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# Antibacterial Effects of Gongronema Latifolium (Utazi) Leaf and Jatrophacurcas (Barbados) Leaf Extracts on Some Clinical Bacterial Isolates

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## ABSTRACT

Methanol and water extracts of J. curcas and G. latifolium were prepared in different concentration of 0.5mg/l to 5mg/l. Methanol extract showed more inhibitory properties over water extract. Gongrenema latifolium exhibited high inhibitory effect than J, curcas. The diameter of zone of inhibition of J. curcas ranges from 6mm to 10mm and 6mm to 14mm for water and methanol extracts respectively while the diameter zone of inhibition for G. latifolium range from 6mm to 10mm and 6mm to 26mm for water and methanol extract respectively. The minimum inhibitory concentration of J. curcas on E. coli was 0.5mg/l and 2.5mg/l on S. aureus and Salmonella typhi. The minimum inhibitory concentration (MIC) of G. latifolium on E. coli was lmg/1 while S. aureus and Salmonella typhi was 2.5mg/l. Therefore, the result obtained indicated that the extracts of Gongronema latifolium and Jatrophacurcas possess antibacterial activities against Staphylococcus aureus, Salmonella typhi and Escherichia coli. Jatrophacurcas are efficacious for the remedy of boils, carbouncle and other entero-infection cause by Staphylococcus aureus, E. coli and Salmonella typhi.

**Keyword:** Antibacterial, Gongronema latifolium (Utazi), Jatrophacurcas

## INTRODUCTION

The treatment and control of disease by the use of available medicinal plants in a locality will continue to play significant role in medical health care implementation in the developing countries [1].The intractable problems of antimicrobial resistance have led to the resurgence of interest in herbal products as sources of noble compound to suppress or possible eradicate the ever-increasing problems of emergence of newer diseases though be brought under control [2].There is a growing interest in plants with antimicrobial activity. Scientist are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects identifying the compounds responsible for the antimicrobial properties [3,4, 1].Traditional medicine using plants extract continues to provide health coverage for over 80% of the world's population, especially in developing countries [5].

Jatropha species belong to the family Euphorbiaceeae and are used in traditional for folk medicine to cure ailments in Africa, Asia and Latin America. Jatrophacurcas is commonly called physic nut Barbados nut and purging. Previous studies have reported th eplants exhibits bioactive activities for fever, mouth infections, jaundice, guinea and joint rheumatic [6,7] reported that anti-parasitic activity of the sap and crushed leaves of J. curcas. The water extract of the also strongly inhibited HIV induced cytopathic effects with low cytotoxicity. Previous works have shown that many Jatropha species possess antimicrobial activities. In general, the leaves are green to pale green alternate to sub-opposite and three to five labeled with a spiral phyllotaxis [8].Herbs that have tannin as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [9].

Gongronema latifolium, known as Utazi in the south-eastern and Adkeke in the South Western part of Nigeria, is a tropical rainforest plants which belongs to the family Asclepiadaceae [10].

Staphylococcus is a group of bacteria that can cause a number of diseases as a result of infection of various tissues of the body. Staphylococcus-related illness can range from mild and requiring no treatment to severe and potentially fatal. Staphylococcus bacteria look like a bunch of grapes or little round berries. They are gram positive, facultative anaerobic, usually un-encapsulated cocci [11]. Over 30 different types of Staphylococci can infect humans, but must infections be caused by Staphylococcus aureus. Staphylococci can be found normally in the nose and on the skin (and less commonly in other locations) of around 35 to 30% of healthy adults and in 25% of hospital workers[12].

