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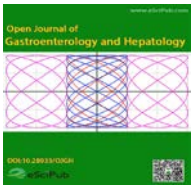

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Phytochemical Antimicrobial Screening of *Costus Afer* Extract and Its Alleviation of Carbon Tetrachloride Induced Toxicity

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ABSTRACT

Plants of medicinal values contain bioactive compounds capable of preventing and combating several oxidative related diseases. Many diseases have been wrongly attended to using several medicinal plants of choice by mere instinct or sunrise. The phytochemical screening, (antimicrobial activity of Costus afer extract and its alleviation of carbon tetrachloride induced toxicity were evaluated. The phytochemical screening of both qualitative and quantitative analyses showed the presence of Tannins, Steroids, Phenols, Phytate, Hydrogen cyanide (HCN). Saponin, Alkaloids and Flavonoids in the aqueous, methanol and n-hexane stem extract. The antimicrobial activity of Costus afer extract using two different solvents showed its bactericical effect and no antibiotic effect on fungi microorganisms at different concentration. The study based on the toxicity of the substance, carbon tetrachloride showed the serum elevation of alanine aminotransferase (ALT, EC 2.6.1.2.), aspirate aminotransferase (AST, EC 2.6.1.1) and alkaline phosphase (ALT, EC 3.1.3.1) in the liver of the rabbits in response to the oral administration of the chemical. The rabbits fed with the Costus afer extract of methanol and N-hexane showed a moderate effect while the rabbits fed with the chemical carbon tetrachloride had a very high elevation on the enzymes. The rabbits, however fed with both the Costus afer and the chemical compound, carbon tetrachloride showed a considerable alleviation on the level of toxicity of the chemical. The rabbits fed with the Costus afer extract and the chemical carbon tetrachloride mixture statistically showed significant ($p<0.05$) difference between the treatment and their liver enzymes. This indicated that biological active compounds of Costus afer are more polar and could serve as a source of bioactive compounds for nutrition and therapeutic purposes.

Keywords: Phytochemical, Antimicrobial-Costus-after, Alleviation, CarbonTetrachloride Toxicity

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Introduction

Many plants are today being Scientifically Studied for bioactive substances and for Medicinal Potentials, *Costus after* appears to be among the least evaluated and as such has scanty published literature on its physiological and pharmacological dynamics. (Obadoni, et al 2001) the plant costus afer plant is among 150 species of stowt, perennial and rhizomatous herbs of the genus costus. It can be found in shady forest belt and river banks in Africa countries like, Nigeria, Ghana, Niger (Nyananyo, 2006) phytochemical analysis of the leaf revealed the presence of alkaloids, saponins, tannins, flavonoids, phenols, glycosides and terpenoids (Anago, et-al, 2004).

Rent studies have found more benefits of saponin to human health and they include the control of cholesterol levels, bone health, cancer and building up the immune system (Anago, et.al 2004) the primary effects of carbon tetrachloride in humans are on the liver, kidneys, and central nervous system (CNS). Human symptoms of acute (short-term) inhalation and oral exposures to carbon tetrachloride include headache, weakness, lethargy nausea and vomiting, while long exposures leads to liver and kidney damage in humans (Anago, et al, 2004).

Guzman, A.I and Guerrero, R.O. (2002) evaluated the liver function of wistar rats fed with combined ethanolic leaf extract of Alchornea cordifolia and costus afer in paracetamol induced toxicity and realized its hepatoprotective effects on paracetamol induced hepatacity in wistar rats. Costus after is useful include the leaves, stems, roots etc, Ae Sap is somewhat rubber facient, and open wounds in burning, yet it is anodynal and healing.

The research is intended to evaluate the phytochemical constituents of costus after, antimicrobial potentials and evaluation of hepatotropic potency of costus after on carbon tetrachloride induced rabbit.

MATERIALS AND METHODS

Materials: Chemical, reagents and equipment used in the research study were of Analytical grade. All were obtained from General Laboratory, department of Applied Microbiology and Brewing.

Study Area: The research was conducted at the Microbiology general laboratory, department of Applied Microbiology and Brewing, Nnamdi Azikiwe University between the Months of October 2015-May, 2017.

