

The phytochemical content, antioxidant, antimicrobial and anti-inflammatory activities of *Lycopersicon esculentum* (Tomato)

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ABSTRACT

In an attempt to explain the scientific basis for the medicinal and nutritional benefits of lycopersicon esculentum (tomato), the phytochemical contents, anti-oxidant, anti-bacteria and anti-inflammatory activity were assessed. In this study the antioxidant properties of lycopersicon esculentum extract were evaluated using Lipid peroxidation scavenging activity and Nitric oxide scavenging activity. The extract were subsequently analysed for their phytochemical constituents, the capability of the extract to inhibit the growth of some isolated bacteria and its anti-inflammatory activity. The result of the phytochemical investigation revealed the presence of carotenoids, phenols, tannin, flavonoids, alkaloid, saponin, phytates and absence of hydrogen cyanide (HCN). The overall lipid peroxidation assay and nitric oxide scavenging activity shows that lycopersicon esculentum possess an antioxidant properties. Agar disk diffusion method was employed to determine the antibacterial activity of lycopersicon esculentum The inhibition of bacteria were found to be concentration dependant from the highest concentration of 250mg to the lowest concentration of 15.5mg. The MIC values were interpreted as the highest dilution (lowest concentration of the sample, which showed clear zone. All tests performed in duplicates. The aqueous extract of lycopersicon esculentum exhibited varying degree of anti-inflammatory activity in dose dependent version and was comparable to that of Aspirin. It could be therefore concluded that the consumption of lycopersicon esculentum would exert both nutritional and health benefits by virtue of their antioxidant, antibacterial and anti-inflammatory activity.

Key words: *Lycopersicon esculentum*, Antioxidant, Antibacterial, Antiinflammatory, Phytochemical

INTRODUCTION

Tomato is one of the most popular of vegetables, used as juice, soup, puree, ketchup or paste. In terms of human health, tomato fruit provide significant quantities of B-carotene, a provitamin A carotenoid, and ascorbic acid [1].

Although densities of ascorbic acid and B-carotene in tomato are modest compared to some other vegetables, tomato ranks high as a source of vitamins A and C in human diets because of high consumption in many countries of the world [2]. In addition to their importance as a provitamin or vitamin, B-carotene and ascorbic acid also serve as antioxidants; B-carotene functions to help prevent and neutralize free radical chain reactions and ascorbic acid is an effective scavenger of superoxide, hydrogen peroxide, singlet oxygen and other free radicals. In living organisms the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including inflammation, Parkinson and coronary heart pathologies, among others [3].. Since the natural antioxidant mechanism in mammals under some circumstances can be inefficient, a dietary intake of antioxidant compounds becomes an alternative, once it has been suggested that there are an inverse relationship between dietary intake of antioxidants and the incidence of disease caused by the deficiency on these substance. In recent years, researchers are kin to ascertain the level of antioxidant activity of some plant, in which

tomato is the typical example. [4] While most often associated with lycopene (a carotenoid phytonutrient widely recognized for its antioxidant properties), tomatoes provide a unique variety of phytonutrients. Included are additional carotenoids (including beta-carotene, lutein, and zeaxanthin); flavonoids (including naringenin, chalconaringenin, rutin, kaempferol, and quercetin); hydroxycinnamic acids (including caffeic, ferulic, and coumaric acid); glycosides (including esculeoside A); and fatty acid derivatives (including 9-oxo-octadecadienoic acid). Tomatoes are also an excellent source of free radical-scavenging vitamin C and vitamin A as well as bone-healthy vitamin K. They are a very good source of enzyme-promoting molybdenum; heart-healthy potassium, vitamin B6, folate, and dietary fiber; blood sugar-balancing manganese. In addition, tomatoes are a good source of heart-healthy magnesium, niacin, and vitamin E; energy-producing iron, vitamin B1, and phosphorus; muscle-building protein, and bone-healthy copper. [5]

Lycopene, the major carotenoid in tomato fruit, is a powerful antioxidant, anti-inflammatory and also has an antimicrobial property and has generated much attention because of the linkage between lycopene-rich diets and lower risks of certain cancers, heart disease, and age-related disease. Although most phenolic compound in tomato fruit have disappeared at maturity, fully ripened fruit contains modest quantities of quercetin-3-O-rutinoside, which like other flavonols is a powerful antioxidant associated with reduced cancer risk. [6].

From this point of view, we report our work on the antioxidant activities through the, Nitric oxide scavenging activity and Anti lipid peroxidation assay. And also the antimicrobial and anti inflammatory activity property of tomato

MATERIALS AND METHODS

SAMPLE PREPARATION:

Fresh ripe tomatoes were bought from our daily market Isigate Umuahia, Abia State and washed thoroughly under running water. Afterwards grind, pour in a beaker and heat to remove excess moisture, and also to activate the lycopene. 2grams of the extract was weighed and mixed in 100ml of distill water [20mg/ml].

PHYTOCHEMICAL INVESTIGATION

The extracts were subjected to quantitative chemical test. Aqueous extracts revealed the presence of carotenoids, tannins, alkaloids, phenol, flavonoids, and absence of hydrogen cyanide (HCN).

Determination for Tannin

Tannin content of the sample was determined by Folin Denis colometric method (Kirk and Sayer 1998). A measured weight of the processed sample (5.0g) was mixed with distilled water in the ratio of 1:10(w/v). The mixture was shaken for 30 minutes at room temperature and filtered to obtain the extract.

A standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric Flasks to serve as standard and reagent blank respectively. Then 2mls of each of the sample extracts was put in their respective labeled Flask.