## MATERIALS AND METHODS

### Collection Of Plant Sample

Fresh leaves of Jatrophacurcas (Barbados leaf) and Gongronema latifolium (Utazi) were collected from the orchard of Michael Okpara University of Agriculture Umudike, Abia State, South-East of Nigeria. The plant materials were

authenticated and identified in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

**Materials Used**

Weighing balance, hot air oven, autoclave, lest tubes, measuring cylinder, petri-dishes, microscope, test tube racks, wire loop, aluminum foil, burnsen burner, masking tape, water bath, incubator, marker, hand gloves, cover slip, cotton wool, filter paper, nose mask, glass slides, electric blender, Durham tubes Gentamycin and Ciprofloxacin

**Media Used**

The media used for the isolation of Escherichia coli Eosin Methylene Blue (EMB), Salmonella typhi was Salmonella Shigella Agar (SSA) and for Staphylococcus aureus was Mannitol salt agar (MSA). They were prepared according to the manufacturer's direction. They were sterilized by autoclaving at 121°C for 15 minutes at 15 psi, except for SSA which was boiled at 45°C before plating out.

**Reagents Used**

Methanol, crystal violet, safranin, hydrogen perioxide, lugols iodine, distilled water, kovacs reagent, lactose, glucose, biju bottles, phenol red indicator and peptone water.

**Preparation Of The Pant Extract**

The freshly collected plants were rinsed in distilled water. The leaves were air dried, initially and finally oven dried at 60°C for 24 hours. The leaves were grounded into powdery form using electric blender. The grounded extracts were stored in air tight sterile containers and kept at room temperature for use.

**Methanol Extract Preparation**

Ten gram of the powder leaves was weighed. The 10g was dispensed into a sterile conical flask (250ml) and 100ml of methanol was poured into it and left for 24 hours with occasional stirring at room temperature. The plant extract was then transferred to a separating funnel from where the filtrate was collected using Whatman No 1 filter paper which was inserted into the funnel. The filtrate was transferred to a water bath where the methanol solvent was evaporated. The extracts were later obtained and lyophilized at 0-4°C until when needed.

**Cold Water Extract Preparation**

Ten gram of the grind leaves was weighed. The 10g was dispensed into a sterile conical flask (250ml) and 100ml of water was poured into it and left for 24 hours with occasional stirring at room temperature, the plant extract was then transferred to a separating funnel from where the filtrate was collected using Whatman No. l filter paper which was inserted into the funnel. The filtrate was transferred to a water bath where the cold-water solvent was evaporated. The extracts were later obtained and lyophilized at 0- 4°C until when needed. Plant 1: Gongronema latifolium.

**Figure 1**: Gongronema latifolium[9]

**Figure 2:** Jatrophacurcas [9]

**Isolation Of Microorganisms**

Escherichia coli, Salmonella typhi and Staphylococcus aureus were collected from the Medical Veterinary Laboratory of National Root Crops Research Institute N.R.C.R.I, Umudike. The isolates were inoculated into appropriate culture media for isolation of the test organism and they were confirmed by carrying out Gram staining and other biochemical tests.

## GRAM STAINING

This test was used to classify all the bacterial isolates into gram positive and gram bacteria.

**Procedure**

Thin smear of overnight bacteria isolates wore made with a loop on a clean slide containing a drop of Physiological saline (Normal Saline). The smear was heat fixed by passing over Bunsen burner flame. It was stained with crystal violet for 60 seconds. This was then washed off with tap water and flooded with Lugol's iodine for 60seconds. This was also decolorized with acetone for 1 sec or immediately and washed off with slow running tap water. The smear was counter stained with safranin for 30 seconds and washed thoroughly with tap water. The slide was later placed on a slide rack to dry. After drying, oil immersion was placed on the stained smear and observed under the microscope with magnification of x 100.

**Biochemical Tests**

**Catalase test:** This is a test used to differentiate these bacteria which produce the enzyme -lase. 2-3mls of 3% freshly prepared hydrogen peroxide solution was poured on a test organism on a slide. A sterile applicator stick was used to pick a colony and immerse in the hydrogen peroxide solution. The presence of effervescence indicates catalase positive while the absence of effervescence indicates catalase negative reactions.