Sample Collection: Fresh plant Samples of *Costus afer* were collected from Ogbor hill, Aba by the river side bank. The plant was first identified by Mr Okonkwo, D by its native name as “Okpete in Igbo”. It was identified properly and authenticated by Dr. Duru M.C with reference to the Herbarium sheets (Voucher number 5051) available at the department of plant/Biotechnology of the polytechnic. The plant samples were stored at the room temperature of 32^0C.

Preparation of Plant Extract: The back of *Costus afer* was pilled off with a sterile knife and chopped into pieces before washing them. The raw barks were washed under running tap water to eliminate dust and other foreign particles. They were shade dried at 35°c for 10 days.

After which the bark were grinded into powder using a blender. The sample was labeled A and kept for extraction at 4^{oc} in the refrigerator.

Preparation of Crude extract: About 50g of bark sample A were electronically weighed into 500ml conical flask; 2 for each extract. And 250mi of N-hexane and 250ml of methanol were added. The methanol and N-hexane extracts were done at 28 ± 1^{oc} for 120 hours the extracts were then decanted and filtered using Whatman filter paper no I. The filtered extract was then sterilized using a membrane filter and evaporated to dryness at 45^{oc} the residues obtained were reconstituted in 100mg/ml. dimethyl Sultoxide (DMSO) stock concentration and the extract were kept stock in the

refrigerator at 4±2°C until they were required for use (Odabasi M. 2008).

Collection and confirmatory test of standard test Organisms

Each of the test organisms *Escherichia coli, Staphylococcus aureus, Candida albican* and *Aspergilus niger* were obtained from pure culture in the plates from the General Laboratory of Applied Microbiology Nnamdi Azikiwe University Awka using (SDA) as the transport medium in a cool chamber. The microorganisms were subcultured on Sabouraud dextrose agar for fungi and on the Mannitol salt agar for the bacteria and pure isolates of the resulting growth were confirmed using gram staining, germ tube test and slide culture for morphological observations of bacteria and fungi.

Serial Dilutions of the Extract

0.6g of the *Costus afer* stem extract was weighed using an electronic weighing balance. This was transferred into a sterilized bijour bottle containing 6ml of dimethyl sulphuric oxide. It was stirred using a glass rod to help it dissolve. This gives a concentration of 100mg/ml. Serial dilution was made by withdrawing 1ml of the concentration from a test tube into another test tube containing 1ml of dimethyl sulphuroxide and mixed to obtain 50mg/ml solution. This procedure was used to prepare concentrations of 25mg/ml. 12.5mg/ml and 6mg/m1.

Susceptibility Testing of the Test Organisms with Costus afer Extracts using Agar Well Diffusion Method.

The Sabouraud Dextrose Agar and Mannitol salt agar were prepared as mentioned above: the plates were inoculated with the suspension or test organisms using the swah method as previously described. The susceptibilities of the test organisms to Costus afer stem extract were tested using agar well diffusion method CLSI 2014. Wells were made aseptically near a Bunsen burner using a sterile cork borer (8mm) and then 0.lml of the solution of different concentrations of Costus afer stem extract was dispensed in the labeled well i.e. (100mg/ml, 50mg/ml, 25mg/mI, 12.5mg/ml, 6mg/ml). It was allowed to stand for a few minutes before incubation (Owoseni and Ogunnusi, 2006) for 24 hours at 37oc for bacteria and at 25oc for 48hours for (Igbinosa, et aI., 2009). After incubation, the plates were examined and inhibition zone diameters measured using a ruler calibrated in (mm) to determine the degree of susceptibility of the test organisms using Kirby Bauer method described in (Prescott et al., 2005).

Tetracycline (150mg/ml) for antibacterial studies and fluconazole (200mg/m l) for antifungal studies were used as positive control.

PHYTOCHEMICAL ANALYSIS

The extracts were analyzed to test for the presence of the active chemical constituents such as alkaloids, Tannins, Steriods, Phenols, Phytate, Saponins and hydrogen cyanide. Photochemical Screening was carried using established protocol as described by Harborne (1998), Sofowara (1993). A stock solution of the extract with a concentration of lmg/ml was prepared and used for the analysis.