The content of each Flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5mls of saturate Na_2CO_2 solution. Thereafter each flask was diluted to the 50ml mark with distilled water and incubated for 90 minutes at room temperature. Their absorbance was measured at 760nm in a spectrophotometer with the reagent blank at zero. The Tannin content was calculated as show below.

$$\% \text{ Tannin} = 100/w \times \text{au}/\text{as} \times c \times \text{vt}/\text{va}$$

w =weight of sample

au =absorbance of test sample

as =absorbance of standard tannin solution

c =concentration of standard tannin solution

va =volume of extract analyzed.

Determination of Alkaloid

The Alkaline precipitation gravimetric method [7] was used.

A measured weight of the processed sample (5g) was dispersed in 100mls of 10% acetic acid in ethanol solution. The mixture was shaken well and allowed stand for 4 hours at room temperature being shaken every 30 minutes. At the end of this period, the mixture was filtered through what man No 42 grade of filter paper.

The filtrate (Extract) was concentrated by evaporation, to a quarter of its original volume the extract was treated with drop wise addition of concentrated NH_3 solution to precipitate the alkaloid. The dilution was done until the NH_3 was in excess.

The alkaloid precipitate was removed by filtration using weighted whatman No 42 Filter paper. After washing with 1% NH_4OH solution, the precipitate in the filter paper was dried at 60°C and weighted after cooling in desiccators. The content was calculated as shown below.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W_t \text{ or sample}} \times \frac{100}{1}$$

Where

W_1 = weight of empty filter paper

W_2 = weight of filter paper + alkaloid ppt.

Determination of Phenol

This was determined by the Folin-ciocateau spectrophotometer [8]. The total phenol was extracted in 200mg of the sample with 10ml concentrated methanol. The mixture was shaken for 30minutes at room temperature. The mixture was centrifuged at 500rpm for 15 minutes and the supernatant (extract) was used for the analysis.

1ml portion of the extract from each sample was treated with equal volume of Folin-ciocateau reagent followed by the addition of 2mls of 2% Na_2CO_3 solution. Mean while, standard phenol solution was prepared and diluted to a desired concentration.

1ml of the standard solution was also treated with the F-D reagent and Na_2CO_3 . The intensity of the resulting blue colouration was measured (absorbance) in a spectrophotometer at 560nm wavelength. Measurement was made with a reagent blank at Zero. The phenol content was calculated using the formula below.

$$\% \text{ phenol} = \frac{100}{W} \times \frac{au}{as} \times c \times \frac{vt}{va}$$

Where

W =weight of sample

au =absorbance of test sample

as =absorbance of standard phenol sample

c =concentration of standard phenol sample

va =volume of extract analyzed.

Determination of Flavonoids

Flavonoid was determined using the method described by Harbone [9] A measured (5g) weight of the processed sample was boiled in 100mls of 2MHCL solution under reflux for 40 minutes. It was allowed to cool before being filtered. The filtrate was treated with equal volume of ethyl acetate (contained in the ethyl acetate portion) was received by filtration using weighted filter paper. The weight was obtained after drying in the oven and cooling in a desiccator. The weight was expressed as a percentage of the weight analyzed. It was calculated as shown below:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{W_t \text{ of sample}} \times \frac{100}{1}$$

W_2 =weight of filter paper x flavonoid precipitate

W_1 =weight of filter paper alone

Determination of Hydrogen Cyanide (HCN)

This was determined by alkaline pikrate colourimeter method 1.02g of the sample was dispersed in 50ml of distilled water in a 25.0ml conical flask. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks.

The set up were incubated overnight and each pikrate paper was eluted (or dipped) into a 60ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and the standard were measured spectrophotometrically at 540nm wavelength with the reagent blank at zero.

The cyanide content was determined by the formula shown below:

$$\text{HCN mg/kg} = 1000/w \times \text{au/as} \times c \times D$$

Where

W=weight of sample analyzed

au = absorbance of standard HCN solution

c= concentration of the standard in mg (d)

D=dilution factor where applicable.

NITRIC OXIDE SCAVENGING ASSAY:

Nitric oxide scavenging activity was measured spectrophotometrically. The prepared extract was added to different test-tubes in varying concentrations [2.5, 2.0, 1.25, 1.0, 0.5 mg/ml] 1ml of each the concentrations above was pipette into another different five test-tube. 1ml of Sodium nitroprusside (5mM) in phosphate buffer was added to each test-tube bringing the volume to 2ml. solution was incubated at room temperature (25°C) for 30minutes. Thereafter 2ml of Griess reagent [1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid] was added to each test tube. The absorbance was measured immediately at 530 and percentage of scavenging activity was measured with reference to ascorbic acid as standard. Distilled water was used as the control.

ANTI-LIPID PEROXIDATION ASSAY:

The study has been performed on the goat liver using common laboratory marker of lipid peroxidation like measurement of the malondialdehyde (MDA) content of tissue.. Goat liver perfused with normal saline through hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were then transferred in a sterile vessel containing phosphate buffer (PH 7.4) solution. After draining the buffer solution as completely as possible, the liver was immediately grinded to make a tissue homogenate (1g/ml) using freshly prepared phosphate buffer (PH 7.4.)