**Coagulate test**

This test is used to identify the organism which produces the enzyme coagulase. A drop of sterile distilled water was placed on a clean glass slide. A colony of the test organism was emulsified with the drop of distilled water to make a thick suspension. A loopful of human plasma was stirred into the suspension on the slide. Clumping visible to the eyes within 5-10 seconds indicates positive result.

**Sugar fermentation test**

About l.0g each of different types of sugar like glucose, lactose, mannitol and maltose were dissolved separately. To each 10ml of peptone water was added in test tube. This was followed by the addition of 2 drops of 0.1% phenol red. Durham tubes were also placed on to the test tubes inverted in order to detect the production of gas and acid. The tubes were autoclaved at 121°C for 15mins. And after cooling, the tubes were inoculated with bacterial culture using sterilized wire loop under aseptical condition. Un-inoculated tubes were used as the control

tubes were then incubated for 24/48h at 37°C. A colour change to yellow showed acid production and was recorded as positive fermentation.

The hanging drop method was used for this test. A loopful of the bacterial isolate was introduced into a clean cover slip held between two fingers. A circle was marked with Vaseline on a glass slide. The slide was then placed on top of the cover slip very well without touching the isolate. The slide was viewed under high power (x40) and oil immersion (x100) objective lens [13].

**Urease test**

The test was used to determine the bacteria that produce urease, an enzyme which breakdown urea to release ammonia. A loopful of the organisms was streaked on the surface of the urea medium plate. The plate was incubated at 37°C for 24 hours. Urease production and subsequent hydrolysis of urea results in the production of ammonia which increase pH of the medium. It changes from yellow to bright pink colour for positive reaction.

**Indole test**

The indole test is a biochemical test performed on bacterial specie to determine the ability of the organisms to convert tryptophan into indole. This test is used to identify Escherichia coli which produced the ammo acid tryptophan and also differentiate it from other Gram-negative organisms. Here 100ml of kovac's reagent was added to a peptone broth contain the organism. It was gently shacked and allow to stand so as to permit their agent to rise to the top. A positive result is indicated by a pink colour which develops on top of the solution layer.

**Triple sugar iron Agar test**

This test is used for the identification and confirmation of enteric bacteria (Enterobacteriaceae) from other Gram-negative intestinal bacilli by the ability to catabolism glucose, lactose or sucrose, and to liberate sulfides from ferrous ammonium sulfate or sodium thiosulphate.

TSI Agar slant contains 1% lactose, 1% sucrose and 0.1% glucose concentrations. The pH indicator (phenol red) is also incorporated into the medium to detect acid production from carbohydrate fermentation.

The sterile medium is inoculated by a zig-zag streaking and deep stabbing. The agar is incubated for 24 hours at 35°c,

E. coli will produce acid in butt, acid in the slant, gas but no hydrogen sulphide ($H_2S$).

Salmonella typhi will produce acidic butt, alkaline in slant, $H_2S$ will be produced and no gas. Reaction and Result of TSI Test is given bellow (TSI – 3).

**Sterility Test Of The Jatropha Crucas And Gongronema Latifolium Leaves Extracts**

Each of the above extracts (cold water and methanol extract) was tested for sterility. This was carried out by inoculating of each of them into Nutrient agar and incubated at37°C for 24 hrs. The plates were observed for growth. No growth in the plate after incubation indicated that the extracts were sterile. The extracts were then

assessed for antibacterial activity.

**Preparation Of Disc**

Whatman No 1 filter paper was cut into circular disc using perforator giving a diameter of 5mm. the discs were placed in glass Petri dishes and sterilized in hot air oven at160°C for 1 hour and stored in a cool dry place.

**Disc diffusion method**

This is done to determine the susceptibility pattern of Escherichia coli, Salmonella typhi and Staphylococcus aureus against extracts of Jatrophacurcas and Gongronema latifolium using a sterile Whatman No 1 filter paper disc. Each of the paper disc receive 0.2ml of the extract stock solution to give the concentration of 0.5%, 10%, 2.5%, 5% and raw of the crude extract per disc. They were kept I sterile freeze-dry temperature until needed the extracts were after inoculated on the surface of the streaked agar plate containing the test organisms and incubated at 37°C for 24 hours. The diameter of the zone of inhibition was observed and measured using a transparent ruler.