Test for Tannins

This was carried out using the ferric chloride test described by Harborne (1973). The 10mls of the

aqueous extract was mixed with 40mls of distilled water. The mixture was allowed to stand for 30 mins at room temperature being shaken every l0mins, and filtered through whatman no.42 filter paper. An aliquot (2ml) of the filtrate was measured into a test tube and 3ml of distilled water were added to it. This was shaken gently to mix well and 2 drops of ferric chloride solution added. A blue or greenish - black precipitate was indicative of the presence of tannins in the test sample. The test was conducted against a blank control consisting of water and ferric chloride.

Test for Steroids

This was done using the Lieberman -Burchard test as described test as described by Harborne (1973). 2mls of acetic anhydride was added to 5mls of the sample extract, followed by the addition of 2mls of concentrated sulphuric acid down the wall of the test tube to form a layer underneath. A brownish ring in the middle of the sample was formed indicating the presence of steroids.

Test for Phenols

An aliquot of the extract was mixed with 5mls of water. The mixture was heated and allowed to stand for 30mins before filtering. Neutral ferric chloride solution was added to the filtrate and observed for black colouration.

Test for Phytate

10m1s of the aqueous extract was mixed with 40mls of diluted HCL. It was allowed to stand for 30mins then filtered. The filtrate was placed in a conical flask and 5cm^3 of 0.3% ammonium thiocyanate (NH_4SCN) solution was added as an indicator. Distilled water was also added to reach the proper acidity.

Test for Hydrogen cyanide (HCN)

10mls of the aqueous extract was mixed with 90mls of water in a beaker and allowed to stand for 18hrs. It was filtered. Picric solution changing from yellow to orange is indicative of Hydrogen cyanide (HCN)

Test for Saponins

This was done using the saponin froth test (Harborne, 1973).

An aliquot of the aqueous extract was measured into a test tube containing 5mls of distilled water. The mixture was shaken vigorously. Little foaming or steady froth observed was indicative of the presence of saponins.

Test for Alkaloids

The presence of alkaloids in the test samples was investigated using the Mayer's colourimetric method as described by Harborne (1973).

Ethanolic extract of the sample was obtained by shaking 2g of the sample in 20mls of ethanol for 30mins before filtration. The filtrate was used as extract. 2mls of the extract was mixed with few drops of Mayer's reagent in a test tube. The formation of an orange colour showed the presence of alkaloids.

Test for Flavonoids

Few drops of ammonia (NI-h) were placed into a test tube containing a little quantity of the aqueous extract sample and ferric chloride (cl_3fe). A yellow colouration indicated the presence of flavonoids.

The presence of flavonoid was further confirmed by adding a few drops of concentrated hydrochloride acid (HCL) to the yellow solution obtained above. The decolourization of the solution confirmed the presence of flavonoids.

QUANTITATIVE DETERMINATION OF THE PHYTOCHEMICALS.

Determination of flavonoids

This was determined gravimetrically using the method described by Harborne (1973). 5g of the sample was boiled in 100ml of 2M HCL solution for 30 mins. The boiled mixture was allowed to cool and then filtered through Whatman No. 42 filter paper. The filtrate was treated with ethyl acetate starting with drop wise addition until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper, and dried in an oven at 80°c, cooled in a dessicator and reweighed. The difference in weight gave the weight of flavonoid which was expressed as a percentage of the sample weight analyzed. Given by the formula,

$$\% \text{ Flavonoids} = \frac{W_2 - W_1}{1\ W} \times \frac{100}{1}$$

Where:

W = Weight of sample

W1 = Weight of empty filter paper

W2 = Weight of filter paper + flavonoid precipitate.

Saponin content of the samplers) was determined by the double solvent extraction gravimetric method as described by Harborne, (1973).

5mls of the extract sample was weighed out and mixed with 50mIs of 20% aqueous ethanol solution. The mixture was heated with periodic agitation on a water bath for 90mins at 55^0c. It was filtered through Whatman filter paper and the residue re-extracted with 50mis of the 20% ethanol, both extracts were combined together. The combined extract was reduced to 40ml over a water bath at 90°c. The concentrate was transferred into a 250ml separating funnel where 40m1s of diethyl ether was added and shaken vigorously. Separation was by partition during which the aqueous layer was recovered and the ether layer was discarded. Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour. The saponins were extracted with 60m[s of normal butanol. The combined n-butanol extracts were washed with 5% aqueous NaCL (sodium chloride) solution and evaporated to dryness in a pre-weighed evaporating dish. It was dried at 60°c in the oven and weighed. The experiment was repeated two more times to get an average. The Saponin content was determined and expressed as percentage of weight analyzed. Given by the formula:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Determination of Alkaloids

