Day 0 vs Day 3 – c; Day 1 vs Day 2 – d; Day 1 vs Day 3 – e; Day 2 vs Day 3 – f, Control vs CACL - x; Control vs CACA – y; CACL vs CACA – z