**Determination of antibacterial properties of the extracts**

Using medium diffusion method of [14],a lawn of bacterial isolate under investigation was prepared and sensitivity disc prepared from filter paper was impregnated with the extract was aseptically transferred unto the lawns. The lawns were incubated at 37°C for 24 hours; cleared zones of inhibition were seen, measured and reported in millimeter.

**Determination of minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) defined as the lowest concentration of the extracts that was able to inhibit any visible growth of microorganism. Dilution sensitivity method of Cheesebrough (2000) was employed. Different dilution concentration of the plant extract was prepared in a sterile test tube of 5%, 2.5%, 1% and 0.5%. Different dilutions were inoculated with 1ml of newly incubated isolate in 25mJ of the extract, the set were incubated for 24 hours. After incubation, the inoculum was taken from different diluents and plated out on Nutrient agar. The concentration that shows the MIC was recorded as minimum inhibitory concentration.

**Phytochemical Analysis**

The crude plants extract of Jatropah curcas and Gongronema latifolium was subjected to qualitative chemical screening for identification of various classes of active chemical constituents such as Tannin, Saponin, Flavonoid, Alkaloid and Phenol.

**Alkaloids**

2ml of extract was measured in a test tube to which ferric acid solution was added. An orange colouration indicated the presence of alkaloids.

**Saponin**

About 1ml solution of the extract was diluted with distilled water to 200ml and shaken in a graduated cylinder for 15min. foams were observed which indicated presence of saponins.

**Flavonoids**

Crude extracts were dissolved in a minimum amount of methanol. A few drops of concentrated hydrochloric acid were added, immediately a red colour developed. This indicates the presence of flavonoid.

**Tannin**

5ml solution of the extracts was taken in a test tube. Then 2ml of EeCLS and 2ml of potassium ferro-cyanide was added. Green colour was observed and this indicate the presence of tannins

**Phenol**

5ml of the extracts was added to a 50 ml, then 10 ml distilled water was used, followed by 2ml of ammonium hydroxide solution and 5ml of concentrated development. The absorbent of the solution was read at 505 nm.

**Interpretation Of Zone Of The Inhibition**

The result from the cultures shows that the organisms were sensitive to the plant extracts. The methanol Jatrophacurcas and Gongronema latifolium showed inhibition on the three test organisms (Escherichia coli, Salmonella typhi and Staphylococcus aureus.

The cold-water extract of J. Curcas has inhibitory range of 6.0m to 10mm one coli, 6.0mm to 10mm on Salmonella typhi and 6.omm to 10.0mm on Staphylococcus aureus. Gongronema latifolium had an inhibiting range of 6.0mm to 10mm on E. coli 6.0mm to 10mm on Salmonella typhi and 6.0mm to 10mm on 10mm on Salmonella and 6.6mm to10mm on Staphylococcus aureus.

The methanol extract of the J. curcas had a diameter zone of inhibition of 6.0mm to 14mm on E. coli, 60 mm to 10mm on salmonella and 6mm to 12mm on S. aureus, G, latifolium had a diameter of zone of inhibition of 6mm to 26mm on E coli, 6mm to 8mm on salmonella and 8mm to 12mm to 12 mm on S. aureus.

## RESULTS

The **(Table 3, 4)** below shows that the methanol and water extracts of J. curcas and G. latifolium inhibited different concentrations. At 2.5% concentration the two extract were able to inhibit all the tested isolates.