This was done b the alkaline precipitation gravimetric method described by Harborne. (1973). A measured 10mIs of the extract was added into 50mls of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand at room temperature for 4hours. It was later filtered via Whatman No. 42 grade of filter paper. The filtrate was concentrated to a quarter of its original volume by evaporation over a steam bath. 5mls of NH_4OH was added in a drop wise order to precipitate alkaloid until full turbidity was obtained. The filter paper was initially weighed on a weighing machine to be -0. 18kg. After filtration, the filter paper was dried in an oven at 80Dc for an hour. It was cooled in a desicator, re-weighed to be 1. 19kg and expressed as a percentage of the sample analyzed, using the formula;

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Determination of Phenols

Harborne (1973) was used. An aliquot (2ml) of the aqueous sample was dispersed in 10mls of methanol and shaken. The mixture was allowed to stand for 30mins at room temperature before it was filtered through Whatman filter paper. 1ml of the extract was placed in a test tube and 1ml of foilins reagent was added to it with 5ml of distilled water. The colour was allowed to develop for about 3 to 4 hours at room temperature. The absorbance of the developed colour was measured at 760nm wavelength. The procedure was repeated two more times to get an average. The phenol content was calculated, thus;

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

W = Weight of sample analyzed, ALL= Absorbance of test sample, AS= Absorbance of standard solution, C= Concentration of standard on mg/ml, VF = Total filtrate volume, Va = Volume of filtrate analyzed, D=Dilution factor where applicable.

Determination of Steroids

This was determined by the method described of Harborne (1973). A measured weight of each sample (5ml) was put in 100mi freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered and the filtrate was eluted with normal ammonium hydroxide solution (PH 9). 1ml of the eluate was put into a flask (250ml) and mixed with 2ml of chloroform. 3ml of ice-cold acetic anhydride was added to the mixture in the flask and 3 drops of Cone. H_2SO_4 was cautiously added to cool. Standard

sterol solution was prepared and treated as described above to serve as standard and blank.

The absorbance of standard and prepared sample was measured in a spectrophotometer at 420nm wavelength, using the reagent blank to calibrate the instrument at zero. The experiment was repeated two more times to get an average. The steroid content was calculated as shown below:

$$\% \text{ Steroid} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va}$$

Determination of Tannins

The follins -Dennis spectrophotometric method as described by Pearson (1976) was used. An aliquot (5ml) of the sample was dispersed in 50mls of distilled water and shaken. The mixture was allowed to stand for 30mins at room temperature being shaken every 10mins. At the end of the 30mins, the mixture was filtered through Whatman filter paper and filtrate was used for the experiment.

2mls of the extract was measured into a 50ml Volumetric Flask. Similarly, 5mls of standard tannic acid solution and 5mls of distilled water were measured into separate flasks to serve as standard and blank respectively. They were also diluted with 35mls of distilled water separately 1ml of follins -Dennis reagent was added to each of the flasks, followed by

2.5mls of saturated sodium carbonate solution (Na_2C0_3). The content of each flask was made up to mark (50mls) with distilled water and incubated for 90mins at rooms temperature. The absorbance of the developed colour was measured at 620nm wavelength in a spectrophotometer. Readings were taken with the reagent blank-at zero. The experiment was repeated two more times to get an average.

The tannin content was calculated as shown below:

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \; D$$

Determination of Hydrogen cyanide

10mls of the aqueous extract was mixed in 90mls of water and allowed to stand for 18hrs before filtering. 0.10g of picric acid was measured out and dissolved in 99.9mls of water. 2mls was measured out from the mixture and put in a test tube containing 2mls of the filtrate.

2mls of picric acid dissolved in water-was measured out and dispersed into another test tube containing a small quantity of water and shaken to homogenize. They were read on the photoelectric colorimeter after zeroing it with water in a small test tube. The test tube with the filtrate read = 0.01 under 530nm. The other showed no reading, which indicates a blank reading).