Anti-lipid peroxidation scavenging activity was measured spectrophotometrically. The prepared extract was added to different test-tubes in varying concentration [2.5, 2.0, 1.25, 1.0, 0.5 mg/ml] 1ml of each the concentrations above was pipette into another different five test-tube. 3ml of liver homogenate was added 1ml of 15Mm ferric chloride solution and was shaken for 30mins. From collected mixture, 1ml was added with 1ml of different concentration of the plant extract in different test-tubes. The same procedure was followed for control and blank. Water was used as a control and ascorbic acid (100mg/ml) as standard. All the test-tubes were incubated for 120mins at 37°C. After incubation, Trichoroacetic acid (TCA) was added to all the tubes containing the mixture in 1:1 ratio and centrifuged for 30mins. The supernatant liquid was collected thiobarbituric acid (TBA) was added in 1:1 ratio and heated for 60mins in water bath, cooled and absorbance was measured at 530nm.

By using the following formula the percentage of anti-lipid peroxidation activity was calculated

$$\% \text{antilipid peroxidation} = \frac{(\text{control-sample})}{(\text{Control})} \times 100$$

ANTI BACTERIA ACTIVITY OF TOMATOES

the concentrations of ciprofloxacin and tomato extract used were as follows; 250mg, 125mg, 62.5mg, 31.5mg and 15.5mg

DETERMINATION OF ANTI-BACTERIAL ACTIVITY

Test microorganism

For this study, *streptococcus pneumonia*, *staphylococcus aureus* *Escherichia coli*, *pretoeus mirabilis*, *pseudomonas aeruginosa* were used as test organism. All these organisms were obtained from the culture collection of the department of microbiology, Michael Okpara University of Agriculture, Umudike.

Sterile 1ml dropper pipettes were used to deliver 0.1ml of each concentration onto punched sterilized circular (3mm diameter) filter papers (what mann number 1). The extract impregnated disc were dried at 40°C, packed into sterile

bottles, labeled and stored at 8⁰c prior to use Thus, the final concentrations of extract per disc in descending order was 250mg, 125mg, 61.5ml, 31.25mg and 15.5mg (Egwari, 1999 10).

SCREENING FOR ANTIBACTERIAL ACTIVITY OF EXTRACTS

The disc diffusion method was used in this study. The test organisms (1:100 dilution of an 18hr broth cultures) were inoculated onto Mueller Hinton agar plates with sterile cotton swabs (sterlin) soaked in the inoculate. Discs of different extract concentrations were placed firmly on the surface of the inoculated agar plates and incubated at 37⁰c for 18hr under aerobic conditions. Zones of inhibition were measured and recorded in millimeters [10].

C) Minimum Inhibitory Concentration

The MIC values were interpreted as the highest dilution (lowest concentration of the sample, which showed clear zone. All tests performed in duplicates.

ANTI-INFLAMMATORY ACTIVITY OF TOMATO

A total of 24 adult white Wister albino rats of both sexes and of average weight of 220g were used. They were placed in cages and grouped into six (A-F) of 4 per a group. They were then left to acclimatize to laboratory environment for four days. The animals were deprived of feed for 12hrs prior to the experiment but were allowed access to pure drinking water. The animals were weighed and crude extract and aspirin were separately administered intraperitoneally. Group A was used as negative control thus, received neither Aspirin nor the crude extract. Group B received intraperitoneally 50mg/kg of the extract. Group C received 100mg/kg. Group D received 200mg/kg of the crude extract intraperitoneally, while Group E received 250mg/kg of crude extract intraperitoneally. Then Group F was used as positive control thus, received 100mg of aspirin intraperitoneally. The animals were then left for 30mins after which 1ml of fresh egg albumen was injected into the sub-plantar of the right hind paw of each of the rat. Using a 10ml-measuring cylinder containing water up to 9ml mark the volume of water displaced per drug/crude extract administrations and post injection of fresh egg albumen were measured and recorded. The difference in the displaced volume of water was taken as the weight of the oedema. Scoring the degree of erythema was done based on the degree of spread of erythema towards the ankle. Precautions were taken to avoid the water from touching the hairs of the rats while the paws were allowed free fall into the water. The weight of the paw-oedema was taken at 30mins interval for 4hrs and taken lastly at 12hrs. Percent inflammations were calculated with non-treated animals as control.

Thus: % inflammation = $\frac{ct}{Co} \times 100$

RESULTS

NITIC OXIDE SCAVENING ACTIVITY

Table 1

CONC.(mg/ml)	% INHIBITION	IC ₅₀
2.5	79.9 ± 0.56	
2.0	63.9 ± 0.36	
1.25	54.4 ± 0.80	1.21
1.0	30.8 ± 0.32	
0.5	15.9 ± 3.15	
STD[vit c]1mg	40.3 ± 0.74	