**Table 1:** Triple Sugar Iron Agar Test

| **Bacterium (organism)** | **Butt** | **Slant** | **$H_2S$** | **Gas** |
|---|---|---|---|---|
| Salmonella typhi | A | K | + | - |
| E. coli | A | A | - | + |

**Key:** A = Acid**,** K = Alkaline, - = Negative, + = Positive, K = Yellow**,** A = Red

**Table 2:** Morphological And Biochemical Characterization

| Morphological appearance | Cell shape | Gram stain | Catalase | Coagulase | Motility | Indole | Urease | Glucose | Lactose | Mannitol | Maltose | Probable |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Greenish metallic chain on EMB | Rod | - | + | - | + | + | - | AG | AG | AG | AG | Escherichia coli |
| Irregular colonies with black spot at the center on SS | Rod | - | + | - | + | - | - | AO | - | - | AO | Salmonella typhi |
| Yellow colonies with yellow zones in the media (MSA) | Cocci | + | + | + | + | - | + | AO | AG | AG | - | Staphylococcus aureus |

**Key words:** - = Negative, + = Positive reaction**,** AG = Acid and gas production**,** AO = Acid no gas**,** EMB = Eosin methylene blue Agar, SSA = Salmonella Shigella Agar**,** MSA = Mannitol Salt Agar

**Table 3:** Diameter Zone Of Inhibition Of Different Concentration Of The Extract.

| | Water extract of Jatrophacurcas | | | Methanol extracts of Jatrophacurcas(mm) | | |
|---|---|---|---|---|---|---|
| **Concentration of the extract** | **E.coli** | **S. typhi** | **S. aureus** | **E. coli** | **Salmonella typhi** | **S. aureus** |
| 5% | 6 | 6 | 6 | 6 | 6 | 6 |
| 2.5% | 10 | 10 | 10 | 14 | 6 | 12 |
| 1% | 10 | 10 | 6 | 8 | 6 | 8 |
| 0.5% | 8 | 10 | 6 | 8 | 10 | R |
| Raw | R | R | R | 6 | 6 | 10 |
| Gentamycin | R | R | 15 | R | R | 15 |
| Ciprofloxacin | R | 20 | R | R | 20 | R |

**Table 4:** Diameter Zone Of Inhibition Of Different Concentration Of The Extract.

| | Water extract of Gongronema latifolium (mm) | | | Methanol extracts of Gongronema latifolium (mm) | | |
|---|---|---|---|---|---|---|
| **Concentration of the extract** | **E.coli** | **S. typhi** | **S. aureus** | **E. coli** | **Salmonella typhi** | **S. aureus** |
| 5% | 6 | 6 | 10 | 16 | 6 | 10 |
| 2.5% | 10 | 6 | R | 26 | 7 | 10 |
| 1% | 7 | 6 | 6 | 10 | 6 | 10 |
| 0.5% | 6 | 8 | 10 | 8 | 8 | 12 |
| Raw | R | R | R | 6 | 8 | 8 |
| Gentamycin | R | R | 15 | R | R | 15 |
| Ciprofloxacin | R | 20 | R | R | 20 | R |

**Table 5:** Antibiotic Of Plant Extract On Tested Bacterial Isolates.

| **Extract concentration** | **E. coli** | **Salmonella typhi** | **Staphylococcus aureus** |
|---|---|---|---|
| **G. latifolium methanol 5%** | S | R | S |
| **G. latifolium water 5%** | R | R | S |
| **J. curcas methanol 5%** | R | R | R |
| **J. curcas water 5%** | R | R | R |

| | | | |
|---|---|---|---|
| **G. latifolium methanol 2.5%** | S | R | S |
| **G. latifolium water 2.5%** | S | R | R |
| **J. curcas methanol 2.5%** | S | R | S |
| **J. curcas water 2.5%** | S | S | S |
| **G. latifolium methanol 1%** | S | R | S |
| **G. latifolium water 1%** | R | S | S |
| **J. curcas methanol 1%** | S | S | R |
| **J. curcas water 1%** | S | S | R |
| **G. latifolium methanol 0.5%** | S | S | S |
| **G. latifolium water 0.5%** | R | S | S |
| **J. curcas methanol 0.5%** | S | R | S |
| **J. curcas water 0.5%** | S | S | R |

Keys: R = Resistant; S = Sensitive

In methanol extraction' of Gongronema latifolium no visible growth was seen at 2.5% concentration on Staphylococcus aureus and Salmonella typhi, no visible growth was seen at 1.0% concentration on Escherichia coli.