The Hydrogen cyanide content was calculated as shown below:

$$\%\text{Hydrogen cyanide} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \; D$$

Procurement of the Rabbits

Five male rabbits and Five female rabbits between 7 months to 9months and weight 2kg±20g of species *Poelagus marjorita* in the family *Leporidae* were bought from the village of Umunze in Umudioka Anambra state Nigeria. The animals were transported in a cage to Nnamdi Azikiwe University animal house.

The Animals and their Groupings

The animals were kept in separate apartments of the animal house on a 12 hour light/dark cycle at a room temperature of 32±2°C and locked in a wooden cage. The animals were; left for 3 days for acclimatization to the new environment while being fed with vegetable stems, cabbages, paw paw, leaves, palm fruits leaves and hay grasses. They were kept afterwards for just 1 day without feeding to suppress their immunity during which they were watched while they exhibited restlessness till they settled to the new system a bit.

The rabbits were split into five groups; group 1,2,3,4 and 5. Group one comprised of one male and one female rabbits treated with Costus *afer* extracted with Methanol. Group two

comprised of one male and one female rabbits treated with *Costus afer* extracted with N hexane. Group three comprised of one male and one female rabbits to be treated with only carbon tetrachloride. Group four comprised of one male and one female rabbits treated with the mixture of the *Costus afer* extract and carbon tetrachloride. Group Five comprised of one male and one female rabbits which served as control.

Blood and Tissue Collection

Twenty four hours (24hrs) after the last treatment, each rabbit was subjected to light anaesthesia in a chloroform saturated chamber, cleaned of fur and dissected with sterile bladek. The thoracic region was opened and blood drained through direct cardiac punctures and delivered into sterile sample bottles having no anticoagulant. Blood was allowed to stand for 10 minutes and centrifuged for 10 minutes at 4000 rpm in order to collect the serum. The serum was transported in ice chip to Federal Medical Centre, Umuahia for liver function test.

Determination of Aspartate Aminotransferase (AST)

Clean test tube were set up and labeled according to the blood sample collected from each animal. The fifteenth tube served as the blank.

Reagent	**Blank**	**Sample**
Serum Sample		0.1ml
Reagent/Buffer:Phosphate buffer L- aspartate α-oxoglutarate	0.5ml	0.5ml
Distilled water		0.1ml

This was mixed and incubated for 30 minutes at 37^0c

Reagent 2 2,4 –dinitro phenylhydrazine	0.5ml	0.5ml

This was mixed and allowed to stand for 20 minutes at 20^0c

Reagent 3 Sodium hydroxide	5.0ml	5.0ml

The tubes was shaken repeatedly to mix properly and allowed to stand for 5 minutes.

After the time elapsed, the absorbance reading of the sample will be taken against blank at 540nm wavelength.

Determination of Alanine Aminotransferase (ALT)

15 clean test tubes was set up and labeled according to the blood sample collected from each animal. The fifteenth tube served as the blank. The following reagents were pipetted into the respective test tubes as follows.

Reagent	**Blank**	**Test Sample**
Serum Sample		0.1ml
Reagent/Buffer: Phosphate buffer L-aspartate α-oxoglutarate	0.5ml	0.5ml
Distilled water		1.0ml

This was mixed and incubated for 30 minutes at 37^0c

Reagent 2 2,4-dinitro phenylhydrazine	0.5ml	0.5ml

This was mixed and allowed to stand for 20 minutes at 20^0c

Reagent 3 Sodium hydroxide	5.0ml	5.0ml

The tubes were shaken repeatedly to mix properly and allowed to stand for 5 minutes.

After the time elapsed, the absorbance reading of the sample was taken against blank at 540nm wavelength.

Alkaline phosphatase

15 clean test tubes were set up and labeled according to the blood samples collected from each animal and last one was labeled as blank.

The following reagents were pipetted into the respective test tubes as follows.

Test sample	**Blank**	
Reagent/Buffer Diethanolamine buffer $MgCl_2$	3.0ml	3.0ml

Serum sample 0.05ml

Distilled water 0.05ml

The solution was mixed and the initial absorbance read at 410nm. The time was started simultaneously. The absorbance was read again after one minute interval for 3 minutes.