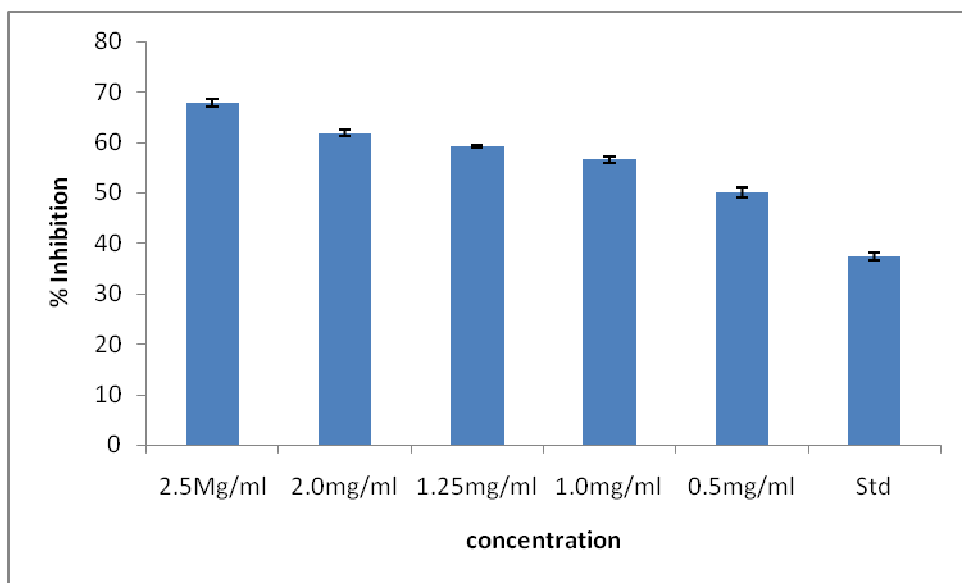


Fig. 1a

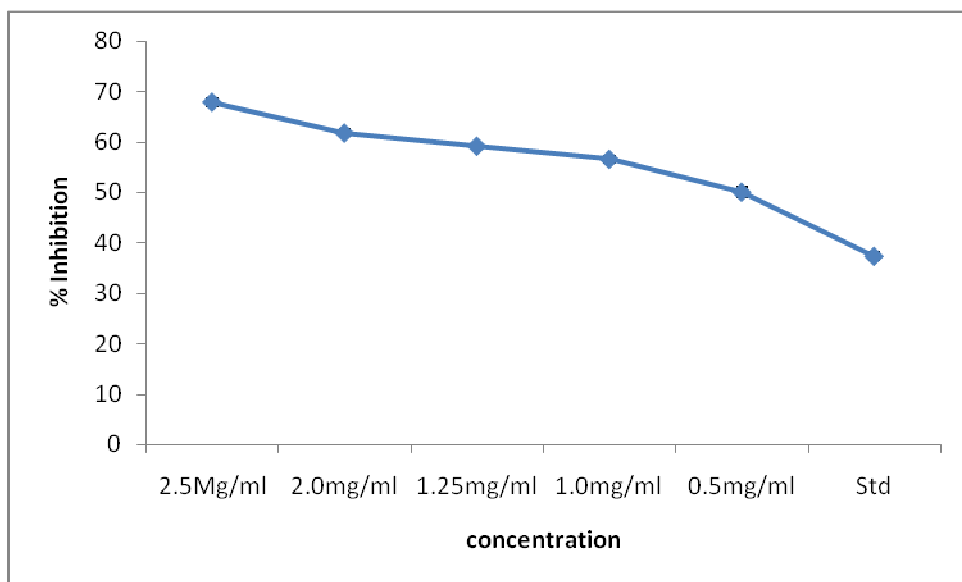


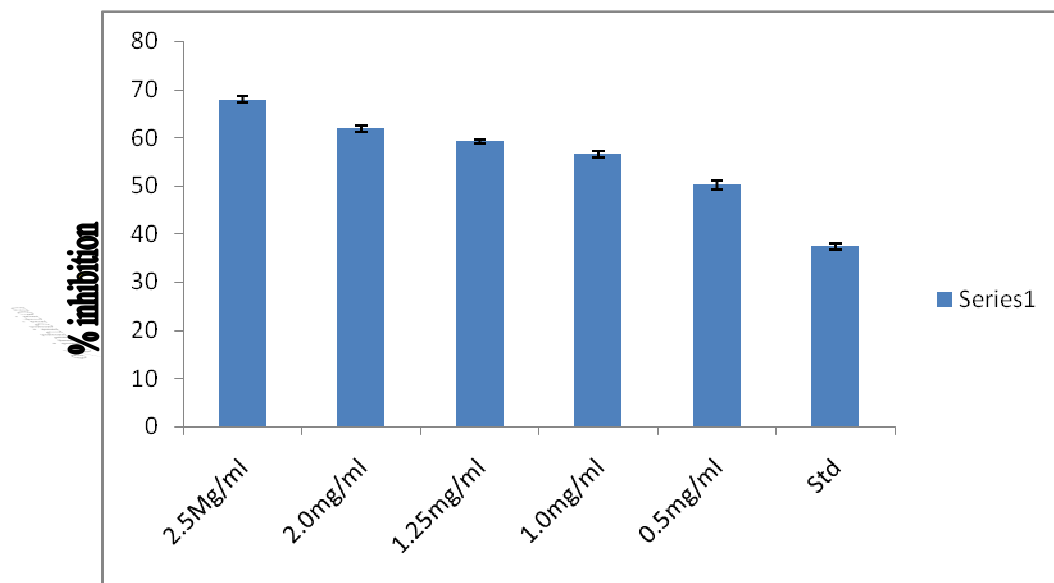
Fig. 1b

ANTI-LIDIP PEROXIDATION ACTIVITY

Table 2

CONC.(Mg/Ml)	% INHIBITION	IC ₅₀
2.5	67.1 ± 0.70	
2.0	61.8 ± 0.60	
1.25	59.2 ± 0.35	
1.0	56.6 ± 0.70	
0.5	50.1 ± 0.96	0.3
STD	37.4 ± 0.69	