In methanol extraction of Jatropha curcas, no growth was observed at 2.5%concentration of Staphylococcus aureus and Salmonella typhi. Water extraction of J. curcas the minimum inhibitory concentration, MIC on E coli was 0.5% concentration and other disc not inhibit the organisms tested.

**Table 6:** Phytochemical Screening Of Jatrophacurcas And Gongronema Latifolium.

| **Test** | **Jatrophacurcas** | **Gongronema latifolium** |
|---|---|---|
| **Tannin** | - | + |
| **Flavonoid** | + | + |
| **Saponin** | + | + |
| **Alkaloid** | + | + |
| **Phenols** | + | + |

Key: + = positive reaction, - = negative reaction

## DISCUSSION

The results of this research shows that the methanol and aqueous extracts of J. curcas and G. latifolium have varying inhibiting effect on the test organism E. colt, salmonella and S. aureus. The extracts on bacterial isolate compared favourably with those of two standard antibiotics (Gentamycin and Ciprofloxacin).

Extracts from leaves were active against the strain of E. coli, but this bacterial isolate was resistant to synthetic

antibiotics ciprofloxacin and gentamycin. On the other hand, S. aureus was sensitive to gentamycin but ciprofloxacin couldn't inhabit their growth. Also, salmonella was sensitive to ciprofloxacin but gentamycin couldn't inhibit their growth. Unfortunately, this is due to the fact that microorganism gains resistance to some synthetic antibiotics with long term use while less resistance was found in medicinal plant. [18]. Aqueous and methanol leaf extracts of J. curcas and G. latifolium has been reported to exhibit antibacterial effect on a number of bacteria including E. coli, Salmonella sp and S. aureus [19,20].

The methanol extracts of leaves were found to be more effective against both grams tested **(Table 1,2)**. The inhibitory activities of plant extract are largely dependents on the concentration and the microbes tested[15].

The methanol extract had the highest activity against bacterial isolates; this may be attributed to the presence of soluble phenol compounds. Phytochemical studies have also shown that the antibacterial properties depend on certain active ingredients especially the oils such as saponins, tannins, flavonoids, phenol, phenolic, HCN and alkaloids. J. aureus and G. latifolium contain saponins and these have been known to be responsible for its antioxidant and antimicrobial properties [16] methanol has a high polarity index than water and thus is able to extract more phenolic and flavonoids compounds. Flavonoids are known to be inhibitory to Staphylococcus aureus and it has been used in treatment of inflamed tissues [17].

## CONCLUSION

In conclusion leaf extracts of J. curcas and G latifolium showed varying degree of antibacterial activity against E. coli, Salmonella typhi and S. aureiis. This spices act through their natural inhibiting mechanisms, either by antiseptic or killing the pathogens. This study has provided the basis for the use of these two plants in the treatments of inflamed gum, stomach pains and ailments caused by S. aureus, E. coli and Salmonella typhi. The potential antibacterial effects of the plants could be enhanced by extracting with methanol instead of water as applied in the traditional practices.

About 2.5% concentration of the plant extract was able to inhibit all the test bacterial spices, therefore J. curcas and G. latifolium is recommended at 2.5% concentration for the treatment of traveler's dysentery with stain blood stool, which is caused by E. coli, carbuncle, rashes or boil which is caused by S. aureus and typhoid fever cause by Salmonella typhi.