RESULTS

Phytochemical analysis of the *Costus afer* as in table 4.1 below shows that the plant contains all screened bioactive agents between the range of 0.76% to 20.07%. The plant extract contains HCN as the highest phytochemical with steroids as the lowest. From Table 4.3 and 4.4, it showed how bioactive the *Costus afer* stem extract is. From the tables it is clear that *Costus afer* stem extract is bactericidal at certain concentrations but has no effect or antifungal activity. Table 4.5 below shows the effect of *Costus afer* extracts and carbon tetrachloride on liver enzymes. The table shows that the control group representing "E" has the lowest value of ALT, AST and ALP. While sample D (Serum from rabbits treated with CCL4) only has the highest ALT, AST and ALP values.

Table 1 Phytochenical Content of *Costus afer*

	Flavonoid %	Saponin %	Alkaloid %	Tannin %	Phytate %	Phenol %	HCN %	Steroids %
A	0.42	0.74	0.52	0.268	0.321	0.641	21.00	0.079
B	0.46	0.76	0.52	0.270	0.318	0.638	20.92	0.075
C	0.40	0.76	0.52	0.273	0.318	0.640	21.00	0.075
ẍ	0.43	0.75	0.52	0.270	0.319	0.640	20.67	0.076
Sd	0.03	0.02	0	0.003	0.001	0.002	0.046	0.002

Values show Mean of duplicate against ± Standard Deviation.

Table 2 Organisms and Morphological Appearances

Organisms	Morphological Appearance
Escherichia coli	Greenish-metallic sheen in Eosine methylene agar
Staphylococcus aureus	Yellow colour in pink Mannitol salt agar
Candida albican	Hyphal extending from the yeast cell with no constriction
Aspergillus niger	Black circular conidia

Table 3: Mean Zone inhibition of the Conc. Of N-Hexane Extract of *Costus after* stem on the standard organisms measured in Millimeter (mm)

Test Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6mg/ml
Escherichia coli	14.6 ±0.44	9.2±0.55	5.5±0.87	1.64±0.56	0.73±0.35
Staphylococcus Aureus	16.4±0.31	12.6±0.31	7.9±0.07	3.4±1.52	0.4±0.29
Candida albican	0.2±0	0.1±0	0.6±0.2	0.2±0	0.1±0
Aspergillus niger	0.1±0	0.1±0	0.3±0.16	0.4±0.10	0.1±10

Table 4: Mean zone inhibition of the Conc. Of Methanol Extract of *Costus after* stem on the standard organisms measured in Millimeter (mm).

Test Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6mg/ml
Escherichia coli	15.2±0.31	9.3±0.74	5.2±0.32	1.6±0.31	0.3±0.28
Staphylococcus Aureus	17.1±0.42	14.5±0.24	6.8±0.07	4.2±0.38	1.4±0.42
Candida albican	0.2±0	0.1±0.29	0.2±0	0.1±0	0.3±0.16
Aspergillus niger	0.1±0	0.3±0.16	0.1±	0.2±0	0.7±0

Table 5: Effect of *Costus after extracts and Carbon tetrachloride on Liver enzymes*

Sample	ALT (U/L	AST (U/L)	ALP (U/L)
A	48	12	190
B	48	12	145
C	45	13	200
D	98	19	235
E	6	9	60

(p≥0.05) 9.488

(X^2 cal>X^2 tab) = (25.28>9.488).

This means that there is statistically significant difference between the treatment given to the rabbits and their liver enzymes.

Sample A = group of rabbits treated with methanol.

Sample B= group of rabbits treated with N-hexane.

Sample C= group of rabbits treated with the mixture of CCL_4 and *Costus afer* extract.

Sample D = group of rabbits treated with CCL_4 only.

Sample E = group of rabbits serving as control.