BAR-CHART PRESENTATION



Concentration
Fig 2a

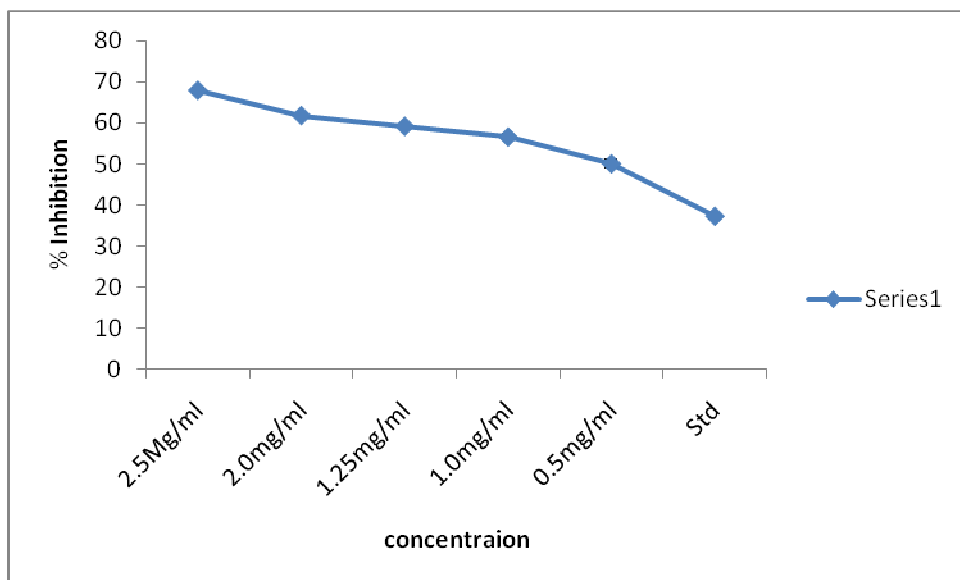


Fig.2b

ANTIBACTERIA RESULT

Table 3a

Bacteria	Zone of inhibition in mm					MIC
	250mg	125mg	62.5mg	31.25mg	15.5mg	
<i>Straph. aureus</i>	26.50 ± 0.71	19.50 ±0.71	11.50 ± 0.71	5 ± 1.41	0	31.25
<i>Strep. Pneu</i>	28.50 ±0.71	21.50 ±0.71	9 ± 1.41	1.5± 0.71	0	31.25
<i>E. coli</i>	23.50 ±0.71	18.50 ±0.71	12 ± 1.41	0.5± 0.71	0	31.25
<i>Proteus Mirabilis</i>	25 ± 1.41	21 ± 1.41	8.50 ± 0.71	0	0.5 ±0.71	15.5
<i>Pseudomonas aeruginosa</i>	17 ± 1.41	14.50 ±0.71	5 ± 1.41	0	0	62.5

Anti-Bacteria Activity of Ciprofloxacin

Table 3b

Bacteria	Zone of inhibition in mm					MIC(mg)
	250mg	125mg	62.5mg	31.25mg	15.5mg	
<i>Straph. aureus</i>	43± 1.41	30.50± 0.71	17.50± 0.71	10.50± 0.71	2.50± 0.71	15.5
<i>Strep. Pneu</i>	39.50±0.71	29± 1.41	18± 1.41	11.50± 0.71	3.50± 0.71	15.5
<i>E. coli</i>	39± 1.41	25± 1.41	18.50± 2.12	8.50± 1.41	2± 0.00	15.5
<i>Proteus Mirabilis</i>	40.50± 0.71	39± 1.41	21.50± 0.71	9± 1.41	3.50± 0.71	15.5
<i>Pseudomonas aeruginosa</i>	28.50± 0.71	26.50± 0.71	17± 1.41	5.50± 0.71	1.5± 0.71	15.5

ANTI-INFLAMMATORY RESULT

Table 4a

Doses	30mins	60mins	90mins	120mins	150mins	180mins
Normal saline. Neg.control	14.91± 0.41	15.38± 0.76	19.28± 0.45	22.02± 0.29	18.81± 0.27	18.45± 0.64
250mg/ml	9.68± 0.60	8.16± 0.09	7.06± 0.09	6.32± 0.06	5.60± 0.04	4.46± 0.06
200mg/ml	11.21± 0.04	10.21± 0.30	9.05± 0.07	8.06± 0.08	7.69± 0.18	6.90± 0.14
100mg/ml	12.73± 0.18	11.35± 0.21	10.27± 0.07	9.15± 0.07	8.91± 0.01	8.28± 0.11
50mg/ml	13.46± 0.51	12.58± 0.03	11.21± 0.30	10.05± 0.07	10.69± 0.01	9.83± 0.04
Aspirin 100mg/ml	6.90± 0.42	6.25± 0.07	5.30± 0.14	4.16± 0.08	2.44± 0.02	1.35± 0.07

Table 4b result showing the calculated percentage inhibition (co-ct/co x 100)

Doses	30mins	60mins	90mins	120mins	150mins	180mins
250mg/ml	35.08	46.94	63.38	71.30	70.23	75.83
200mg/ml	24.82	33.62	53.06	63.40	59.12	62.60
100mg/ml	14.62	26.20	46.73	58.45	52.63	55.12
50mg/ml	9.73	18.21	41.86	54.36	43.17	46.72
Aspirin 100mg/ml	53.72	59.36	72.51	81.11	87.03	92.68