## REFERENCES

1. Alaebo PO, Onuoha UD, Ukpabi-Ugo JC, Enya E, Okakpu JC, et al. Phyto-chemical analysis and antimicrobial evaluation of aloe vera gel against some human and plant pathogens. International Journal of Phytology Research. 2013;3(2):1-6.
2. Wuhke W. Phototherapy in the treatment of mastodynia, premenstrual symptoms and mental cycle disorders. Gynekoloque 2000;33(1)36-39.
3. Abinu L, Adenipekun T, Adetowotan T, Ogunsanya T. Evaluation of the antimicrobial properties of different plants of citrus aurantifolia (lime fruits) as useilocally. African Journal Traditional Cambridge; 2007;4:85-90.

4. Ndukwe KC, Dkeke IN, Lamikanra A, Adesina SK, Aloderin O. Antibacterial activity of aqueous extracts of selected chewing sticks. Journal contemp; Deut Practical. 2007;3:86-94.
5. WHO. Traditional medicine growing need and potential. Perspectives on medicines, World Health Organization. Geneva 2002; 1-6.
6. Oliver BB. Medicinal plants in tropical West Africa, Cambridge University Press London. 2000;34
7. Aiyelalgbe OO. Antibacterial activity of jatrophamultiflda roots. African Journal Pharmacology. 2001; 12;3-7
8. Nahar K, Ozores-Hampton M. Link: Jatropha. An alternative substitute to fossil fuel University of florida. IFAS publication, Plant Biotechnology. 2011;1-19.
9. Kan AU. History of decline and present status of natural tropical forest in punjab. Practical In Tropical Biology 2002;63(3):210-250.
10. Ugochukwu NC, Babady NE, Camborne M, Gasset SR. The effect of Gonogronemalatifoliumleaf extracts on serum Lipid profile and oxidative stress of hepatocytes of diabetic rats. Journal of Biological Science 2003;28.1-5.
11. Onuoha UD, Alaebo PO, Ukpabi-ugo JC, Enya E, Okakpu JC, et al. In vitro Antimicrobial Activities of Methanol Crude Leaf Extract of Psidium guajava L. On Clinical Isolates of Multidrug Resistant Staphylococcus aureus. Asian Journal of Biotechnology and Genetic Engineering. 2003;6(1):39-45.
12. Enright M, Robinson D, Gaynor R, Edward F, Hajo G, Brian S. The evolutionary History of methicillin resistant Staphylococcus aureus (MRSA). Proceeding of the National Academy of Science of the United States of America 2002;99(2):7687-7692.
13. Onyeagba A. Laboratory guide for microbiology crystal publishers, Okigwe, Imo state. 2004; 95-97.
14. Okigbo RN, Ajalie AN. Inhibition of some human pathogens with tropical plant extract. International Journal of Molecular Medicine and Advanced Science. 2005;1(1):34-41.
15. Kalimuuthu IC, Vilauakamar S, Senthilkuma R. Antimicrobial infection of the biochemical Jatroph Curccs. International Journal of Pharmacology and Biological Sciences 2010;1:1-5.
16. Morebise O, Fafunod MA, Makinde JM, Olajide OA, Awe, EG. Antinflammtory property of Gongronema latifolium. Phytotherapentic Research. 2002;16(15):575-577.
17. Aires-de-sousa M, Concercoa T, De-Lencastre H. Unusually high prevalence of nosocomial panton valentine leukocidine positive Staphylococcus aureusisolates in Cape Verde Island. Journal Clinical Microbiology 2006;37:3787-3793.
18. Acar G, Dogan NM, Duru ME, Kivrak L. Phenolic Profiles, Antimicrobial and antioxidant activity of the various extracts of crocus species in anatolia. Africa Journal Microbiology Research 2010;14:115-1161.
19. Eleyimi AF. Chemical composition of antibacterial activity of Gongronema latifolium. Journal Zhejiang University Science 2007;8:352-358.
20. Oshodi AA, Amoo IA, Eleyinmi AF. Antimicrobial activity of aqueous extracts of Vernoniaamydalina, blends on some beer spoilage organisms. Technical quarterly of the master. Brewers Association of the American 2004;41(4); 398-402