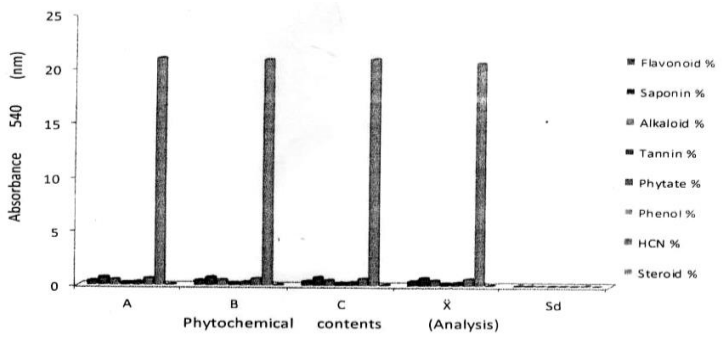

FIG 1. Phytochemical content of *Costus afer*

A, B, C = Values of duplicate result

Ẍ = Mean of Duplicate

Sd = Standard Deviation

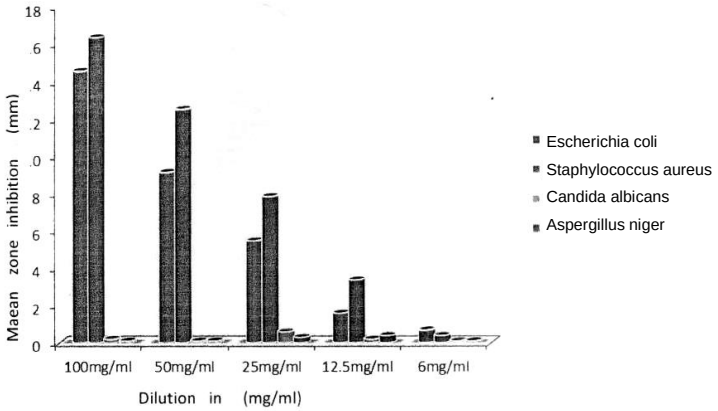

FIG 2. Antimicrobial activity of *Costus afer* stem extract using N-hexane

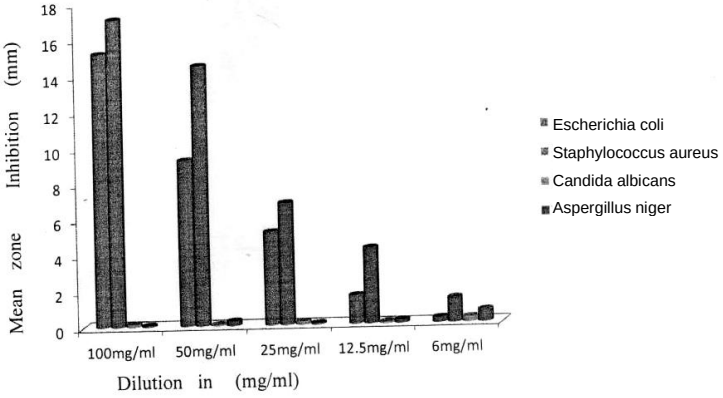

FIG 3. Antimicrobial Activity of *Costus Afer* Stem Extract Using Methanol

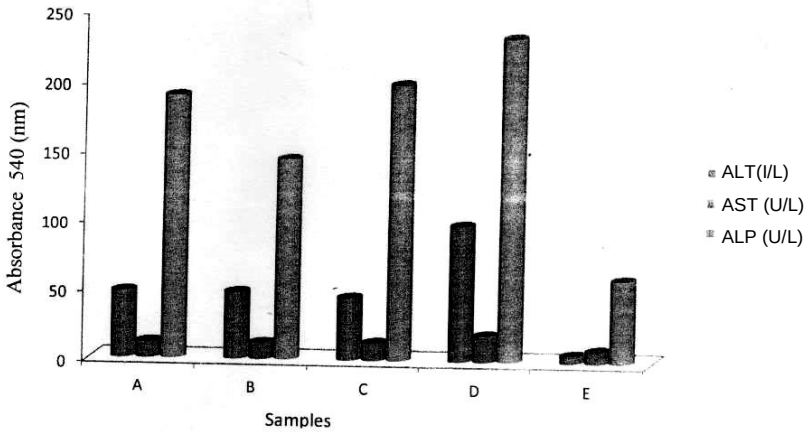

FIG 4. The Effect of Costus Afer Extracts And Carbon Tetrachloride On Liver Enzymes

Sample A = group of rabbits treated with Methanol. Sample B= group of rabbits treated with N-hexane. Sample C = group of rabbits treated with the mixture of CCL_4 and *Costus afer* extract.

Sample D = group of rabbits treated with CCL_4 only. Sample E = group of rabbits serving as control. ALT, AST, ALP = LIVER ENZYMES

DISCUSSION

The use of plant materials in the treatment of illnesses and diseases been reported by many authors. Among which is Hazan and Atta (2005). Little wonder traditional medicine has become integral part of the people's culture.