PERCENTAGE INHIBITION

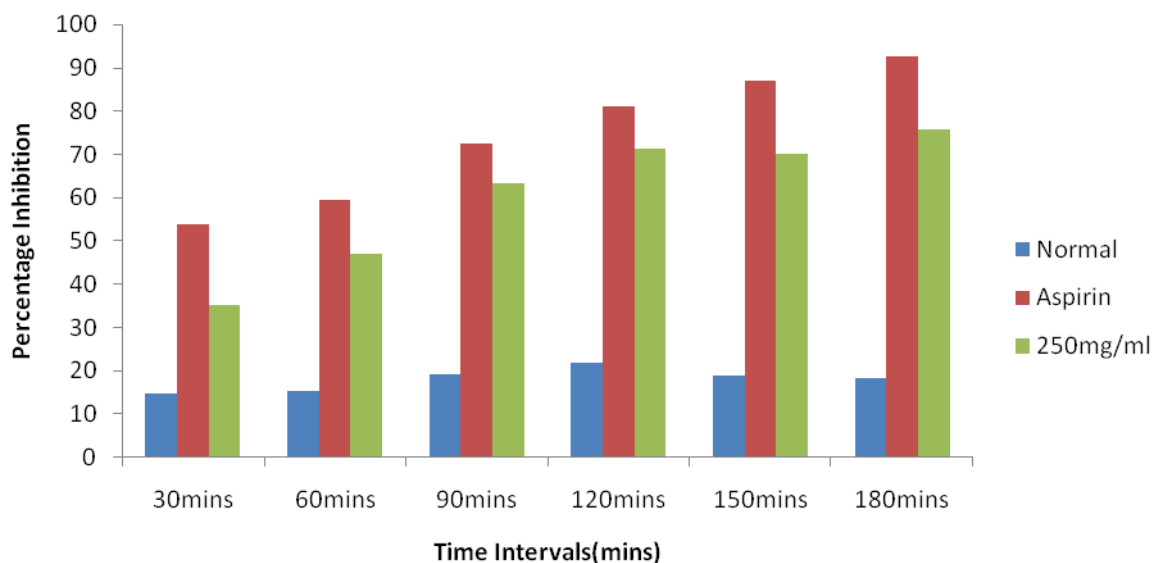


Fig.4a

Percentage inhibition when 250mg/ml of extract was administered.

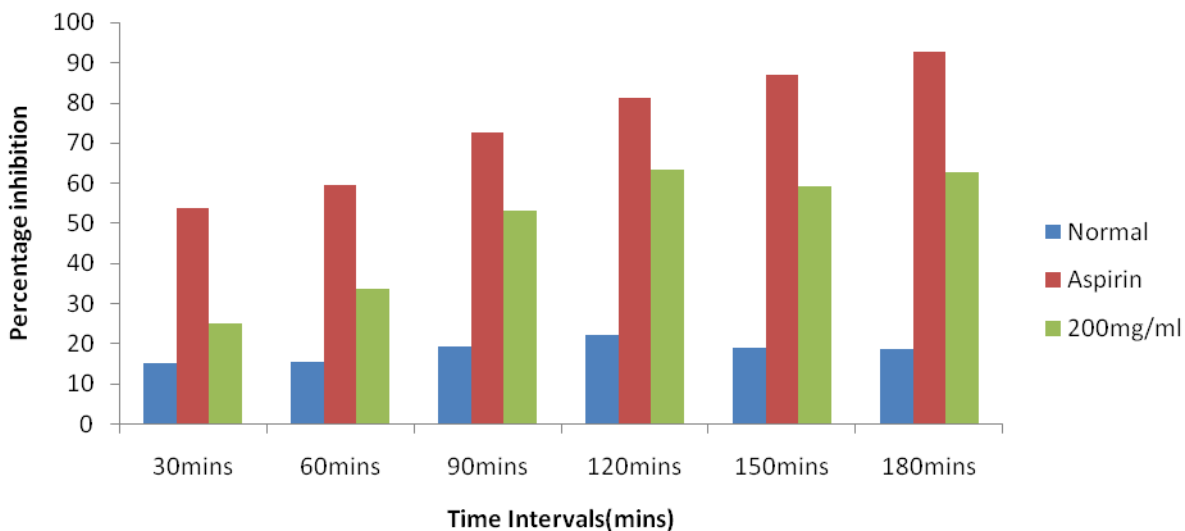


Fig.4b

Percentage inhibition when 200mg/ml of extract was administered.