The bioactive substance screened in the plant extracts are flavonoid. Saponin. Alkaloid. Tannin, Phytate, phenol, hydrogen cyanide and Steroids. The presence of the phytochemicals could account for the hepatotoxic alleviation as observed in table 1. The morphology of the standard organisms are observed in table 2.

These screened phytochemical agents have many mechanisms of hepatotoxicity alleviation of carbon tetrachloride. Hydrogen cyanide transforms acute hepatotoxin-caused by carbon tetrachloride to free radical trichloromethyl by cytochrome p450 -2EI in the microsomal compartment of the liver. Trichlorornethyl and trichloromethyl peroxyl radical interact with membrane lipids leading to their peroxidation which is resolved in melondialdehyde among other metabolites. This accounts for the highest value of hydrogen cyanide as seen in table 1.

Also alkaloids are known to have anti-inflamatory, antifungal and anti-microbial effects as well as anti-hypertensive agent (Sofoworo, 1993) while tannins have been reported to have healing effects (Okwu and Okwu, 2004).

The therapeutic potential of antioxidants in controlling degenerative disease with marked oxidative damage from reactive oxygen species or free radicals have been documented (Anyasor, *et al.,* 2010).

Moreover, phytochemical constituents have been shown to protect against various forms of oxidative related disease via the indications of detoxifying enzymes such as epoxide hydroxylase, glutathione tansferase among others. Also the phytochemicals various intracellular signaling cascaders. Arora (2008).

From the quantitative analysis there was statistically significant difference between the serum samples with the liver enzymes of the rabbits.

The antimicrobial activity of *Costus afer* showed how potential the plant is in terms of eradicating microorganisms. The bioactive potency however is mostly bactericidal rather than fungicidal.

From Table 3 and Table 4.4, it clearly showed maximum bactericidal effect from both *Costus afer* extracts using N-hexane and Methanol solvents in the mean zone inhibition on the standard organisms and its corresponding results in standard deviation.

The elevation of the .levels of AL T (98), AST(19) and ALP (235) in response to the oral administration of carbon tetrachloride showed its hepatotoxic nature. This lends credence to (Doherty 2000).

The toxicity of carbon tetrachloride may require the activation to a highly reactive metabolite via reactions catalyzed by cytochrome p450 (Kennedy, 2011).

Early reports concerning pathological effect of carbon tetrachloride attested that the mitochondria were altered after carbon tetrachloride poisoning of rats Recknagel et al (2002). This toxicity of carbon tetrachloride has been linked to the production of free radicals.

Lipid peroxidation is an autocatalytic process which could cause necrosis in various tissues in the body. Liver injury is defined as an alanine aminotransferase (ALT) level of more than three times the upper limit of the normal range and an alkaline phosphatase (ALP) level of more than twice the upper limit of normal range (Anago et al 2004). Hence, the oral administration of ccl_4, caused liver injury on the animals Table 5.

Liver injury is further characterized as patho-cellular when there is a predominant initial elevation of the ALT level or as cholestatic when there is a predominant initial elevation of the ALP level, a mixed pattern comprises elevations of both the ALT and ALP levels.

From the results, *Costus afer* has the potentials to approximately half the AL T, AST and ALP levels raised by carbon tetrachloride. However, *Costus afe* also raises the liver enzymes beyond normal which is a great concern to both biochemist and anatomists. Nevertheless the mechanism of action remains unknown.

CONCLUSION

The *Costus afer* analyzed contains various phytochemical agents which include Saponin. Alkaloids, Phenol, Flavonoids, Tannins, Phytate, Steroids and HeN with the later having the highest percentage.

The study shows that these phytochemicals act synergistically to reduce the toxicity induced by carbon tetrachloride. These suggest its potential in the treatment and prevention of various oxidative related diseases. The *Costus afer* shows further that it is bacteriocidal in nature with little or no effect on the fungi of study.

Costus afer extracts could be exploited as sources of radical scavengers and bioactive metabolites for nutritional, medicinal purposes. Though the findings in this work are promising, there should be advanced toxicological and pharmacological studies.

RECOMMENDATON

Costus afer extracts could be exploited as sources of radical scavengers and bioactive metabolites for nutritional, medicinal purposes.

Though the findings in this work are promising, there should be advanced toxicological and pharmacological studies.

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