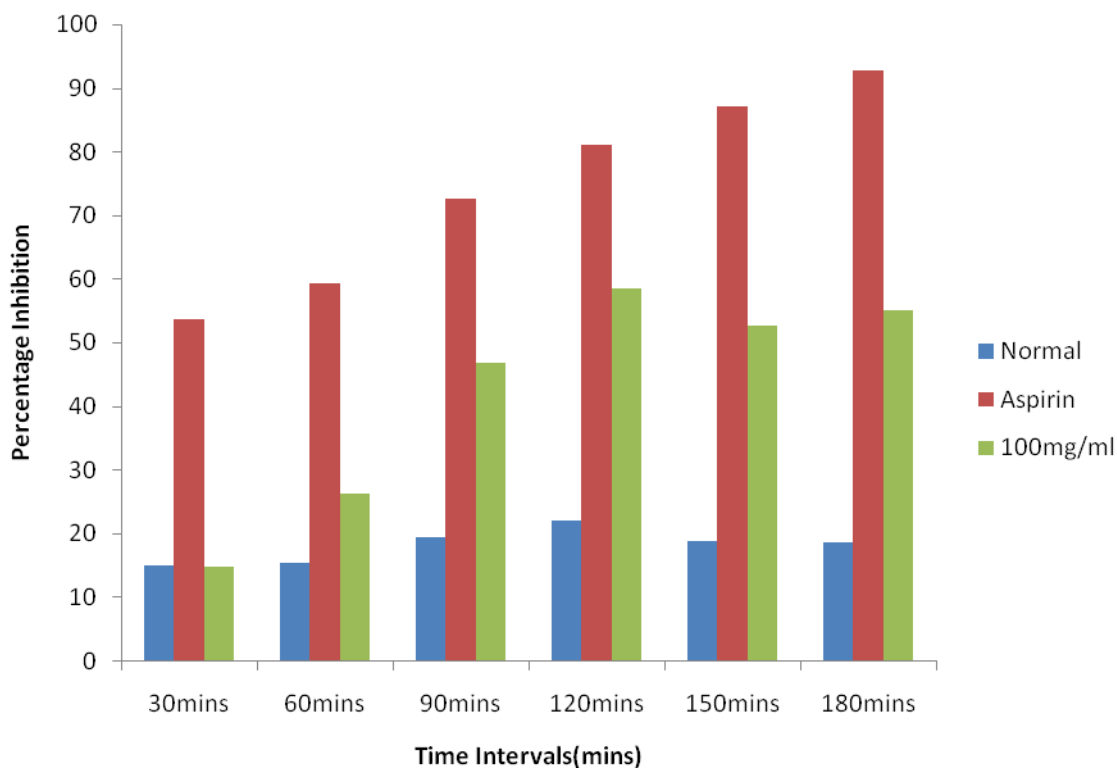


Fig.4c

Percentage inhibition when 100mg/ml of extract was administered.

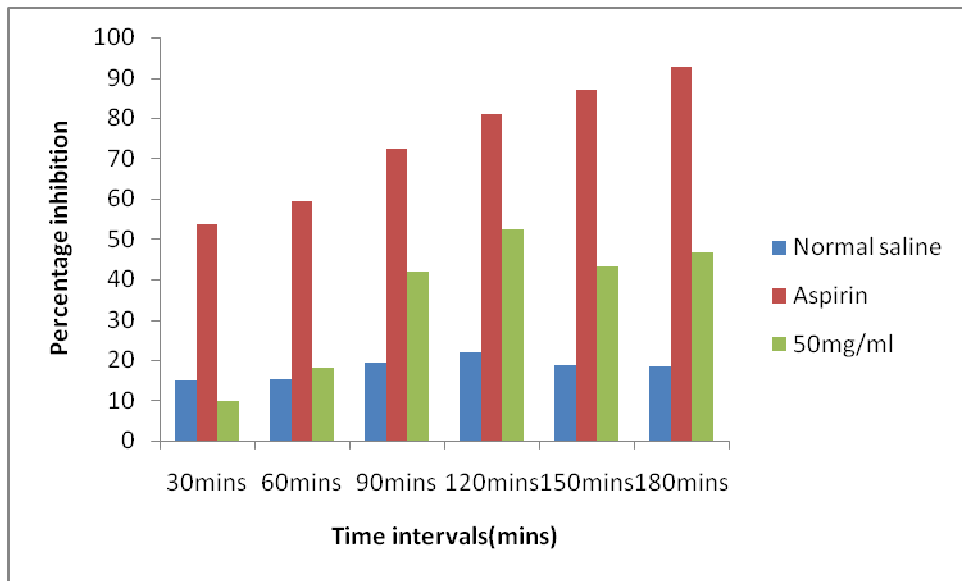
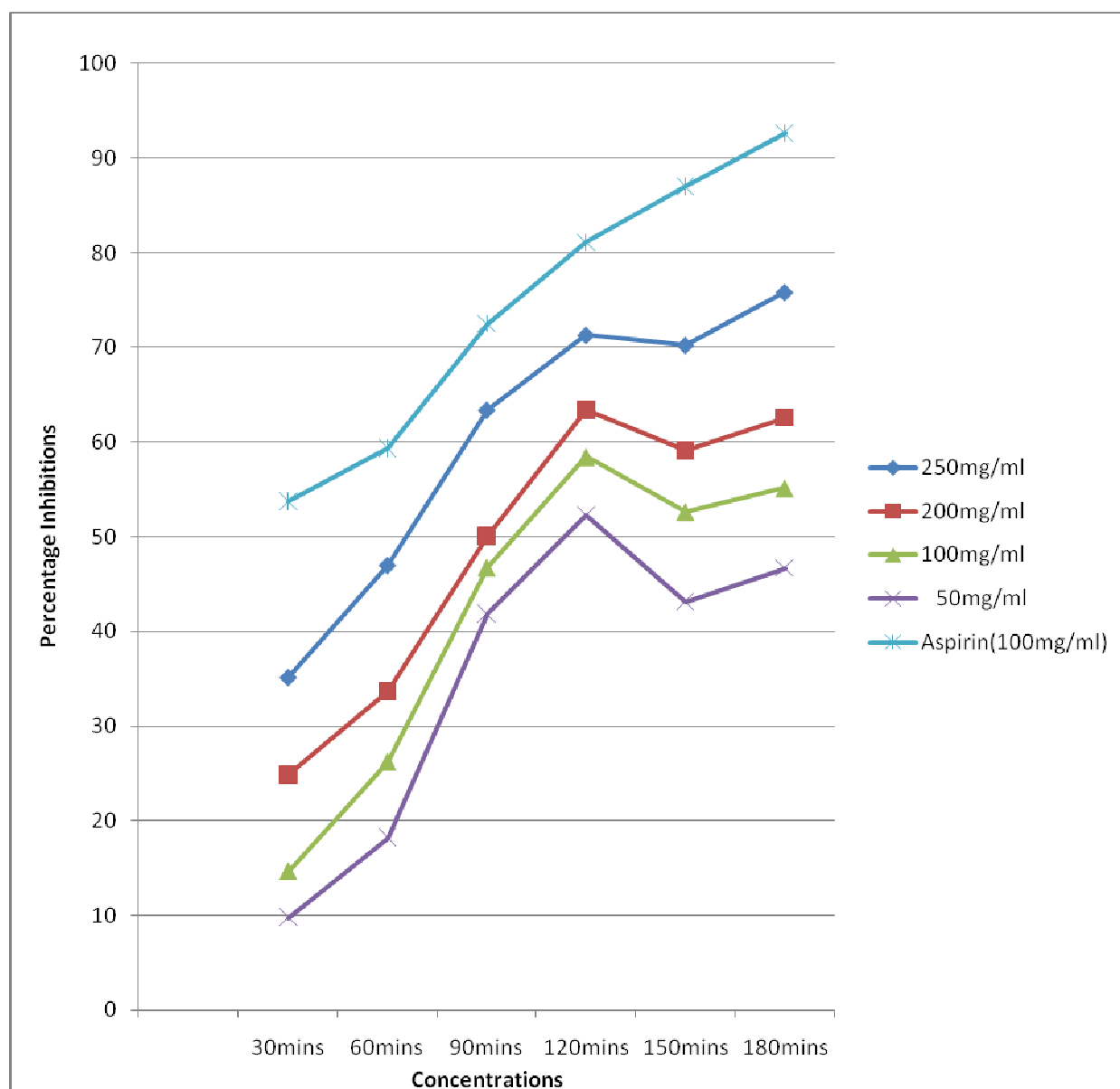


Fig.4d

Percentage inhibition when 50mg/ml of extract was administered.



Graph presentation showing Percentage inhibition when different concentrations of extract were administered.

DISCUSSION

A majority of prospective and case-control study epidemiology studies support the hypothesis that diets rich in tomatoes and tomato products are associated with reduced risk of chronic disease. In vitro studies and animal trials using tomato phytochemicals have provided further data supporting these epidemiology associations.

Oxidation stress has been implicated in the pathology of many disease and conditions including cardiovascular disease, diabetes, inflammatory conditions, cancer and aging. Anti-oxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per-oxidation and other mechanisms[11]. The lycopersicon esculentum extract were able to reduce thiobarbituric acid reactive substance (TBARS) significantly when compared with a standard antioxidant ascorbic acid(table1and figure1a and1b). The sample is found to inhibit lipid peroxidation significantly in goat liver homogenate which indicate the strong free radical scavenging and anti-lipid peroxidation properties(table 2 and figure2a and 2b). This research work demonstrates that daily consumption of lycopersicon esculentum might be helpful in preventing or suppressing the progress of various oxidative stress related disease. And also investigation of antibacterial activity of tomato extract on some of the isolated microbes ranging from *streptococcus pneumonia*, *staphylococcus aureus* *Escherichia coli*, *pretoeus mirabilis*, *pseudomonas aeruginosa* really show that tomatoes has an antibacterial properties when compare with Ciprofloxacin an antibacterial drug base on their minimum inhibitory concentration parameter(table3a and 3a figure3a and 3b). Both

the tomatoes extract and the standard (Ciprofloxacin) in the following concentrations of 250mg, 125mg, 61.5ml, 32.25mg and 15.5mg in descending order. Inhibition effects of tomato extract were noticeable on the 250mg, 125mg, 62.5mg and 31.25mg, only on 15.5mg parameter there was no inhibition on the microbes. Minimum inhibitory concentration (MIC) for *staphylococcus aureus*, *streptococcus pneumonia* and *Escherichia coli* was observed at 31.25mg and for *pretoeus mirabilis* and *pseudomonas aeruginosa*. The antibacterial activity are more pronounced at higher concentrations than at the lower concentration Against the standard (Ciprofloxacin) all the microbes are inhibited even at the minimum concentration of 15.5mg. Consumption of tomatoes daily can be of needed help for effectively control of bacterial pathogen.

Inflammation can be defined as a reaction of a living cell or tissue to injury, infection or irritation/infiltration. Inflammation is characterized by pains, swelling, redness and heat/fever. Inflammation could be induced by conditions that bring about the release of inflammatory mediators such as Histamine, Prostaglandins, Nitric oxide and cytokines. Prostaglandins are released by a host of mechanical, thermal, chemical, bacterial and other insults, and they contribute importantly to the signs and symptoms of inflammation. A wide range of immunological disorders also result in abnormal inflammatory reactions. Notably among are inflammatory bowel disease, asthma/other respiratory tract diseases, rheumatic fever and recently in age-related Macular degenerative disease.[12, 13]

The tomatoes extract exhibited varying degree of anti-inflammatory activity in dose dependent version and was comparable to that of Aspirin(tab or fig). Mechanism that possibly undermine this anti-inflammatory activity include inhibition of the actions of inflammatory mediators such as Histamine, Prostaglandins, Nitric oxide, Cytokines, effect on adrenocorticoid hormone and immunosuppression. Morphine is an established analgesic as well as an inflammatory drug and since its like alkaloids is found in tomato.

CONCLUSION

This research established the fact that tomatoes can be used to treat diverse diseases ranging from cancer, artherosclerosis, hypertension and etc. This conclusion is as a result of the abundant of antioxidant present in tomatoes. Its antimicrobial effects on some selected clinical isolates confirmed its usefulness as antibiotic in the treatment of infectious diseases. This study further shows that tomatoes extract possesses anti-inflammatory properties, it can therefore be used to treat rheumatoid arthritis. We conclude that tomatoes possess pharmacological properties which if properly harnessed can be used in the management of diseases.

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